GLEPAGLUTIDE

CLINICAL TRIAL PROTOCOL ZP1848-17111

EASE SBS 1

A PHASE 3, INTERNATIONAL, MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED TRIAL TO EVALUATE THE EFFICACY AND SAFETY OF GLEPAGLUTIDE IN PATIENTS WITH SHORT BOWEL SYNDROME (SBS)

Sponsor	Zealand Pharma A/S Sydmarken 11 DK-2860 Søborg Denmark
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Protocol version 1	31-May-2018

The trial will be conducted according to the protocol and in compliance with Good Clinical Practice (GCP), the Declaration of Helsinki and with other applicable regulatory requirements.

PROTOCOL APPROVAL / SIGNATURE PAGE

Protocol ZP1848-17111 Version 10.0 Dated: 27-Jan-2022

EASE SBS 1

A Phase 3, international, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of glepaglutide in patients with short bowel syndrome (SBS)

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1 Synopsis

Title of Trial: A Phase 3, international, multicenter, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of glepaglutide in patients with short bowel syndrome (SBS) **Short Title**: EASE SBS 1 (Efficacy And Safety Evaluation of Glepaglutide in treatment of SBS)	
Trial center(s): Approximately 33 sites in Europe and North America	
Studied period (years): Estimated date first patient enrolled: Q3 2018 Estimated date last patient completed: Q3 2022	**Phase of development:** 3
Objectives: **Primary Objective:** To confirm the efficacy of glepaglutide in reducing parenteral support (PS) volume in SBS patients **Secondary Objectives:** • To evaluate the efficacy of glepaglutide on other efficacy endpoints in patients with SBS • To evaluate the safety and tolerability of glepaglutide in patients with SBS	
Methodology: This is a multicenter, placebo-controlled, randomized, parallel-group, double-blind, fixed dose, Phase 3 trial to demonstrate the superiority of once weekly and twice weekly subcutaneous (SC) injections of 10 mg glepaglutide versus placebo in stable SBS patients. After providing informed consent and initial confirmation of eligibility during the 2-week Screening period, patients will enter a PS Optimization and Stabilization Phase before baseline measurements are performed. An individual drinking menu will be defined by the patient and the Investigator during the Screening period and until the end of the Optimization Phase. All patients will be equipped with an electronic diary (eDiary) for recording of trial relevant data/information. Unless otherwise specified, baseline is defined as Day 1, prior to first dosing of trial product. Optimization Phase During the Optimization Phase, the Investigator may change the PS volume and content if the patient is considered unstable or not optimized. Any changes in PS volume or content will be administered according to institutional standard practice. The effect of any PS optimizations must be investigated after 2 weeks. Prior to an Optimization Phase visit, the patient must measure his/her urine over 48 hours, while adhering to the pre-defined drinking menu, and report the urine volume and oral fluid intake in the eDiary. PS optimization consist of 2 rounds, which limits the Optimization Phase to a maximum duration of 4 weeks (± 4 days).). If optimization cannot be shown during the 4-week period, a second Optimization Phase of up to 4 weeks (± 4 days) is allowed. The last Optimization Phase visit can be combined with the first visit in the Stabilization period if the patient is considered optimized. Stabilization Phase The Stabilization Phase has a minimum duration of 2 weeks and a maximum duration of 4 weeks (± 4 days). The last visit of the Optimization Phase can also be the first visit of the Stabilization Phase. Prior to the Stabilization Phase visit, the patient must measure his/her urine over 48 hours, while adhering to the pre-defined drinking menu, and report the urine volume and oral fluid intake in the eDiary. Patients will be evaluated every 2 weeks during the Stabilization Phase and will need to fulfill the pre-specified stability criteria before the patient can be randomized. If stability cannot be shown during the 4-week period due to unforeseen events such as infections, illness or similar, a second Stabilization Phase of up to 4 weeks (± 4 days) is allowed.	

A patient will be considered stable if all the following criteria are met:

- Actual PS usage (volume and content) matches prescribed PS (± 10% deviation in volume is acceptable) and
- 48-hour urine volumes at 2 consecutive visits within a 2-week interval (± 4 days, i.e., visits should be 10 to 18 days apart) are similar (a maximum of ± 25% deviation is acceptable), while the oral fluid intake is constant (the two 48-hour oral intakes differ less than 10%) and maximum 3.5 L per day and
- Urine volume is on average ≥ 1 L and ≤ 2.5 L per day.

The Investigator and Medical Monitor must both agree and approve that the patient has met the criteria to be considered stable after completing the Stabilization Phase.

The baseline PS volume (L/week) will be defined as the actual PS volume received during the 7-day period prior to Visit 1 (Day 1). The baseline daily urine volume (L per day) will be defined as the average of the last two 48-hour urine volume measures from the Stabilization Phase. For scheduling of the 48-hour measurement periods throughout the trial, see Table 4.

Main trial period:

Visit 1 is done within 2 weeks after the last Stabilization Phase visit. All eligible patients who complete the Optimization and Stabilization Phases will be randomized in a 1:1:1 manner to receive either: a) glepaglutide 10 mg twice weekly, b) glepaglutide 10 mg once weekly and placebo once weekly, or c) placebo SC twice weekly for the following 24 weeks.

During the 24-week Treatment Phase, PS need will be evaluated by 48-hour balance periods involving urine measurements and during which patients will be required to keep to an individually pre-defined drinking menu (timing, volume, and content) and document this in the eDiary.

The actual volume of PS will be recorded on an ongoing basis in electronic diaries (eDiaries) by the patients. The Investigator will record the type, content, and volume of the PS being used. Once trial drug treatment is initiated, PS volume can be adjusted at trial visits (at Weeks 1, 2, 4, 8, 12, 16, 20, and 24) if the criteria for adjustment are met and according to a predefined algorithm.

Algorithm for PS volume reduction:

IF: daily average urine volume of the current visit is at least 10% higher than baseline urine volume.

THEN: New PS volume (weekly) = Current PS volume (weekly) – 7 x absolute increase in daily urine volume from baseline

The Investigator may arrange unscheduled visits (preceded by a 48-hour balance period) if he or she considers the visits to be needed based on medical judgement to assess PS volume needs.

It is acknowledged that intake of oral liquids and PS might have to be changed between scheduled visits to avoid edema, especially if treatment is effective. In such cases changes to the PS is at the discretion of the Investigator and the reason needs to be documented in the eCRF.

Any changes to the content of PS are left to the discretion of the Investigator and the reason is documented in the eCRF.

After completing the Treatment Phase, all patients (patients in all 3 treatment groups) will be eligible to enter an Extension Trial and receive glepaglutide. In addition, patients who were dosed but discontinued from trial treatment due to reasons other than an unacceptable adverse event (AE) related to the trial product or withdrawal of consent may be invited to enter the Extension Trial when completing the 24-week Treatment Phase schedule. For patients not entering the Extension Trial, a Follow-up Visit will be conducted 4 weeks after completion of the Treatment Phase.

Target Patient Population:

Key patient inclusion criteria include:

- Diagnosis of SBS defined as remaining small bowel in continuity of estimated less than 200 cm [equal to 79 inches] with the latest intestinal resection being at least 6 months prior to Screening and where the patient is considered stable with regard to PS needs. No restorative surgery planned in the trial period.
- PS requirement of at least 3 days per week as assessed prior to Screening and at the end of the Optimization and Stabilization Phases.
- Willing to adhere to an individual pre-defined drinking menu and urine measurement during the 48-hour measuring intervals.
- Age ≥ 18 years and ≤ 90 years at Screening.
- At randomization: Maintains a stable PS volume for at least 2 weeks prior to randomization.

Trial products, dosage and mode of administration:

Glepaglutide: Provided in single-use vials containing 1 mL (an extractable volume of 0.5 mL) of a clear, essentially colorless solution for injection, containing 20 mg/mL glepaglutide. Patients randomized to active treatment will inject 10 mg (0.5 mL) glepaglutide either a) twice weekly or b) once weekly and placebo once weekly.

Reference therapy: Placebo is provided in single-use vials containing 1 mL (an extractable volume of 0.5 mL) of clear, essentially colorless solution for injection. Patients randomized to placebo treatment will inject (0.5 mL) placebo solution twice weekly.

Intervention groups and duration:

Duration of treatment is 24 weeks across treatment groups.

- Glepaglutide twice weekly: 10 mg glepaglutide on Day 1 and on Day 4 or 5 (same days of the week every week)
- Glepaglutide once weekly: 10 mg glepaglutide or placebo on Days 1 and on Day 4 or 5 (same days of the week every week)
- Placebo: placebo on Day 1 and on Day 4 or 5 (same days of the week every week)

Endpoints:

Primary Endpoint:

Reduction in weekly PS volume from baseline to Week 24

Key secondary endpoints:

- Clinical response, defined as achieving at least 20% reduction in weekly PS volume from baseline to both Weeks 20 and 24
- Reduction in days on PS ≥ 1 day/week from baseline to Week 24
- Reduction in weekly PS volume from baseline to Week 12
- Reduction in weekly PS volume of 100% (weaned off) at Week 24

Secondary efficacy endpoints:

- Reduction of at least 20% in PS volume from baseline to both Weeks 12 and 24
- Change in fluid composite effect (FCE) from baseline to Week 24
- Reduction in calculated energy content of parenteral macronutrients from baseline to Week 24
- Reduction in number of days on PS per week from baseline to Week 24
- Reduction of at least 40% in PS volume from baseline to both Weeks 20 and 24
- PGIC improvement at Weeks 4, 12, 20, and 24

- Change in weight from baseline to Week 24

Other efficacy endpoints:

- Reduction in days on PS ≥ 2 days/week from baseline to Week 24
- Reduction in days on PS ≥ 3 days/week from baseline to Week 24
- Reduction in duration of PS infusions per week from baseline
- Concentration trough levels of glepaglutide and metabolites
- Change in plasma citrulline level from baseline to Week 24
- Change in weekly need for parenteral micronutrients (sodium, potassium, magnesium and calcium) from baseline to Week 24
- Change in patient-reported outcomes (SBS-I and EQ-5D-5L) from baseline to Week 24
    - Reduction in bowel movements or stoma bag emptying from baseline to Week 24 Safety endpoints:
- Incidence and types of AEs and serious adverse events (SAEs)
- Change in clinical evaluations:
- Vital signs
- Electrocardiogram (ECG)
- Change in safety laboratory assessments:
- Hematology
- Biochemistry
- Urinalysis
- Standard bone markers
- Immunogenicity

Statistical methodology:

Inferential statistical analyses of the primary and secondary efficacy endpoints will be performed. All comparisons will be between each glepaglutide treatment group and placebo. No statistical interim analysis is planned.

Analysis Sets

- The Full Analysis Set (FAS) will consist of all randomized patients, who received at least one dose of trial drug (glepaglutide or placebo). All efficacy analyses will be based on the FAS.
- The Per-protocol Analysis Set will consist of all FAS patients who do not experience any major protocol deviations. Final judgments on exclusion from the Per-protocol Analysis Set will be made prior to database lock. The Per-protocol Analysis Set will be used for supplementary analyses of the primary and key secondary efficacy endpoints.
- The Safety Analysis Set will consist of all randomized patients, who received at least 1 dose of trial drug (glepaglutide or placebo). This is the same definition as for the FAS, but the two can deviate in special circumstances. All safety analyses will be based on the Safety Analysis Set.

Analysis of primary and key secondary endpoints

The primary analysis of the primary efficacy endpoint uses a restricted maximum likelihood–based repeated-measures approach to compare treatment groups with respect to the mean change from baseline in weekly PS volume at Week 24. The model will use weekly PS volume assessments at Week 1, 2, 4, 8, 12, 16, 20, and 24, and will include the covariates for treatment group, baseline weekly PS volume, visit (categorical variable), stratification factor (weekly PS volume requirements <12 L/week versus >12 L/week), and visit-by-treatment group interaction. Missing values will be

imputed using multiple imputation methods. For the primary analysis, a Copy Reference approach is used with placebo treatment as reference, while for sensitivity analyses, a Jump to Reference and a Copy Incremental from Reference approaches will be applied. As a supplementary analysis, the analysis will be repeated using the Per-protocol Analysis Set.

The 4 key secondary efficacy endpoints will be analyzed to assess the treatment effect using the FAS. Continuous endpoints will be analyzed in a similar manner as the primary efficacy endpoint. The binary endpoint of clinical response will be tested using the Cochran-Mantel-Haenszel test with stratification on the randomization stratification factor (weekly PS volume requirements <12 L/week versus >12 L/week). As a supplementary analysis, the 4 key secondary endpoints analyses will be repeated using the Per-protocol Analysis Set.

Gatekeeping Procedure

A parallel gatekeeping testing procedure will be used to protect the overall type I error rate of α testing the primary endpoint together with the key secondary endpoints between each glepaglutide treatment group versus placebo. The twice weekly treatment group and once weekly treatment group comparisons to placebo will be tested by splitting α into 2 α/2 comparisons.

Secondary endpoints

The secondary efficacy endpoints will be analyzed to assess the treatment effect using the FAS. The tests will be non-hierarchical and type I error will not be adjusted for multiple testing. The other efficacy endpoints will be summarized to assess the treatment effect using the FAS. Only descriptive statistics will be presented for these other efficacy endpoints and no inferential statistics will be performed.

Safety Analysis

All safety analyses will be conducted using the Safety Analysis Set. No inferential tests of safety data will be performed. Descriptive summaries of safety data will be presented. Adverse events (treatment-emergent unless otherwise specified) will be presented by system organ class (SOC) and preferred term (PT) for each treatment group.

Discontinuations and Data Handling Rules

The analysis for the primary efficacy endpoint as well as for the continuous key secondary efficacy endpoints will handle missing values using a CR-imputed data set generated from the multiple imputation approach described for the primary efficacy endpoint. For all responder analyses, patients who discontinue for any reason will be considered as non-responders from the time of discontinuation onwards. No pooling of sites is planned for the efficacy analyses.

Number of patients (planned):

Approximately 108 patients are planned to be randomized into the trial 1:1:1.

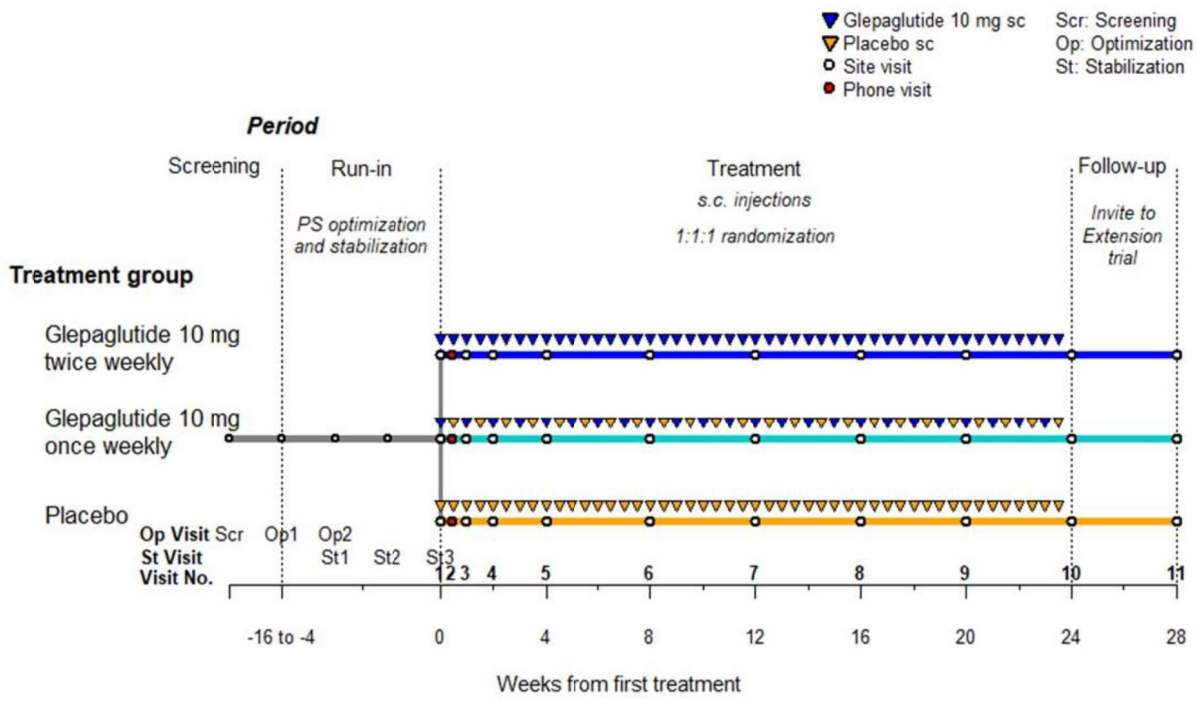

Figure 1: Trial Design

Table 1: Schedule of Assessments

Phase	Screening	Run in: PS optimization phase	Run-in: PS stabilization phase	Treatment phase									EOT	FU[19]
Visit day or week Time window (days)	up to 14 d prior to Op1	Duration: 2 to 4 weeks ±4d*	Duration: 2 to 4 weeks ±4d*	D1[18]	D3 ±1	D8 ±2 (W1)	D15 ±3 (W2)	D29 ±5 (W4)	D57 ±5 (W8)	D85 ±5 (W12)	D113 ±7 (W16)	D141 ±7 (W20)	D169 ±7 (W24)	D197 ±7 (W28)
Visit #	Sc	Op1, Op2 etc.	St1, St2 etc.	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	FU
Visit type (site, phone)	S	S	S	S	P	S	S	S	S	S	S	S	S	S
Informed consent	X[1]													
Inclusion/exclusion criteria	X			X										
Demographics (age, gender, race and ethnicity [if allowed in the participating country])	X													
Medical history and concomitant illness	X[2]													
SBS characteristics	X													
PS regimen (day, volume, and content)[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Definition of individual drinking menu (volume, content & timing)[4]	X	X												
Body weight/height (height at Sc only)[5]	X	X	X	X		X	X	X	X	X	X	X	X	X
Concomitant medications/procedures	X	X	X	X		X	X	X	X	X	X	X	X	X
ECG	X			X						X			X	
Vital signs (heart rate, blood pressure, body temp)	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
Physical examination (Full PE at Sc; SBS symptom-driven at all other visits)	X (full)			X		X	X	X	X	X	X	X	X	X
Colonoscopy	X[6]													
Laboratory														
Urine sample[7]	X	X	X	X		X	X	X	X	X	X	X	X	X
Pregnancy test for females of childbearing potential only	X	X	X	X				X	X	X	X	X	X	X
Hematology and Biochemistry [8, 9, 10]	X	X	X	X		X	X	X	X	X	X	X	X	X
Citrulline[11]				X				X					X	X
PK[11]				X		X	X	X	X	X	X	X	X	X

Phase	Screening	Run in: PS optimization phase	Run-in: PS stabilization phase	Treatment phase									EOT	FU[19]
Visit day or week Time window (days)	up to 14 d prior to Op1	Duration: 2 to 4 weeks ±4d*	Duration: 2 to 4 weeks ±4d*	D1[18]	D3 ±1	D8 ±2 (W1)	D15 ±3 (W2)	D29 ±5 (W4)	D57 ±5 (W8)	D85 ±5 (W12)	D113 ±7 (W16)	D141 ±7 (W20)	D169 ±7 (W24)	D197 ±7 (W28)
Visit #	Sc	Op1, Op2 etc.	St1, St2 etc.	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	FU
Visit type (site, phone)	S	S	S	S	P	S	S	S	S	S	S	S	S	S
Anti-drug Antibodies[11]				X			X	X	X	X			X	X
Bone Markers[12]				X									X	
HIV, hepatitis B, hepatitis C	X													
Diary: 48-hour oral fluid intake, fixed drinking menu		X	X	X**		X	X	X	X	X	X	X	X	X
Diary: 48-hour urine volume		X	X	X**		X	X	X	X	X	X	X	X	X
Diary 48-hour: Colon-in-continuity patients: Number of bowel movements Stoma patients: Number of stoma bag emptying			X	X**		X	X	X	X	X	X	X	X	X
Diary: PS use		X	X	X	X	X	X	X	X	X	X	X	X	X
Diary: Trial product administration (date and time of the day) + injection site (abdomen, thigh)				X										
SBS-I[13]		X	X	X		X	X	X	X	X	X	X	X	X
EQ-5D-5L[13]		X	X	X						X			X	X
PGIC[13]								X		X		X	X	
Exit interviews (Danish, French, German, UK and US sites only)													X	
Randomization				X										
Decision on dosing days schedule				X	(X)[14]									
Dispense trial product				X[15]				X	X	X	X	X		
Trial product return and accountability[16]						X	X	X	X	X	X	X	X	
Compliance check[17]				X	X	X	X	X	X	X	X	X	X	
Final Visit[19]													X	X

Abbreviations: d=day; FU=Follow-up; Op=Optimization Phase visit; SBS=short bowel syndrome; Sc=Screening visit; St=Stabilization Phase visit; V=visit; W=week

* If optimization/stabilization cannot be shown during the 4-week period, a second Optimization/Stabilization Phase of up to 4 weeks (± 4 days) is allowed.

** In case the patient is considered stable at the last two St Visits, it is not needed to conduct another 48-hour period prior to Visit 1.

1. Informed consent must be obtained before any trial related assessments incl. the start of the 48-hour oral fluid intake and urine volume measurement. Informed consent may be obtained prior to the Screening Visit.
2. Including detailed information on whether the patient has a history of encephalopathy, ascites, cholestasis, steatosis, and/or cirrhosis. If yes, the outcome / histopathologic diagnosis and date of histopathologic diagnosis is reported. Any history of drug/alcohol abuse is reported. Information on smoking and current use of alcohol will be reported.
3. PS regimen will be based on information from the eDiary.
4. Define 24-hour drinking menu, which will be repeated twice during the 48 hour balance periods. It can be adjusted until the end of the Optimization Phase. After this, it may not be changed. Provide information and instructions to patients for documentation in eDiary.
5. Patients are encouraged to measure their body weight at home weekly to detect fluid retention early. If the weight changes, patients should call the trial site for guidance.
6. For patients with remnant colon, colonoscopy should be performed and evaluated before start of Optimization Phase. Colonoscopies performed as part of routine clinical practice (and prior to provision of informed consent) up to 6 months prior to Screening (Sc) are acceptable. In case a remnant colon is not connected to the passage of foods and thereby dormant, a computerized tomography (CT) scan or magnetic resonance imaging (MRI) (if standard of care at site) will suffice at the discretion of the Investigator to document the absence of concerns regarding malignancy.
7. Urinalysis: Blood, glucose, leukocytes, pH, osmolality, protein, sodium, and potassium.
8. Hematology: Hemoglobin, hematocrit, red blood cell (RBC) count, white blood cell (WBC) count with differential, and platelet count. Biochemistry: Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, estimated CLcr, glucose, calcium, phosphorous, alkaline phosphatase, alanine aminotransferase (ALT), aspartate aminotransferase (AST), international normalized ratio (INR), gamma-glutamyl transferase (GGT), lactic dehydrogenase, conjugated bilirubin, total bilirubin, total protein, albumin, amylase, uric acid, C-reactive protein (CRP). In case of suspected liver injury based on increased ALT, AST, alkaline phosphatase, or total bilirubin, the tests should be repeated at 48-72 hours for evaluation of the event course/confirmation.
9. In addition, cholesterol and triglycerides will be measured orally fasting at visits 1 (Day 1) and Visit 10 (Day 169/Week 24).
10. In addition, magnesium and zinc will be measured at visits 1 (Day 1), 7 (Day 85/Week 12) and 10 (Day 169/Week 24).
11. Blood draws for PK, ADA, and citrulline sampling must be done prior to dosing, if dosing occurs on the day of the visit. In case of treatment discontinuation, the patient will be asked to come for ADA sampling at EOT (End of Treatment) as well as approximately four weeks after treatment discontinuation.
12. Bone markers include: 25OH vitamin D, parathyroid hormone (PTH), thyroid stimulating hormone (TSH; thyrotropin), P-CTx (collagen I, C-terminal telopeptide-fragments), and P-PINP (Pro-collagen, N-terminal pro-peptide).
13. Questionnaires must be completed at site visits prior to any other trial related assessment. It is recommended that the PGIC is completed first, followed by the SBS-I, then the EQ-5D-5L. The SBS-I and EQ-5D-5L are to be completed once during PS optimization phase, at the start of and after the stabilization phase, and all PROs are to be completed during treatment as indicated in the Schedule of Assessments. The exit interviews (Danish, French, German, UK and US sites only sites only) will be conducted no longer than 7 days after EOT visit.
14. Remind patient of the next dosing day.
15. Train the patient to self-inject.
16. Patient should be instructed to return all used vials on an ongoing basis.
17. Treatment compliance should be discussed with the patient to ensure that the medication is being taken correctly and that a new vial is used for each injection.
18. Visit 1 should be done within 2 weeks after the last Stabilization Phase. If done on the same day, Visit 1 lab samples should be drawn.
19. Patients entering the Extension Trial will have Final visit at Visit 10. Patients not entering the Extension Trial should come to the follow-up, including handing in the eDiary.

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3 List of Abbreviations and Definitions of Terms

The following abbreviations and specialist terms are used in this trial protocol.

Table 2: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
ADA	Anti-drug antibody
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
C_{avg}	Average steady-state plasma concentration in the dosing interval
CiC	Colon-in-continuity
CLCr	Clearance creatinine
CMH	Cochran-Mantel-Haenszel
CT	Computerized tomography
DILI	Drug-induced liver injury
DPP-4	Dipeptidyl peptidase-4
DUN	Dispensing Unit Number
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic data capture
eDiary(ies)	Electronic diary(ies)
EMA	European Medicines Agency
EOT	End of Treatment
EQ-5D-5L	European Quality of Life-5 Dimension 5 Level
ESPEN	European Society for Clinical Nutrition and Metabolism
FAS	Full Analysis Set
FCE	Fluid Composite Effect
FDA	Food and Drug Administration
FSH	Follicle Stimulating Hormone
GCP	Good Clinical Practice
GGT	Gamma glutamyl transferase
GLP	Glucagon-like peptide
HGH	Human growth hormone

Abbreviation or Specialist Term	Explanation
hCG	Human chorionic gonadotropin
HRQoL	Health Related Quality of Life
IBD	Inflammatory bowel disease
ICF	Informed consent form
ICH	International Council for Harmonisation
IEC	Independent Ethics Committee
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous
IWRS	Interactive web Response System
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
NOAEL	No observed adverse effect level
NYHA	New York Heart Association
PGIC	Patient Global Impression of Change scale
PK	Pharmacokinetic
PRO	Patient-reported outcome
PS	Parenteral support
QTc	Corrected QT interval
QTcF	QT interval corrected using Fridericia's formula
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBS	Short bowel syndrome
SBS-I	SBS-Impact Scale. A disease specific patient reported outcome questionnaire developed by Zealand Pharma
SC	Subcutaneous
SD	Standard deviation
SUSAR	Suspected unexpected serious adverse reaction
TNF-α	Tumor necrosis factor-alpha
ULN	Upper limit of normal

4 Introduction

4.1 Background

Patients with short bowel syndrome (SBS) are characterized by reduced intestinal absorptive surface area due to extensive surgical bowel resection or congenital diseases, such as recurrent Crohn's disease or mesenteric vascular disease[1,2,3,4], resulting in decreased absorptive capacity of the gut.[5] This causes a reduced uptake of nutrients, fluids, electrolytes, vitamins, and trace elements, leading to difficulties with maintaining metabolic balances when receiving a conventional diet.[6] The malabsorption can lead to dehydration, malnutrition, and weight loss if left untreated.[7]

Whereas less severely affected SBS patients are able to compensate for their malabsorption by increasing oral intake (hyperphagia) and adapt metabolically or pharmacologically, more severely affected patients depend upon the safe and well-adjusted provision of parenteral support of nutrients, fluids, electrolytes, vitamins, and trace elements to maintain body function, growth, homeostasis, and health.[8] For those dependent on parenteral support (PS), it is life-sustaining but at the same time associated with life-threatening complications. Among those are blood stream infections or sepsis,[9] central vein thrombosis, liver damage[10], and renal impairment.[11] In addition, the treatment burden of parenteral support is substantial. Often 10-18 liters of parenteral support per week are required. In addition to 10- to 12-hour over-night parenteral support infusions in such patients, additional hours during daytime may be required to compensate for losses. While liberating patients during daytime, night-time infusions disturb sleep and exacerbate the need for nocturnal urination. Adding to the treatment burden, patients on parenteral support often need frequent follow-up checks at the hospital, and for many there is a need for help from a homecare nurse.

Glucagon-like peptide (GLP)-2 is a specific intestinal growth factor that plays a role in enhancing small intestinal mucosal morphology, function, and integrity both under normal as well as pathophysiological conditions. Exogenous GLP-2 induces significant growth of the small intestinal mucosal epithelium via the stimulation of stem cell proliferation in the crypts and inhibition of apoptosis on the villi.[12] This trophic effect of GLP-2 has been observed in numerous species, including humans.[13] Additional effects of GLP-2 include inhibition of gastric emptying and gastric acid secretion, stimulation of nutrient absorption, enhancement of intestinal barrier function, and increase in intestinal blood flow.[13,14,15,16,17,18,19] The short half-life of native GLP-2 of 5-7 minutes in circulation is a major drawback for its use in a therapeutic setting, and with the approval of teduglutide (US: Gattex® EU: Revestive®) for the treatment of adult patients with SBS to improve intestinal absorption of fluids and nutrients, the therapeutic relevance of a GLP-2 analog in SBS has been established.

4.2 Key Characteristics of Glepaglutide

Glepaglutide is a potent, long-acting GLP-2 analog comprised of 39 L-amino acids, all of which are naturally occurring. Glepaglutide has 9 amino acid substitutions compared with native GLP-2 and a C-terminal structural inducing probe (SIP) tail consisting of 6 lysines. The sequence similarity of the back bone is about 64% compared with native GLP-2.

When glepaglutide is injected into the subcutaneous (SC) compartment, 2 well-described and functionally active metabolites are formed ($ZP1848_{1-34}$ and $ZP1848_{1-35}$). Under steady state conditions, the majority of the functional effect on the GLP-2 receptor derives from the main

metabolite ZP1848$_{1-34}$, which accumulates during continued use. Thus, the pharmacokinetic (PK) profile and the pharmacological effects of glepaglutide are described and should be understood as a composite effect of the drug substance and its 2 metabolites.

Glepaglutide shows significantly protracted PK. The half-life after SC injection has been shown to differ for glepaglutide and its 2 main metabolites, with the longest half-life observed for the main metabolite ZP1848$_{1-34}$. This suggests that a SC depot is formed and that release of glepaglutide and metabolites from the depot into the systemic circulation is the rate-limiting step that governs the plasma half-life.

The efficacy of 3 weeks of treatment with glepaglutide was demonstrated in a Phase 2 trial (Trial ZP1848-15073) in a small number of stable SBS patients.

This Phase 3 trial is being conducted to confirm the findings from the Phase 2 trial and to compare the safety and efficacy of glepaglutide versus placebo on the amount of PS needed for patients to maintain a satisfactory hydration level.

Based on data from the Phase 2 trial (ZP1848-15173) and the PK data from the Phase 1 PK trial (ZP1848-16182), 2 dosing regimens of glepaglutide – 10 mg once weekly and 10 mg twice weekly – will be investigated in order to find the optimal dosing regimen. See Section 6.1.1 for the rationale for these doses.

4.3 Risk-Benefit Assessment

4.3.1 Benefits

For the conducted dose-finding trial ZP1848-15073 testing glepaglutide in SBS patients with or without the need of parenteral support, the primary endpoint of change in wet weight of ostomy/diarrhea output ('wet weight output') was chosen as the most directly measure of the impact of glepaglutide on intestinal absorption. The trial met its primary efficacy endpoint by showing statistically significant and clinically relevant reductions in wet weight of ostomy/diarrhea output ('wet weight output') with glepaglutide dosed 1 mg/day (estimated reduction of 592 g/day; p=0.002) and 10 mg/day (estimated reduction of 833 g/day; p=0.0002). Results for wet weight absorption and urine weight supported the results for the primary endpoint, with statistically significant improvements demonstrated for 1 mg/day and 10 mg/day glepaglutide. In addition, absorption of macronutrients increased in the combined 1+10 mg and 1 mg dose groups, and improvements were observed for absolute absorption of sodium and potassium at the higher glepaglutide dose levels. In conclusion, the Phase 2, dose-finding trial of glepaglutide in SBS patients showed consistent and clinically relevant benefit for 1 mg/day and 10 mg/day glepaglutide in improving intestinal function. For further efficacy results please see the Investigator's Brochure for glepaglutide.

Patients receiving glepaglutide treatment in the present Phase 3 trial are likely to experience similar improvements in intestinal function, with reduced dependence on parenteral support as result. Trial patients completing the trial period (including those receiving placebo and patients who were dosed but discontinued from trial treatment due to reasons other than an AE related to the trial product or withdrawal of consent) will receive the opportunity to receive long-term glepaglutide treatment in an Extension Trial to the present trial.

4.3.2 Risks

Overall risk profile

The results from clinical and non-clinical studies and the safety profile described to date do not give rise to specific safety concerns.

Specifically, the completed non-clinical chronic toxicity program raises no concerns in relation to the extended treatment period of the present trial. The evaluation of chronic toxicity included a study in rats receiving up to 1, 3, and 10 mg/kg/day glepaglutide for 26 weeks and a study in Beagle dogs receiving 0.25, 1, and 5 mg/kg/day glepaglutide for 39 weeks. In both studies, glepaglutide caused a range of findings in the intestinal tract that were attributable to its pharmacological action. In the study in rats, changes occurred in the liver and kidney, which were likely physiological adaptations to high dose levels of the test material. The systemic no-observed-adverse-effect-level (NOAEL) in this study was therefore determined to 10 mg/kg/day. In the study in Beagle dogs, reduced weight gain was noted in females receiving the highest dose level of 5 mg/kg/day glepaglutide, and the systemic NOAEL in this study was therefore determined to 5 mg/kg/day in males and 1 mg/kg/day in females. Local irritation at the injection sites occurred at all dose levels in both studies. The identified NOAEL exposure level in rats and dogs is ≥86 and ≥48 times higher, respectively, than the expected maximum exposure level in this trial.

Glepaglutide was well tolerated at daily doses of up to 10 mg in the Phase 2 trial ZP1848-15073 conducted in SBS patients. Consistent with the clinical setting, the most frequently reported adverse events (AEs; reported in >20% of patients) in the phase 2 trial were nausea, abdominal pain, abdominal distension, vomiting, stoma complication, fatigue, dizziness, polyuria, decreased appetite, peripheral edema and cough. Treatment-emergent serious adverse events (SAEs) comprised 8 events, with no dose dependency or clustering of events being observed. Injection site reactions were dose dependent, mild to moderate in severity and transient by nature. The most frequently reported symptoms were itching and redness. No deaths were reported in this or any other trials with glepaglutide.

No specific safety issues were raised from the Phase 1 clinical trial program; for further details please see the Investigator's Brochure.

In addition to in the gastrointestinal tract, there are also GLP-2 receptors in the lung, brain, and hypothalamus[22]. So far, clinically significant off-intestinal targeted effects resulting from these additional receptor sites have not been seen.

Experiences with native GLP-2 and teduglutide suggest that expected common AEs for this class of compounds include abdominal pain and distension, injection site reactions, nausea, headache, upper respiratory tract infection, and (in some studies) vomiting, and fluid overload.[23]

Immunogenicity

Based on the current non-clinical and clinical knowledge of glepaglutide, the risk of immunogenicity (development of anti-drug antibodies [ADA]) following administration of glepaglutide is considered high. However, longer-term clinical treatment is required to investigate whether such a response will be transient or persistent. As no acute or non-acute AEs or effects on PK or pharmacodynamics have been linked to the immune response towards glepaglutide in the completed clinical trials, the effects and potential consequences of the anti-

glepaglutide response are so far considered of minor criticality. Glepaglutide ADA will be monitored in this trial, including their glepaglutide neutralizing potential and cross-reactivity to the main glepaglutide metabolite ($ZP1848_{1-34}$) as well as to native GLP-2.

Cardiovascular safety

No cardiovascular safety issues have been identified for glepaglutide. A concentration-response analysis of the potential of glepaglutide to cause QT prolongation ruled out any clinically concerning effect at the intended dose level, on which grounds a waiver for a dedicated TQT study was granted by the FDA in April 2018.

Patients with severe and acute cardiac disease are excluded from trial participation (see Section 7.3).

Neoplasms

GLP-2 stimulates development of colonic adenomas in rodent models.[20,21] Increases in plasma citrulline concentrations as seen with GLP-2 analog treatment might promote growth of existing tumors in patients during long-term treatment. Although the risk of malignancy is hypothetical in humans and colonoscopy can be difficult in these patients, a baseline colonoscopy has been suggested for patients taking GLP-2 analogs who have residual colons.[20] Therefore a screening colonoscopy (within 6 months prior to screening) is a requirement for patients in the present trial, and patients with a pre-existing recent history of cancer (except for select, treated, and highly curable *in situ* cancers) are excluded from the trial. These are considered adequate precautionary measures. Neoplasms (malignant and benign) are defined as AEs of special interest (AESIs) for the trial.

Risk of underdosing

The PK results and exposure-response analyses for glepaglutide substantiates that both once weekly and twice-weekly dosing of 10 mg glepaglutide result in glepaglutide concentrations within the therapeutically effective dose range. Regardless, a risk of inadequate dosing in individual patients receiving 10 mg glepaglutide once-weekly cannot be excluded.

4.3.3 Overall Benefit-risk Conclusion for the Trial

In conclusion, the benefit-risk ratio for the proposed glepaglutide treatment regimens is considered favorable for the intended trial population, and potential risks are considered appropriately handled and mitigated.

5 Trial Objectives

5.1 Primary Objective

To confirm the efficacy of glepaglutide in reducing PS volume in SBS patients.

5.2 Secondary Objectives

To evaluate the efficacy of glepaglutide on other efficacy endpoints in patients with SBS.

To evaluate the safety and tolerability of glepaglutide in patients with SBS.

5.3 Estimand

Translating the trial objective into a precise description of the treatment effect to be estimated, leads to four components that together define the estimand of interest:

1. The population is defined as patients with SBS randomized into the trial and having received treatment
2. The endpoint is the reduction from baseline in actual weekly PS volume after 24 weeks
3. The effect of twice weekly and once weekly glepaglutide 10 mg, regardless of discontinuing treatment or not, is of interest
4. The summary measure is the difference in endpoint means between active (twice weekly and once weekly glepaglutide 10 mg) and placebo

Estimand: Difference between mean reduction from baseline in actual weekly PS volume at 24 weeks in the SBS population regardless of whether treatment is discontinued.

The estimand is constructed based on the treatment policy strategy (ICH E9 (R1) addendum).

It is required to collect data after treatment discontinuation to get a reliable estimate of this estimand.

6 Investigational Plan

6.1 Trial Design Rationale

A double-blind, randomized trial design permits the unbiased assessment of the safety and efficacy of glepaglutide compared with placebo. Placebo was chosen as the comparator for this trial to allow for a direct comparison of glepaglutide with local standard of care for PS.

A 3-treatment, parallel-group design with a 1:1:1 randomization scheme (2 active treatment groups [once and twice weekly] and placebo) was chosen to compare the dosing regimen. The trial design is presented in Figure 1.

Originally, the trial was designed with a fixed samples size of 129 SBS patients (43 patients planned for each of the three treatment groups) without any interim analysis. Because the recruitment of new patients into the phase 3 trial ZP1848-17111 is impacted by COVID-19, the trial design has been amended to a reduced fixed sample size of approximately 108 SBS patients (36 patients planned for each of the three treatment groups).

A reduction of the weekly PS volume is the primary aim of the treatment to be confirmed in the present trial. The primary comparison to placebo will be made using the actual weekly PS reduction in L/week, which is a direct assessment of the decrease in burden for the patients. A reduction of at least 20% in the weekly need for PS volume is considered a clinically meaningful endpoint in the SBS patients and is included as a key secondary endpoint. This 20% reduction has previously been used in the teduglutide development program as the primary endpoint. A reduction in the need for parenteral support may further result in clinically meaningful benefits such as an increase in the number of days off of PS per week, decreased nocturia and less interrupted sleep, reduced infusion time per day, decreased stomal output or diarrhea, and reduced costs and resources associated with managing patients dependent on PS.

The target population consists of SBS patients with a stable need for PS at least 3 days per week. The exclusion criteria ensure that randomized patients are not put at any undue risk and that there are no concomitant diseases, conditions, or treatments that potentially could interfere with the interpretation of the data and results.

Key clinical safety risks include the potential for acceleration of neoplastic growth and enhanced growth of colorectal polyps, intestinal obstruction/stenosis, cholecystitis, pancreatitis, fluid overload, and immunogenicity. Patients will be screened to exclude those recently hospitalized due to SBS, those with uncontrolled inflammatory bowel disease, those with a history of cancer (except resected cutaneous basal or squamous cell carcinoma and *in situ* cervical cancer) unless it can be documented that the patient has been in a disease-free state for at least 5 years) and other disorders that may put the patient at an increased risk. Patients with any history of colon cancer are not allowed to be entered. Additionally, patients with a remnant colon are required to have a colonoscopy according to local standard practice performed as part of the screening procedures and before start of the stabilization phase. If the patient had a colonoscopy performed within the last 6 months prior to Screening as part of the standard of care of the disease, results from this will be acceptable. If a remnant colon is present, but not connected to the passage of foods and thereby dormant, a colonoscopy may not be appropriate and in this case a computerized tomography (CT) scan or magnetic resonance imaging (MRI) (if standard of care at site) will suffice at the discretion of the Investigator to document the absence of concerns regarding malignancy. During treatment with trial drug, patients will be carefully monitored for AEs, vital signs, and laboratory abnormalities. As no acute or non-acute AEs have been linked to the immune response to glepaglutide so far, samples for ADA will be analyzed following the last patient's last visit. In the event that a patient discontinues early, the patient will be asked to come for a site visit for ADA sampling at EOT (End of Treatment) and approximately four weeks after treatment discontinuation, so that a potential ADA response can be characterized and results related to the clinical picture. Additionally, an internal Safety Committee will routinely review blinded safety data to monitor and ensure patient safety.

6.1.1 Glepaglutide Dose Rationale

The selected doses for Phase 3 are 10 mg, once- or twice-weekly. The selected doses are considered supported with respect to both safety and efficacy, as described below.

Safety

The safety of the proposed doses is supported by the outcome of the completed clinical trials, which showed that SC administrations of 10 mg (or 20 mg) and total weekly doses of 10 mg, 70 mg or 140 mg all appeared well tolerated. No safety issues were raised in these trials, which included maximum plasma concentration (C_{max}) and overall exposure levels well above those proposed for phase 3. From a safety perspective, once- or twice-weekly SC injection of 10 mg glepaglutide is therefore considered to be appropriate dose levels for testing in Phase 3.

Efficacy

Using the data from the glepaglutide PK trial ZP1848-16182, the average steady-state plasma concentration in the dosing interval (C_{avg}) and the associated 90% prediction interval can be calculated for 10 mg glepaglutide dosed once weekly (based on observed data) as well as for twice-weekly dosing (based on estimated data). These average plasma concentrations and associated 90% prediction intervals are shown superimposed on the exposure-response curve

established for the Phase 2 primary endpoint in supporting that both once-weekly and twice-weekly dosing of 10 mg glepaglutide result in glepaglutide concentrations within the therapeutically effective dose range (see Figure 2).

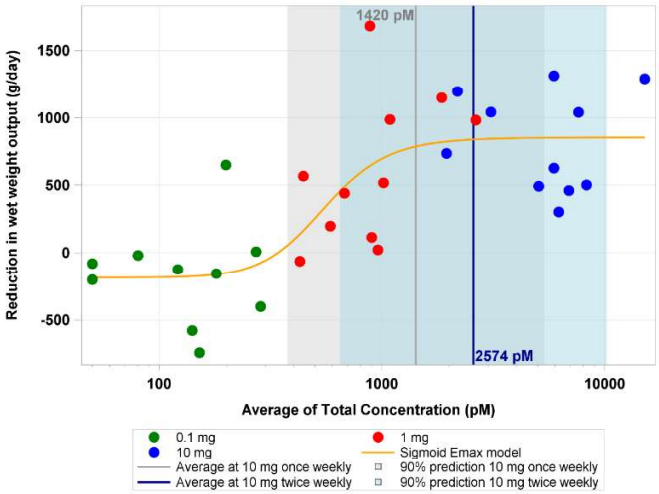

Figure 2: Exposure-Response Curve

Investigating the assumption that a minimum plasma concentration is needed to drive efficacy, the analysis predicts that a once-weekly dosing regimen of 10 mg glepaglutide would result in plasma concentrations within the therapeutically effective range for several days after dosing, but possibly falling below the therapeutically effective level during the latter phase of each weekly dosing interval. This dosing regimen is expected to result in a therapeutically relevant overall effect based on the current knowledge of biological activities related to GLP-2 receptor activation.

In conclusion for dose selection, the PK results and exposure-response analyses for glepaglutide substantiates that both once-weekly and twice-weekly dosing of 10 mg glepaglutide result in glepaglutide concentrations within the therapeutically effective dose range. To establish the optimal dosing interval, both a 10 mg once-weekly glepaglutide arm and a 10 mg twice-weekly glepaglutide arm are tested in this trial.

6.2 Overall Trial Design

After providing informed consent, and initial confirmation of eligibility during the 2-week Screening period, patients will enter a PS Optimization and Stabilization Phase before baseline measurements are performed. An individual drinking menu will be defined by the patient and the Investigator during the Screening Period and until the end of the Optimization Phase. All patients will be equipped with an electronic diary (eDiary) for recording trial relevant data/information. Unless otherwise specified, baseline is defined as Day 1, prior to first dosing of trial product. PS optimization consist of 2 rounds, which limits the Optimization Phase to a maximum duration of 4 weeks (± 4 days). If optimization cannot be shown during the 4-week period, a second Optimization Phase of up to 4 weeks (± 4 days) is allowed. The last Optimization Phase visit can be combined with the first visit in the Stabilization Phase if the patient is considered optimized.

During the Optimization Phase, the Investigator and the patient may redefine and optimize the individual drinking menu to best fit the patient's needs. Once the drinking menu has been set at

the end of the Optimization Phase, no additional modification to the drinking menu will be allowed and the patient will be required to adhere to the drinking menu during the 48-hour balance periods throughout the remainder of the trial.

Stabilization Phase

The Stabilization Phase has a minimum duration of 2 weeks and a maximum duration of 4 weeks (± 4 days). The last visit of the Optimization Phase can also be the first visit of the Stabilization Phase. Prior to the Stabilization Phase visit, the patient must measure his/her urine volume over 48 hours while adhering to the pre-defined drinking menu and report the urine volume and oral fluid intake in the eDiary. Patients will be evaluated every 2 weeks (± 4 days) during the Stabilization Phase and will need to fulfill the stability criteria (see below) before the patient can be randomized. If stability cannot be shown during the 4-week period due to unforeseen events such as infections, illness or similar, a second Stabilization Phase of up to 4 weeks (± 4 days) is allowed.

The baseline value for urine volume (L per day) will be defined as the average of the last two 48-hour urine volumes from the Stabilization Phase (e.g., visits St1 and St2 or visits St2 and St3).

The baseline PS volume (L/week) will be defined as the actual PS volume received during the 7-day period prior to Visit 1 (Day 1). No changes in the prescribed weekly PS volume or schedule is allowed during the Stabilization phase.

A patient will be considered stable if all of the following criteria are met:

- Actual PS usage (volume and content) matches prescribed PS (± 10% deviation in total volume is acceptable) and
- 48-hour urine volumes at 2 consecutive visits within a 2-week interval (± 4 days, i.e., visits should be 10 to 18 days apart) are similar (a maximum of ± 25% deviation is acceptable), while the oral fluid intake is constant (the two 48-hour oral intakes differ less than 10%) and maximum 3.5 L per day, and
- Urine volume is on average ≥ 1 L per day and ≤ 2.5 L per day.

The Investigator and Medical Monitor must both agree and approve that the patient has met the criteria to be considered stable after completing the Stabilization Phase. See Section 8.7 for more details.

Main Trial Period

Visit 1 is done within 2 weeks after the last Stabilization Phase visit. If done on the same day, Visit 1 lab samples should be drawn. All eligible patients who complete the Optimization and Stabilization Phases will be randomized in a 1:1:1 manner to receive 2 SC injections weekly of either: a) glepaglutide 10 mg twice weekly, b) glepaglutide 10 mg once weekly and placebo once weekly, or c) placebo twice weekly for the following 24 weeks.

During the 24-week Treatment Phase, PS need will be evaluated based on the 48-hour balance periods with a fixed drinking menu (individually pre-defined during the Optimization Phase) and measurements of urine volume done prior to each trial visit (except for Visit 2-phone assessment).

The actual volume of PS will be recorded on an ongoing basis in the eDiaries by the patients. The Investigator will record in the eCRF the type, content, and volume of the PS being used. Once trial drug treatment is initiated, PS volume can be adjusted at trial visits (at Weeks 1, 2, 4, 8, 12, 16, 20, and 24) if the criteria for adjustment are met and according to a predefined algorithm (Section 8.7.4).

The Investigator may arrange unscheduled visits (preceded by a 48-hour balance period) if he or she considers the visits to be needed based on medical judgement to assess PS volume needs.

It is acknowledged that intake of oral liquids and PS might have to be changed between scheduled visits to avoid edema, especially if treatment is effective. In such cases changes to the PS is at the discretion of the Investigator and the reason needs to be documented in the eCRF.

Any changes to the content of PS are left to the discretion of the Investigator and the reason documented in the eCRF.

After completing the Treatment Phase, all patients (patients in all 3 treatment groups) will be eligible to enter an Extension Trial and receive glepaglutide. In addition, patients who were dosed but discontinued from trial treatment due to reason other than an AE related to trial product or withdrawal of consent may be invited to enter the Extension Trial when completing the 24-week Treatment Phase schedule.

For patients not entering the Extension Trial, a Follow-up Visit will be conducted 4 weeks after completion of the Treatment Phase. Any post-treatment Follow-up Visit will be considered redundant if the patient is enrolled in the Extension Trial at the time of the Follow-up Visit.

6.3 Trial Endpoints

6.3.1 Primary Endpoint

Reduction in weekly PS volume from baseline to Week 24

6.3.2 Key Secondary Endpoints

- Clinical response, defined as achieving at least 20% reduction in weekly PS volume from baseline to both Weeks 20 and 24
- Reduction in days on PS ≥ 1 day/week from baseline to Week 24
- Reduction in weekly PS volume from baseline to Week 12

6.3.3 Reduction in weekly PS volume of 100% (weaned off) at Week 24Secondary Efficacy Endpoints

- Reduction of at least 20% in PS volume from baseline to both Weeks 12 and 24
- Change in fluid composite effect (FCE) from baseline to Week 24
- Reduction in calculated energy content of parenteral macronutrients from baseline to Week 24
- Reduction in number of days on PS per week from baseline to Week 24
- Reduction of at least 40% in PS volume from baseline to both Weeks 20 and 24
- PGIC improvement at Weeks 4, 12, 20, and 24

- Change in weight from baseline to Week 24

6.3.4 Other Efficacy Endpoints

- Reduction in days on PS ≥ 2 days/week from baseline to Week 24
- Reduction in days on PS ≥ 3 days/week from baseline to Week 24
- Reduction in duration of PS infusions per week from baseline
- Concentration trough levels of glepaglutide and metabolites
- Change in plasma citrulline level from baseline to Week 24
- Change in weekly need for parenteral micronutrients (sodium, potassium, magnesium, and calcium) from baseline to Week 24
- Change in patient-reported outcomes (SBS-I and EQ-5D-5L) from baseline to Week 24
- Reduction in bowel movements or stoma bag emptying from baseline to Week 24

6.3.5 Safety Endpoints

- Incidence and type of AEs and SAEs
- Changes in clinical evaluations:
  - Vital signs
  - Electrocardiogram (ECG)
- Changes in safety laboratory assessments:
  - Hematology
  - Biochemistry
  - Urinalysis
  - Standard bone markers
- Immunogenicity

6.4 End of Trial Definition

A patient will be considered as having completed the trial when:

- Patient completed EOT visit and exit interview, if consented (Danish, French, German, UK and US, sites only) and will continue participation in Extension Trial.
- Patient completed Follow-up (FU)/Final Visit and exit interview, if consented (Danish, French, German, UK, and US, sites only) and will not enter the Extension Trial.

6.5 Criteria for Premature Termination of the Trial and/or Trial Site

The Sponsor, the Institutional Review Boards (IRBs)/Independent Ethics Committees (IECs), or a Regulatory Authority may decide to stop the trial, part of the trial, or a trial site at any time.

The trial will be prematurely terminated overall or at a particular trial site at any time in the emergence of any safety information that could significantly affect continuation of the trial, i.e.

adverse events or other safety information that are considered unacceptable taken the indication into account and resulting in an unacceptable benefit-risk ratio. Guidance for when the Safety Committee will take action is provided in Section 11.4.6.

If the trial is suspended or prematurely terminated, the Investigator must inform the patients promptly and ensure appropriate therapy and follow-up. The Investigator and/or Sponsor must also promptly inform the Regulatory Authorities and IRBs/IECs and provide a detailed written explanation.

If, after the termination of the trial, the benefit-risk analysis changes, the new evaluation must be provided to the IRBs/IECs in case it has an impact on the planned follow-up of patients who have participated in the trial. If it has an impact, the actions needed to inform and protect the patients should be described.

7 Trial Patients

7.1 Number of Patients

A total of approximately 108 patients are planned to be randomized into the trial; approximately 72 to the glepaglutide treatment groups (36 in each of the 2 active treatment groups) and approximately 36 to the placebo treatment group, after the PS Stabilization Phase is completed. A screen failure rate of 15% is expected, hence, approximately 152 patients may need to be screened.

7.2 Inclusion Criteria

The patient must meet all of the following inclusion criteria:

1. Informed consent obtained before any trial-related activity.
2. Age ≥ 18 years and ≤ 90 years at Screening.
3. Diagnosis of SBS defined as remaining small bowel in continuity of estimated less than 200 cm [equal to 79 inches] and with the latest intestinal resection being at least 6 months prior to Screening and considered stable with regard to PS need. No restorative surgery planned in the trial period.
4. Requiring PS at least 3 days per week.
5. Willing to adhere to an individual pre-defined drinking menu during 48-hours measuring intervals.
6. Willing to maintain a stable weight (± 5%) for the duration of the trial (24 weeks).
7. Having:
    a. A stoma Or
    b. Colon-in-Continuity (CiC) with documented colonoscopy performed during Screening and which does not give rise to any safety concerns.
    1. Note: A colonoscopy performed within 6 months prior to Screening and not giving rise to any safety concerns is accepted. For patients with a remnant colon, which is not connected to the passage of foods and is thereby dormant, a computerized

tomography (CT) scan or magnetic resonance imaging (MRI) (if standard of care at site) will suffice at the discretion of the investigator.

8. Having a) a stoma Or b) colon-in-continuity (CiC) and able to separate stool and urine during the 48 hours measuring intervals.

7.3 Exclusion Criteria

The patient must be excluded from the trial if he or she meets any of the following criteria:

1. More than 2 SBS-related or PS-related hospitalizations (e.g., catheter related bacteremia/sepsis, bowel obstruction, severe water-electrolytes disturbances, etc.) within 6 months prior to Screening.
2. Poorly controlled inflammatory bowel disease (IBD) that is moderately or severely active or fistula interfering with measurements or examinations required in the trial.
3. Bowel obstruction.
4. Known radiation enteritis or significant villous atrophy, e.g., due to active celiac disease.
5. Cardiac disease defined as: decompensated heart failure (New York Heart Association [NYHA] Class III-IV), unstable angina pectoris, and/or myocardial infarction within the last 6 months prior to Screening.
6. Clinically significant abnormal ECG as judged by the Investigator.
7. Repeated (2 or more consecutive measurements separated by at least 15 minutes) systolic blood pressure measurements > 180 mm Hg.
8. HIV (human immunodeficiency virus) positive, acute liver disease, or unstable chronic liver disease.
9. Any history of colon cancer. History of any other cancers (except margin-free resected cutaneous basal or squamous cell carcinoma or adequately treated *in situ* cervical cancer) unless disease-free state for at least 5 years.
10. Estimated creatinine clearance (CLcr; by the Cockcroft-Gault formula) < 30 mL/min.
11. Hepatic impairment defined as:
    a. Total bilirubin ≥ 2 × the upper limit of normal (ULN), or
    b. Aspartate aminotransferase (AST) ≥ 5 × ULN, or
    c. Alanine aminotransferase (ALT) ≥ 5× ULN
12. Use of GLP-1, GLP-2, human growth hormone (HGH), somatostatin, or analogs thereof, within 3 months prior to Screening.
13. Use of dipeptidyl peptidase (DPP)-4 inhibitors within 3 months prior to Screening.
14. Systemic immunosuppressive therapy that has been introduced or has been unstable within 3 months prior to Screening.
15. Unstable biological therapy (e.g. anti-TNF-α, natalizumab, etc.) within 6 months prior to Screening, including significant changes in doses or switch of drug.

16. Females of childbearing potential, who are pregnant, breast-feeding, intend to become pregnant or are not using highly effective contraceptive methods. Highly effective contraception methods and definition of child-bearing potential are described in Section 11.4.4.
17. Known or suspected hypersensitivity to glepaglutide or related products.
18. Previous exposure to glepaglutide.
19. Previous participation (randomization) in this trial.
20. Current, or within 30 days prior to Screening, participation in another interventional clinical trial that includes administration of an active compound.
21. Mental incapacity or language barriers which preclude adequate understanding or cooperation, or unwillingness to comply with trial requirements.
22. Any condition or disease or circumstance that in the Investigator's opinion would put the patient at any undue risk, prevent completion of the trial, or interfere with the analysis of the trial results.
23. Committed to an institution by virtue of an order issued either by the judicial or the administrative authorities.
24. An employee of the sponsor or Investigator or otherwise dependent on them.

7.4 Randomization Criteria

The patient must meet all of the following criteria at the time of randomization:

2. Requiring PS at least 3 days per week and maintains a stable PS volume for at least 2 weeks. PS volume is considered stable if all of the criteria below are fulfilled:
    - Actual PS usage (volume and content) matches prescribed PS (± 10% deviation in volume is acceptable) and
    - 48-hour urine volumes at 2 consecutive visits within a 2-week interval (± 4 days, i.e., visits should be 10 to 18 days apart) are similar (a maximum of ± 25% deviation is acceptable), while the oral fluid intake is constant (the two 48-hour oral intakes differ less than 10%) and maximum 3.5 L per day and
    - Urine volume is on average ≥ 1 L per day and ≤ 2.5 L per day
3. No SBS-related hospitalizations within 30 days prior to randomization. Note: Hospitalizations related to trial procedures are allowed.
4. Since Screening, no poorly controlled IBD that is moderately or severely active or fistula interfering with measurements or examinations required in the trial.
5. No bowel obstruction since Screening.
6. No cardiac disease defined as: decompensated heart failure (New York Heart Association [NYHA] Class III-IV), unstable angina pectoris, and/or myocardial infarction since Screening.
7. No clinically significant abnormal ECG as judged by the Investigator.

8. Repeated (2 or more consecutive measurements separated by at least 15 minutes) systolic blood pressure measurements ≤180 mm Hg.
9. No use of GLP-1, GLP-2, human growth hormone (HGH), somatostatin, or analogs thereof since Screening.
10. No use of dipeptidyl peptidase (DPP)-4 inhibitors since Screening.
11. No systemic immunosuppressive therapy that has been introduced or has been unstable since Screening.
12. No unstable biological therapy (e.g. anti-TNF-α, natalizumab, etc.) since Screening, including significant changes in doses or switch of drug.
13. No unstable doses (including as needed use) within 2 weeks prior to randomization:
    - Antimotility drugs, e.g., loperamide, diphenoxylate, codeine or other opiates
    - H2 antagonists
    - Anti-diarrheal agents
    - Bile acid sequestering agents
    - Oral glutamine
    - Proton pump inhibitors
    - Diuretics
    - Systemic antibiotics or antibiotics affecting the gastrointestinal tract
    - Oral rehydration fluids
14. Females of childbearing potential, using highly effective contraceptive methods and who are not pregnant, breast-feeding, or intend to become pregnant.

7.5 Patient Withdrawal or Discontinuation from Trial Treatment

The patient is free to withdraw consent at any time during the trial. The patient's request to withdraw from the trial must always be respected.

All data collected from a withdrawn patient prior to withdrawal and during the final visit will be included in the analyses of the trial data. Although a patient is not obliged to give his/her reason(s) for withdrawing consent, the Investigator must make a reasonable effort to ascertain the reason(s), while fully respecting the patient's rights. An end of trial form must be completed and final drug accountability done even when patients are not able to come for a trial visit.

Only patients who withdraw their consent will be considered as withdrawn from the trial.

For patients who permanently discontinue from trial treatment (i.e. there is no plan to resume trial treatment or to enter the Extension Trial) every efforts must be made to ensure patients attend and complete all scheduled visit procedures to the extent possible. Patients should stay in the trial regardless of compliance with trial drug, assessments, or visit schedule.

The investigators should make a special effort to ensure that all patients complete the assessment of actual PS volume in Week 24 (primary endpoint).

Patients who permanently discontinue from trial treatment and the trial (without withdrawal of consent) should be asked to come to the site for an EOT visit during which all general, safety, and laboratory assessments should be performed as detailed in the schedule of assessments. During this visit, the Investigator should also discuss further treatment options for SBS with the patient. Note: if a patient is discontinued during a trial visit, efforts should be made to complete all trial-related assessments for the EOT Visit. If not participating in the Extension Trial, the patient should attend the FU/Final visit.

A patient must be discontinued from trial treatment if:

1. Any safety concerns or AEs that in the opinion of the Investigator might place the patient at unacceptable risk, including any deterioration of a patient's health state, especially in terms of the frequency and volume of total PS.
2. Any malignancy.
3. Increased liver values, defined as:
    a. ALT or AST >3 times baseline value lasting more than 2 weeks*, or
    b. ALT or AST >5 × ULN and total bilirubin >2 x ULN*, or
    c. ALT or AST >5 × ULN and INR >1.5*, or
    d. ALT or AST >5 × ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%) %)*.

    * where no other etiology exists
4. Pregnancy or intention to become pregnant.
5. The patient requires treatment with prohibited medications (see Section 8.6).

Patients can be discontinued from trial treatment if:

1. A patient was randomized despite it was later identified as having violated any of the inclusion, exclusion, or randomization criteria. The Investigator must discuss with the Sponsor's Medical Monitor if it is safe for the patient continue the trial treatment.

If a patient prematurely discontinues trial treatment, the primary reason must be specified in the eCRF and the Investigator must undertake procedures similar to those for the relevant visits as soon as possible. The intent should be to follow the patient to the extent possible according to the planned visit schedule and assessments with the purpose of following the safety and efficacy of the patients. Reason for trial visits not having been performed must be documented.

7.6 Trial Drug Pausation

If a patient experiences an AE, which is considered related to trial drug and which according to the Investigator requires dose pausation, the investigator may decide that the patient should skip the following week's doses (2 doses in total); i.e. the dose interval is increased; however, the injected dose remains the same. In that case, both the doses in the week need to be skipped in order to keep the blind.

If the AE does not abate and it continues to be considered related to trial product, the investigator may decide that the patient should continue to pause treatment but always so that both doses in a week are skipped. If the AE has not abated and the AE continues to be considered related to trial product and is not acceptable based on an overall assessment of benefit and risks, the patient

should be discontinued from the trial treatment but should, to the extent possible, adhere to the planned visit schedule.

The patient may resume treatment if the AE resolves and the Investigator deems it safe and in the patient's best interest to resume treatment. When resuming, the patient should start by taking Vial 1, followed by Vial 2.

If the same type of AE reappears with re-challenge and the nature of the event is not considered acceptable, the dosing should be stopped, but the patient should, to the extent possible, adhere to the planned visit schedule.

An accumulated maximum of 4 weeks of treatment pausation can be allowed. Hereafter, trial treatment should be discontinued (discontinuation due to adverse event) for the remainder of the trial, but patients should be encouraged to attend all remaining visit and complete all required assessments.

7.7 Patient Replacement

Randomized patients who have subsequently withdrawn consent or who have been discontinued from trial treatment will not be replaced.

Rescreening is allowed and can take place after a patient has failed the Optimization and Stabilization Phases (prior to randomization) if the Investigator judges that the patient is clinically stable.

8 Treatment of Patients

8.1 Treatment Assignment and Randomization

Eligible patients will be randomized in a double-blind fashion to 1 of 3 treatment groups in a ratio of 1:1:1:

- Glepaglutide 20 mg per week, administered as 10 mg SC injections twice weekly
- Glepaglutide 10 mg per week, administered as 10 mg SC injection once weekly and placebo SC injection once weekly
- Placebo, administered as SC injections twice weekly

All 3 treatment arms involve twice-weekly dosing (glepaglutide and/or placebo) to maintain the blind. The first dose is taken on Day 1 and the second dose should be taken on either Day 4 or Day 5 of each treatment week (interval chosen at the randomization visit and adhered to throughout the trial period). Please see Table 3 for a schematic overview.

Table 3: Dosing Regimen

Second Dosing 3 Days after Visit 1								
Day	1	4	8	11	15	18	22	…
Dispensing unit	1		2		3		4	
Twice weekly DB treatment	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	…
Second Dosing 4 Days after Visit 1								
Day	1	5	8	12	15	19	22	...
Dispensing unit	1		2		3		4	
Twice weekly DB treatment	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	Vial 2	Vial 1	…

Abbreviations: DB=double-blind

The choice of dosing days will be recorded. Both the patient and Investigator will be blinded to the actual content of each vial (active or placebo); therefore, it is important that the patient selects the correct vial for each administration. Randomization will be performed using a block randomization scheme stratified by patient's weekly PS volume requirements (< 12 L per week versus ≥ 12 L/week).

The randomization scheme will be generated prior to the initiation of the trial by an independent statistician/programmer who will not be a member of the trial team; Investigators will not be aware of the block size of the randomization scheme.
Patients will be randomly assigned to trial treatments using an automatic, interactive web response system (IWRS), which is integrated into the electronic data capture system (EDC) and has been validated for the intended use under Title 21 of the US Code of Federal Regulations (CFR) Part 11 (FDA regulation for Electronic Records and Electronic Signatures) and the International Council for Harmonisation (ICH) Guidance E6 for Industry on Good Clinical Practice.

8.2 Trial Drug Administration

Glepaglutide and placebo are delivered as ready-to use liquid formulation in vials. Patients will use single-use syringes, provided by Zealand Pharma, to withdraw 0.5 mL solution from a vial of drug product and inject it SC. Patients must choose their preferred injection site area; either on their abdomen or their thigh(s). Unless otherwise agreed with the Investigator, patients must use this selected injection site area during the entire trial. The specific injection site must be rotated for each weekly injection such that the injections are administered at least 5 cm away from the last injection administered, and still within the same injection site area, i.e., abdomen or thigh. It is acceptable to inject in 1 thigh or both thighs during the Treatment Phase.

The initial dose of trial drug will be administered by a trial staff member at the first visit (Day 1). The patient will be instructed by trial staff on the preparation, administration, storage, and return of trial drug at the Day 1 visit. Patients will be instructed to report the DUN (Dispensing Unit Number) and date and time of the day of trial drug administration. If dosing is on the same day as the visits (and in the morning), the trial drug should not be administered at home before

visiting the site as the trial drug is taken at the trial site shortly after the PK and ADA and other lab sampling and assessments.

8.3 Missed Doses of Trial Drug

Patients should not omit planned doses. Patients who miss a scheduled dose of trial drug should be instructed to administer the missed dose as soon as they become aware of the mistake; however, two doses should not be administered the same day.

8.4 Treatment Compliance

Treatment compliance is encouraged by patients receiving reminders from their eDiary for trial drug administrations. Patients are requested to enter details regarding trial drug administration (including date and time of the day as well as DUN) and injection site area used (i.e., abdomen, thigh) in the eDiary. Patients are to record PS use (including bag name, volume, infusion date, and duration) in the eDiary on an ongoing basis.

8.5 Concomitant Medications

Since the mechanism of action of glepaglutide may increase absorption of orally administered drugs (e.g., motility medications, warfarin, benzodiazepines, phenothiazines, and thyroid medications), consideration is advised when modifying concomitant oral medication regimens.

Down-titration of concomitant medications should be considered when drugs, including those with a narrow therapeutic range or those requiring titration, are given at dosages that are higher than usual. Investigators should monitor all patients carefully, since some of the patients have not previously been treated with a GLP-2 analog.

8.6 Prohibited Medications

GLP-1, GLP-2 analogs (teduglutide), HGH, DPP-4 inhibitors, and somatostatins are not allowed to be used by patients during the course of the trial.

Medications commonly used to treat SBS symptoms including anti-diarrhea agents are allowed during trial participation; however, changes (start, stop, new brands, or dose changes) should be kept to a minimum and the Medical Monitor be informed on the indication for the change. Any changes to concomitant medications must be documented in the eCRF.

8.7 Parenteral Support

Actual volume of PS will be recorded on an ongoing basis in the eDiaries by the patients. Type and content of the prescribed PS will be recorded in the eCRF by the Investigator. The PS volume and content should always be according to prescription. Patients will develop a personal drinking menu together with the Investigator before initiation of the Optimization Phase. During the Optimization Phase, the Investigator and patient will be allowed to redefine or optimize the drinking menu. Thereafter, no further changes to the drinking menu will be allowed and the patient will be required to adhere to the personal drinking menu for the remainder of the trial during the 48-hour balances periods. Thus, patients must follow this personal drinking menu during all subsequent 48-hour urine measurement days performed during the trial.

Once trial drug treatment is initiated, PS volume can be adjusted at trial visits if the criteria for adjustment are met and according to the algorithm described in Section 8.7.3. However, intake of oral liquids and PS might have to be changed to avoid edema, especially if treatment is effective.

The patient should discuss any changes with the Investigator, as requirement for changes in PS will be at the discretion of the Investigator and will have to be documented in the patient's records and the eCRF.

8.7.1 Algorithm for Parenteral Support Adjustments during the Optimization Phase

A patient is considered stable if:

- Actual PS usage (volume and content) matches prescribed PS (± 10% deviation in volume is acceptable) and
- 48-hour urine volumes at 2 consecutive visits within a 2-week interval (± 4 days, i.e., visits should be 10 to 18 days apart) are similar (a maximum of ± 25% deviation is acceptable), while the oral fluid intake is constant (the two 48-hour oral intakes differ less than 10%) and maximum 3.5 L per day, and
- Urine volume is on average ≥ 1 L per day and ≤ 2.5 L per day

During the Optimization Phase, the Investigator may change the PS volume and content if the patient is considered unstable or not optimized. Any changes in PS volume or content should be done according to institutional standard practice. The effect of any PS optimizations must be investigated after 2 weeks (± 4 days). Prior to an Optimization Phase visit, the patient must measure the volume of his/her urine over 48 hours, while adhering to the 48-hour pre-defined drinking menu. No more than 2 rounds of PS optimization are allowed to be performed, which limits the Optimization Phase to a maximum duration of 4 weeks (± 4 days). If optimization cannot be shown during the 4-week period, a second Optimization Phase of up to 4 weeks (± 4 days) is allowed.

8.7.2 Parenteral Support during Stabilization Phase

The Stabilization Phase has a minimum duration of 2 weeks and a maximum duration of 4 weeks. The last visit of the Optimization Phase can also be the first visit of the Stabilization Phase. Patients will be evaluated every 2 weeks (± 4 days) during the Stabilization Phase and will need to fulfill the stability criteria as listed in Section 8.7.1 before the patient can be randomized. It must be documented that the stability criteria are fulfilled, before a patient can be randomized. If stability cannot be shown during the 4-week period due to unforeseen events such as infections, illness, or similar, a second Stabilization Phase of up to 4 weeks is allowed. The baseline values for urine production will be the average values for 48-hour urine volume measured at the last 2 Stabilization Phase visits.

8.7.3 Algorithm for Parenteral Support Adjustments during Trial Drug Treatment

In general, patients should remain well hydrated during the trial. The urine production should stay above 1 L per day for all patients and the targeted daily urine production should be close to the baseline urine production for the individual patient.

Changes to the content of PS are left to the discretion of the Investigator. Once trial drug treatment is initiated, PS volume can be adjusted at Weeks 1, 2, 4, 8, 12, 16, 20, and 24 if the criteria for adjustment are met and according to the pre-defined algorithm below. Unscheduled visits (preceded by a 48-hour measurement period) may be considered by the Investigator as medically appropriate to adjust PS.

Prerequisite for PS volume reduction:

IF: daily average urine volume of the current visit is at least 10% higher than baseline urine volume.

THEN: New PS volume (weekly) = Current PS volume (weekly) – 7 × absolute increase in daily urine volume from baseline.

Algorithm

New PS volume (weekly) = Current PS volume (weekly) – 7 x (daily current urine volume – daily baseline urine volume)

Note: The current PS volume is defined as the latest prescribed PS volume, i.e. the PS volume that the patient should be following prior to the visit.

Example:

Patient receives 10 L of PS per week. The urine volume has increased from 1.3 L per day to 1.5 L per day. Hence, the urine volume has increased by more than 10% and PS volume should be reduced. The new weekly PS volume should be 8.6 L (detailed as 10 L – 7 × 0.2 L).

In cases where a patient may not have been fully compliant with the adherence to the prescribed PS volume, the drinking menu, and/or may have missed a urine measurement (e.g., in case the patient has missed to report a urine measurement during the 48 hour measurement period), the Investigator may still perform adjustments to the new PS volume at the Investigators discretion, in order to best match the needs of the patient and in adherence with the algorithm stated above. In that case, the reason for the change must be documented in the eCRF.

The patients should be instructed to contact the clinical site in case urine production falls below normal and if their clinical status changes.

Increasing PS volume:

Increasing PS volume should be considered if laboratory parameters indicate safety concerns. If the average urine production falls below 1 L per day, the Investigator should consider increasing PS volume.

8.7.4 Parenteral Support Volume Adjustments due to Clinical Signs

Especially in the beginning of the trial drug Treatment Phase, the Investigator must be in frequent contact with the patient. If the patient presents with clear adverse clinical signs of fluid overload, PS volume may be reduced without 48-hour urine measurements being available. During the next scheduled 48-hour measurement period however, further PS volume adjustments might have to be considered based on the results of the 48-hour urine measurement.

8.7.5 Parenteral Support Volume Adjustments during Unscheduled Visits

PS volume adjustments are allowed during unscheduled visits if the patient has performed a 48-hour urine volume measurement. In these cases, PS volume should be adjusted according to the algorithm described in Section 8.7.3.

9 Investigational Medicinal Product

9.1 Trial Drug

The investigational medicinal products to be used comprise 1 active dose strength of glepaglutide (10 mg) and a placebo formulation.

9.1.1 Glepaglutide

Glepaglutide is provided in a single-use vial containing 1 mL (an extractable volume of 0.5 mL) of a clear, essentially colorless solution for injection, containing 20 mg/mL glepaglutide.

9.1.2 Placebo

Placebo for glepaglutide is provided in a single-use vial containing 1 mL (an extractable volume of 0.5 mL) of clear, essentially colorless solution for injection.

9.2 Blinding and Breaking the Blind

Each unique dispensing unit will be labelled such that treatment blinding is ensured. It will not be possible to conclude from the appearance of the dispensing unit (box containing 2 vials) the treatment sequence allocated.

The Investigator, all site research personnel, trial patients, Sponsor, and Contract Research Organization (CRO) will be blinded to the allocated trial treatments until database lock

For pharmacokinetic and immunogenicity analyses the randomization codes will be supplied to the bioanalytical teams at Charles River (PK analyses) and Syrinx (ADA analyses). Blinding of samples are secured throughout the trial conduct until database lock. All PK and ADA samples are shipped from the central laboratory to the bioanalytical lab, regardless if the patient has been on placebo or active treatment. The randomization list is sent to the bioanalytical teams by an unblinded, trial independent, data system designer at the CRO.

Un-blinded results will only be shared with the Sponsor and the CRO after database lock.

In case of an emergency, where the knowledge of the trial drug treatment is important for the further treatment course, treatment assignment will be available in the IWRS set up within EDC to the blinded personnel. The Investigator may unblind immediately without any restrictions. However, unblinding should only be performed when the knowledge of the actual treatment administered will influence decisions on the future treatment of the patient. Date, reason for code break, and randomization number must be documented in the medical records. However, the Investigator should NOT reveal the actual treatment identification to sponsor or sponsor representatives. The Medical Monitor should be contacted immediately after the blind is broken. An automated notification will be sent to designated personnel at Zealand Pharma and Pharm-Olam if the blind is broken for a patient.

9.3 Trial Drug Packaging, Labeling and Storage

Trial drug vials will be packed and labeled into a treatment box containing two vials (unique dispensing unit), as appropriate according to the 3 treatment groups. The dispensing unit configuration will support administration of trial drug on Day 1 and Day 4/5, see Section 8.1.

Sponsor (or designee) will package and label the trial drug. The trial drug label will describe the storage conditions for the trial drug. The labels will supply no information about the patients.

Each dispensing unit will have a unique DUN for drug allocation, drug accountability, and traceability purposes.

Drug labels will be printed in local language and labeling will be performed according to Annex 13 of the Good Manufacturing Practice guidelines of the European Commission, ICH Good Clinical Practice (GCP) guidelines, local laws, and regulations.

For further information, refer to the Trial Material Manual.

The Investigator must ensure the availability of proper storage conditions. All drug supplies provided for this trial will be stored in a secure area with restricted access at the trial site.

The temperature should be monitored by recording the actual, minimum, and maximum temperatures using a calibrated thermometer or thermocouple, or by continuous recording using a qualified temperature monitoring system. The temperature should be evaluated and documented at least on working days on a temperature log. This log must be included in the Investigator Site File upon trial termination.

The Investigator (or designee) must inform Zealand Pharma or designee and the monitor immediately if any trial product has been stored outside the defined conditions (e.g., outside the temperature range).

Trial drug should be stored at 2°C to 8°C (36˚F to 46˚F).

Patients will be instructed to store the trial drug refrigerated at 2 to 8°C (36˚F to 46˚F); however, they are not required to monitor the storage temperature.

The vials should be maintained in the outer packaging (dispensing carton box) until immediately prior to trial drug use.

9.4 Accessory Supplies

Besides the trial products, patients will be provided with syringes, needles, swabs for disinfection, a safety bin for safe disposal of used syringes. For further details, see the Trial Material Manual.

9.5 Trial Drug Supply and Handling

The Sponsor will provide trial drug to each site for each patient for the duration of his/her participation in the trial. The Investigator, or designee will dispense trial drug at each dispensing visit. A sufficient quantity of trial drug will be dispensed to assure that the patient has sufficient drug supply to last at least until the next scheduled drug dispensing visit. No trial product may be dispensed to any person not enrolled in the trial.

Patients will be instructed to report in the eDiary the administration of the trial drug (DUN and date and time of the day) as well as injection site (abdomen or thigh).

Patients will be familiarized with the handling and use of vials and syringes prior to the first administration of trial drug, and as needed at the site. The Investigator must maintain an accurate record of the shipment and dispensing of trial drug as described in Section 9.6.

In case the Investigator, the site staff, or the monitor suspect that the trial drug is defective or potentially defective, Zealand Pharma should be contacted immediately.

9.6 Trial Drug Accountability

The Investigator must ensure that a designated person receives trial product deliveries from the Sponsor or designee and that all such deliveries are:

- Recorded
- Handled and stored safely and properly
- Only dispensed to trial patients according to the IWRS
- Returned to Sponsor or designee, as required

The Investigator or designee must keep drug inventory and accountability logs of which a copy must be given to the Sponsor at the end of the trial. An accurate record of the date and amount of trial drug dispensed to and returned from each patient must be available for inspection at any time. The inventory log will include details of the trial product received and dispensed to the patient. All used and unused vials and the dispensing cartons must be kept and returned to Sponsor after reconciliation of delivery records and accountability logs. Discrepancies between the amount of trial drug received, dispensed and returned must be reconciled.

Patients should be instructed to bring all their trial drug supply with them to each scheduled trial visit. Investigator or delegated staff must perform drug accountability with the patient at every visit to the clinic. This to enforce strict adherence to schedule and amount withdrawn and dosed from each vial. Returned trial drug (used or unused vials and the dispensing cartons) must be stored separately from non-allocated trial drug. The Investigator must not destroy any used or unused drug supplies. At the conclusion of the trial (or by agreement with Sponsor during the trial if storage capacity at site is limited) the Investigator will return all used and unused trial drug vials and the dispensing cartons to the Sponsor. Returned drug vials may also be destroyed locally according to trial site procedures. However, prior destruction of any vials it has to be approved by the sponsor via completion of the trial drug accountability forms. When the drug is destroyed the destruction certificate must be provided the sponsor. The Investigator's copy of the drug accountability forms must accurately document the return of all trial drug supplies to the Sponsor.

After self-administration, the patient should dispose the used syringe and needle in the container provided.

10 Trial Procedures by Visit

All trial visits will be performed on-site, except Visit 2, which will occur via a phone call from the trial coordinator or Investigator to the patient.

10.1 Screening

The Screening visit will occur 0 to 14 days prior to starting the Optimization Phase.

During the Screening visit, the following assessments will be performed:

- Obtain informed consent; must be signed before the start of any protocol-related assessments
- Confirm eligibility based on inclusion/exclusion criteria

- Document patient demographics
- Record medical history (including history of drug/alcohol abuse)
  - Record current concomitant illnesses and note relatedness to SBS
  - Record information on smoking and current use of alcohol
  - Record the details of whether the patient suffered from any of the following: Encephalopathy, ascites, cholestasis, steatosis, and/or cirrhosis. If yes, the outcome / histopathologic diagnosis and date of histopathologic diagnosis is recorded
- Document SBS characteristics (see Section 11.1.2)
- Record PS regimen (including weekly schedule, volume, and content)
- Record all concomitant medications and relevant previous treatments, including treatment with teduglutide, any other GLP-2 analogs or native GLP-2
- Perform a 12-lead ECG
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Measure body weight and height
- Perform a full physical examination
- Perform colonoscopy if needed (results from colonoscopy performed within 6 months of Screening visit are acceptable). In case of a dormant colon, i.e. a remnant colon is present, but not connected to the passage of foods, a colonoscopy may not be appropriate and in this case a CT scan or MRI (if standard of care at site) will suffice at the discretion of the Investigator
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only) or FSH (to confirm menopause)
  - Hematology
  - Biochemistry
  - HIV
  - Hepatitis B and C
- Agree with patient on individually fixed drinking menu for future 48-hours balance periods (including volume, content, and approximate time)
- Provide information and instructions for documentation in eDiary of:
  - PS use
  - 48-hour oral fluid intake
  - 48-hour urine volume

10.2 Optimization Phase

The first Optimization Phase visit should be scheduled within 2 weeks after the Screening Visit and a possible subsequent Optimization Phase visit should occur 2 weeks hereafter (duration 2 to 4 weeks ± 4 days). Thus the PS optimization consist of 2 rounds, which limits the Optimization Phase to a maximum duration of 4 weeks (± 4 days). If optimization cannot be shown during the 4-week period, a second Optimization Phase of up to 4 weeks (± 4 days) is allowed. The last Optimization Phase visit can be combined with the first visit in the Stabilization period if the patient is considered optimized.

During the Optimization Phase visit(s), the following assessments will be performed:

- Complete patient-reported outcome (PRO) questionnaires (preferably the SBS-I first followed by the EQ-5D-5L) at start of optimization phase
- Record prescribed PS regimen (including weekly schedule, volume, and content)
- Record body weight
- Record any procedures and changes to concomitant medications
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
- Review eDiary information for:
  - PS use
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - Agree with patient if any changes to the individually fixed drinking menu for future 48-hours balance periods are needed (including volume, content, and approximate time). Hereafter, no further changes will be allowed for the remainder of the trial.

10.3 Stabilization Phase

The Stabilization Phase visits should be scheduled approximately 2 to 4 weeks (± 4 days, i.e., visits should occur 10 to 18 days apart) after the Optimization Phase visit. The first Stabilization Phase visit can be combined with the last Optimization Phase visit if the patient is considered optimized. If stability cannot be shown during the 4-week period due to unforeseen events such as infections, illness or similar, a second Stabilization Phase (St4, St5, etc.) of up to 4 weeks (± 4 days) is allowed. Prior to the patient's planned visit, the Investigator should review the patient's eDiary information to ensure that the necessary data are available. If the information was not

entered into the eDiary, the patient's trial visit should be rescheduled to ensure availability of 48-hour balance measurements.

During the Stabilization Phase visit(s), the following assessments will be performed:

- Complete PRO questionnaires (preferably the SBS-I followed by EQ-5D-5L) at start of stabilization phase
- Record body weight
- Record any procedures and changes to concomitant medications
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
- Review eDiary information for:
  - PS use
  - 48-hour oral fluid intake
  - 48-hour urine volume output
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and drinking menu
- Instruct patient in eDiary completion of number of bowel movements (CiC patients) /number of stoma bags emptied (stoma patients) during the 48-hour balance periods

10.4 Treatment Phase (Up to 24 Weeks)

Once the last two Stabilization visits have occurred and both the Medical Monitor and the Investigator have reported that the patient is considered stable, the Visit 1 can be scheduled. The Visit 1 should be planned to occur within 2 weeks after the last Stabilization Phase visit.

Note: Prior to the patient's planned trial visit the Investigator should review the patient's eDiary information to ensure that the necessary data are available. If the information was not entered into the eDiary, the patient's trial visit should be rescheduled.

10.4.1 Visit 1 (Day 1)

During Visit 1, the following assessments will be performed:

- Confirm eligibility based on inclusion/exclusion criteria and that the patient meets the stable PS regimen criteria

- Complete PRO questionnaires (preferably the SBS-I first followed by EQ-5D-5L)
- Record any procedures and changes to concomitant medications
- Perform 12-lead ECG
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Obtain urine sample for urinalysis
- Obtain blood samples prior to dosing for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
  - Citrulline
  - PK
  - ADA
  - Bone markers
- Review eDiary information for (if applicable):
  - PS use
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and drinking menu
- Upon approval from Medical Monitor of the stabilization criteria, randomize patient into treatment group using the IWRS criteria set up within EDC
- Administer trial drug and review self-administration procedures, clean-up procedures, and eDiary assignments with patient
- Dispense trial product to patient and discuss treatment compliance with the patient
- Decide on dosing schedule (Day 1 and 4 or Day 1 and 5) (See Table 3)

10.4.2 Visit 2 (Day 3 ± 1 day)

Visit 2 will occur via a phone call by the Investigator or designee to the patient on Day 3.

During Visit 2, the following assessments will be performed:

- Record adherence to PS regimen (including weekly schedule, volume, and content)
- Record AEs
- Confirm patient is recording the following information in the eDiary:
  - PS use
  - Trial product administration (including injection site, time of administration)
- Remind patient of next dose and discuss treatment compliance with the patient

10.4.3 Visit 3 (Week 1/Day 8 ± 2 days)

During Visit 3, the following assessments will be performed:

- Complete SBS-I
- Record any procedures and changes to concomitant medications
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - Hematology
  - Biochemistry
  - PK (drawn prior to dosing if dosing on same day)
- Review eDiary information for:
  - PS use
  - Trial product administration (including injection site, time of administration)
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)

10.4.4 Visit 4 (Week 2/Day 15 ± 3 days)

During Visit 4, the following assessments will be performed:

- Complete SBS-I
- Record any procedures and changes to concomitant medications

- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - Hematology
  - Biochemistry
  - PK (drawn prior to dosing if dosing on same day)
  - ADA (drawn prior to dosing if dosing on the same day)
- Review eDiary information for:
  - PS use
  - Trial product administration (including, injection site, time of administration)
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)
- Collect and account for returned trial product

10.4.5 Visit 5 (Week 4/Day 29 ± 5 days)

During Visit 5, the following assessments will be performed:

- Complete PGIC and SBS-I
- Record any procedures and changes to concomitant medications
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology

- Biochemistry
- Citrulline (drawn prior to dosing, if dosing on same day)
- PK blood sample (drawn prior to dosing, if dosing on same day)
- ADA (drawn prior to dosing, if dosing on same day)

- Review eDiary information for:
  - PS use
  - Trial product administration (including injection site, time of administration)
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)
- Patient to return all used and unused vials and the dispensing cartons. Account for returned trial product
- Dispense trial product to patient

10.4.6 Visit 6 (Week 8/Day 57 ± 5 days)

During Visit 6, the following assessments will be performed:

- Complete SBS-I
- Record any procedures and changes to concomitant medications
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
  - ADA (drawn prior to dosing, if dosing on same day)
  - PK (drawn prior to dosing if dosing on same day)
- Review eDiary information for:
  - PS use

- Trial product administration (including injection site, time of administration)
- 48-hour oral fluid intake
- 48-hour urine volume
- 48-hour bowel movements/stoma bag emptying

- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)
- Patient to return used and unused vials and the dispensing cartons. Account for returned trial product
- Dispense trial product to patient

10.4.7 Visit 7 (Week 12/Day 85 ± 5 days)

During Visit 7, the following assessments will be performed:

- Complete PROs (PGIC, SBS-I, and EQ-5D-5L)
- Record any procedures and changes to concomitant medications
- Perform 12-lead ECG
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
  - PK (drawn prior to dosing, if dosing on the same day)
  - ADA (drawn prior to dosing if dosing on the same day)
- Review eDiary information for:
  - PS use
  - Trial product administration (including injection site, time of administration)
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying

- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)
- Patient to return used and unused vials and the dispensing cartons. Account for returned trial product
- Dispense trial product to patient

10.4.8 Visit 8 (Week 16/Day 113 ± 7 days)

During Visit 8, the following assessments will be performed:

- Complete SBS-I
- Record any procedures and changes to concomitant medications
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
  - PK (drawn prior to dosing if dosing on the same day)
- Review eDiary information for:
  - PS use
  - Trial product administration (including injection site, time of administration)
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)
- Patient to return all used and unused vials and the dispensing cartons. Account for returned trial product
- Dispense trial product to patient

10.4.9 Visit 9 (Week 20/Day 141 ± 7 days)

During Visit 9, the following assessments will be performed:

- Complete PGIC and SBS-I
- Record any procedures and changes to concomitant medications
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
  - PK blood sample (drawn prior to dosing, if dosing on the same day)
- Review eDiary information for:
  - PS use
  - Trial product administration (including injection site, time of administration)
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)
- Patient to return all used and unused vials and the dispensing cartons. Account for returned trial product
- Dispense trial product to patient
- Schedule patient Exit Interview in Clinigma patient interview portal, if consented (Danish, French, German, UK, and US, sites only), to be conducted no longer than 7 days after EOT visit

10.4.10 Visit 10 (End of Treatment; Week 24/Day 169 ± 7 days)

During Visit 10, the following assessments will be performed:

- Complete PROs (PGIC, SBS-I, and EQ-5D-5L)
- Record any procedures and changes to concomitant medications
- Perform 12-lead ECG
- Record body weight

- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
  - Citrulline (drawn prior to dosing, if dosing on the same day)
  - PK (drawn prior to dosing, if dosing on the same day)
  - ADA (drawn prior to dosing, if dosing on the same day)
  - Bone markers
- Review eDiary information for:
  - PS use
  - Trial product administration (including injection site, time of administration)
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Record adherence to PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3
- Patient to return all used and unused vials and dispensing cartons. Account for returned trial product
- Decision whether the patient will participate in the Extension Trial or not
  - If not participating
    - The patient should resume standard of care and the treating physician is advised to pay careful attention to the PS regimen
    - The patient should attend the Follow-up/Final visit
  - If the patient agrees to enter the Extension Trial:
    - Complete the end-of-trial form in the eCRF
  - Exit Interview (selected countries only)
    - The exit interviews, if consented (Danish, French, German, UK and US sites only sites only) will be conducted no longer than 7 days after EOT visit

- Investigator to review the transcript of exit interview for potential AE reporting within 2 days after receiving the transcript.

10.5 Follow-up/Final Visit

Note: Prior to the patient's planned Follow-up visit the Investigator should review the patient's eDiary information to ensure that the necessary data are available. If the information was not entered into the eDiary, the patient's trial visit should be rescheduled. Follow-up/Final Visit is only applicable for patients who will not enter the Extension Trial.

10.5.1 Follow-up Visit (Week 28/Day 197 ± 7 days)

Patients who are not entering the Extension Trial should return the eDiary. During Follow-up Visit, the following assessments will be performed:

- Complete PROs (SBS-I and EQ-5D-5L)
- Record any procedures and changes to concomitant medications
- Record body weight
- Record vital signs (heart rate, systolic and diastolic blood pressure, and body temperature)
- Record AEs
- Perform short physical examination (driven by SBS symptoms)
- Collect urine sample for urinalysis
- Obtain blood samples for:
  - hCG pregnancy test (for females of childbearing potential only)
  - Hematology
  - Biochemistry
  - Citrulline
  - PK
  - ADA
- Review eDiary information for:
  - PS use
  - 48-hour oral fluid intake
  - 48-hour urine volume
  - 48-hour bowel movements/stoma bag emptying
- Complete end-of-trial form in the eCRF

10.6 Unscheduled Visits

If required for patients' safety, unscheduled visits can be performed, including any of the following as applicable:

- Review eDiary information for:
  - PS use
  - 48-hour oral fluid intake
  - 48-hour urine volume
- Record PS regimen (including weekly schedule, volume, and content) and modify weekly PS volume and schedule in accordance with Section 8.7.3)
- Lab sampling: In case of suspected liver injury based on increased ALT, AST, alkaline phosphatase, or total bilirubin, the tests should be repeated at 48-72 hours for evaluation of the event course/confirmation

11 Trial Assessments

11.1 General Assessments

11.1.1 Demographics and Medical History

Demographic information and medical history will be obtained at Screening. Demographic information to be collected includes age, gender, race, and ethnicity (if allowed in the participating country).

Medical history information to be collected includes all ongoing conditions and relevant/significant medical history (including all major hospitalizations and surgeries). Concomitant illness must also be captured and noted as to relatedness to SBS. Patient's history of drug and/or alcohol abuse, information on tobacco smoking (never smoker (<100 cigarettes or equivalent /lifetime), current smoker, or former smoker) and current alcohol use will also be captured.

11.1.2 SBS Characteristics and Disease History

Information about the patient's SBS will be obtained at Screening and include the following characteristics:

- The underlying cause of SBS
- Date of diagnosis of underlying disease
- Date of SBS diagnosis
- Remaining bowel sections[28]; whether patient has a colon-in-continuity (jejuno-colic anastomosis/SBS anatomical group 2, jejuno-ileo-colic anastomosis/SBS anatomical group 3, or other), an end-jejunostomy/SBS anatomical group 1, ileostomy/SBS anatomical group 1, or colostomy, the surgery dates and reason(s) for resection, this includes the most recent resection
- Bowel lengths; length of the remnant small bowel, the remnant colon in percent (according to Cummings classification[29])
- Severity of SBS based on the ESPEN Functional and Clinical Classification of Chronic Intestinal Failure[8]
- Date that the patient started PS

11.1.3 Concomitant Medications

All prescription and non-prescription medications taken within 7 days prior to and including the Screening visit will be recorded in the eCRF. Any changes to concomitant medications that occur throughout the trial will be recorded.

All relevant previous treatments, including treatment with teduglutide, any other GLP-2 analogs or native GLP-2 are recorded in the eCRF.

11.1.4 Drinking Menu

An individual fixed drinking menu should be decided upon by the patient and the Investigator based on the patients' drinking habits and preferences during the Screening visit.

The drinking menu should be followed throughout the 48-hour urine volume measurement periods (which means that the 24-hour drinking menu will be repeated twice during the 48 hours).

11.2 Assessments of Efficacy

11.2.1 Patient Diaries

Patients will be required to complete eDiaries throughout the course of the trial. The patient will record the following:

- Trial drug administration (including date and time of day) and injection site area used
- PS use
- During 48-hour measurement periods:
    - Oral fluid intake based on the individually pre-defined drinking menu
    - Urine volume
    - Number of bowel movements resulting in defecation/stoma bags emptied
    - The Investigator (or designee) must review each patient's diary entries routinely throughout the entire trial period. If clarification of entries or discrepancies in the diary is needed, the patient must be questioned and a conclusion made in the patient's medical record and in the diary. Care must be taken not to bias the patient.

11.2.2 Parenteral Support Use and Volume

Parenteral support use and volume will be assessed based on trial visit records of weekly average of PS (day, volume, and content), and patient's daily diary entries. Data will be checked at all trial visits.

11.2.3 Parenteral Calories and Nutrients

Parenteral calories (macro-nutrients) and micro-nutrients will be assessed based on the prescribed PS regimen (type, volume, and frequency) and via patient's daily diary entries of patient-reported PS regimen.

11.2.4 48-hour Urine Volume

Urine volume will be recorded by the patient in his or her diary during the defined 48-hour measurement periods prior to a trial visit. During this time the patient must adhere to the

individually pre-defined drinking menu (see Section 11.1.4). The patient will be provided with urine measuring cups.

The 48-hour urine volume measurements should occur as close as possible to and within 7 days of the coming site visit and should be timed with the PS schedule. Patients on PS 7 days weekly must perform the 48-hour measurement on 2 consecutive days on PS. Patients on PS 1-6 days weekly must record the 48-hour measurement at 1 day on PS and 1 day off starting on the day on PS. See Table 4 for further clarity. The grey boxes indicate the possible 48-hour balance periods. If more than one interval is available any 1 of them can be chosen.

The patient should perform the 48-hour measurement on the same days of the week during each period as long as the PS regimen remains the same. The selected days will be recorded in the eDiary. If the PS regimen changes during the trial a new schedule for 48-hour measurement can be chosen and recorded in the eDiary.

Table 4: Timing of 48-hour Measurements

PS days/ Week	48-hour measurement	Day 1*	Day 2*	Day 3*	Day 4*	Day 5*	Day 6*	Day 7*
7 days	**2 days on PS**	1	2	3	4	5	6	7
6 days	**1 day on PS + 1 day off PS**	1	2	3	4	5		6
5 days	**1 day on PS + 1 day off PS**	1		2	3	4		5
4 days	**1 day on PS + 1 day off PS**	1		2		3	4	
3 days	**1 day on PS + 1 day off PS**	1		2		3		
2 days**	**1 day on PS + 1 day off PS**	1				2		
1 day**	**1 day on PS + 1 day off PS**	1						
0 days**	**2 days off PS**							

* Relative to PS week days

** < 3 days of PS per week is only allowed after reduction during trial participation.

The patient will, if possible, be contacted prior to the scheduled start of the 48-hour measurement period and reminded to start measuring complete oral fluid intake and urine volume, and to record these measurements in the eDiary.

11.2.5 Plasma Citrulline Levels

Plasma citrulline levels will be measured at Visit 1 (prior to first dose of trial drug), Visit 5 (Day 29/Week 4), Visit 10 (Day 169/Week 24), and at Follow-up Visit (Day 197/Week 28), if

applicable. After at least 8 hours of orally fasting, plasma citrulline blood samples will be taken in the morning and analyzed at a central laboratory.

11.2.6 Patient-Reported Outcomes

The PGIC, SBS-I and EQ-5D-5L[24] PROs will be used to investigate the effects of treatment on health-related quality of life (HRQoL).

Questionnaires must be completed in paper format at site visits prior to any other trial related assessment. The PROs will be completed by the patient without assistance of site personnel. When the PGIC, SBS-I, and the EQ-5D-5L questionnaires are to be completed, patients is recommended to complete the PGIC first. When only the SBS-I and EQ-5D-5L are to be completed, it is recommended that the SBS-I is completed first. The PROs must not be completed at home before the patient attends the visit. Patients will be instructed to complete the PRO in a private area without influence from trial team members or accompanied by family or friends. No one is allowed to answer or interpret items for the patient. The Investigator or a delegated trial team member is allowed to read items/answers options to the patient aloud if the patient is unable to read. The Investigator or delegated trial team member will instruct the patient to complete every item in the PROs and explain that there are no right or wrong answers. The Investigator or a delegated trial team member will instruct the patient to give the best answer they can and explain that all responses will remain confidential.

Immediately after completion, the PROs will be reviewed by the Investigator (or designee) for completeness and potential AEs. When reviewing the PROs for AEs the Investigator should not influence nor question the patient on the content of their response to PRO questions. Review of the PROs must be documented. If entries are missing in the PROs, the patient should be asked to answer all questions. Care should be taken not to bias the patient.

The Investigator and/or delegated trial team members will receive training and instruction in completion of the PROs prior to the conduct of the trial.

Patients will report in the eDiary their number of bowel movements/stoma bag emptying during the 48-hour balance periods.

11.2.7 Health Economic Assessments

The health economic assessments will collect the number and duration of hospitalizations due to SBS-related issues during the 24- to 28-week trial period (including follow-up if not continuing in Extension Trial). Information will include reason for admission, dates and duration of stay and medications given and procedures performed.

11.2.8 Exit interview

A telephone exit interview will be conducted by an external provider on a subset of patients upon completion of the placebo-controlled, double-blind treatment period. The purpose of the exit interview will be to gain insight and understanding of patients' experiences with treatment and to assess the clinical meaningfulness of reducing parenteral support. See the separate Exit Interview Manual for full research.

11.3 Assessment of Safety

11.3.1 Safety Parameters

Safety will be assessed by the following parameters:

- AEs
- Weight
- Vital signs
- ECGs
- Clinical laboratory tests (hematology, biochemistry, and urinalysis)
- Immunogenicity

11.3.2 Vital Signs

The following vital signs will be collected at every trial visit (except Visit 2-phone assessment): body temperature (ºC or ºF) (measured according to the site's usual procedure), heart rate (beats/min), and seated diastolic and systolic blood pressure (mm Hg). During treatment period visits, vital signs will be collected before trial drug injection.

11.3.3 Weight and Height

Body weight (kg or lb) will be measured at Screening, during the Optimization and Stabilization Phases, and at every trial visit (except Visit 2-phone assessment). During weight measurements patients should wear light clothing and no shoes. Stoma bags should be emptied prior to the measurement. At Screening Visit height (cm or inches) will be measured and the patient's body mass index (BMI) will be calculated.

Patients should be encouraged to measure their body weight at home weekly to detect potential signs of fluid retention early (before edema becomes readily visible). If the weight changes, patients should be instructed to call the trial site for guidance.

11.3.4 Physical Examination

Patients will undergo a full physical exam at Screening. The physical exam will consist of an examination of the following: General Appearance, Skin and mucosae, Head, Ears, Eyes, Nose and Throat incl. thyroid gland, Heart, Lung, Chest (incl. breast), Abdomen (incl. genitourinary system), Nervous System, Lymph Nodes, and Musculoskeletal. Abnormalities on physical examination will be recorded in the patient's medical notes. Clinically significant changes from baseline examination that are noted during follow-up will be recorded as an AE on the appropriate eCRF pages.

For all subsequent site visits during Treatment Phase, an abbreviated physical exam will be completed. The body systems included in these exams will be based on Investigator judgment and/or patient symptoms.

11.3.5 Electrocardiogram (ECG)

A 12-lead ECG will be obtained at Screening and at Visits 1 (Day 1; prior to the start of the first trial drug), 7 (Day 85/Week 12; prior to dosing of trial drug in case drug is administered this

day), and 10 (Day 169/Week 24). Additional ECGs will be performed for cause as needed to evaluate AEs.

ECG parameters (heart rate, PR, QRS, QT, QTcF, RR) and any abnormality will be recorded and described in the eCRF including the Investigator's assessment of clinical significance ('abnormal, not clinically significant' or 'abnormal, clinically significant'). Any clinically significant deterioration of a pre-existing condition as well as any new clinically significant findings will be recorded as AEs (see Section 11.4).

11.3.6 Laboratory Assessments

Safety laboratory tests, including hematology, biochemistry, and bone markers will be performed as specified below, and in the Schedule of Assessments (Table 1). Blood samples should be obtained prior to trial drug dosing. In total approximately 280 mL blood is drawn.

Safety laboratory tests are to be performed and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner). Safety laboratory samples will be analyzed via central laboratories.

11.3.6.1 Hematology

Hemoglobin, hematocrit, red blood cell (RBC) count, white blood cell (WBC) count with differential, and platelet count will be measured at Screening, at the Optimization and Stabilization Phase visits, and at every trial visit (with the exception of Visit 2-phone assessment).

11.3.6.2 Biochemistry

The following parameters will be measured (or calculated) at every trial visit (with the exception of Visit 2-phone assessment): Sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, CLcr (estimated), glucose, calcium, phosphorous, alkaline phosphatase, ALT, AST, international normalized ratio (INR), gamma-glutamyl transferase (GGT), lactic dehydrogenase, conjugated bilirubin, total bilirubin, total protein, albumin, amylase, uric acid, and C-reactive protein.

In case of suspected liver injury based on increased ALT, AST, alkaline phosphatase, or total bilirubin, the tests should be repeated at 48-72 hours for evaluation of the event course/confirmation. When drug-induced liver injury (DILI) is suspected based on the transferases values, the liver functionality needs to be evaluated by the bilirubin and INR for confirmation/information of DILI.

Cholesterol and triglycerides will be measured orally fasting at Visits 1 (Day 1) and 10 (Day 169/Week 24).

Magnesium and zinc will be measured at Visits 1 (Day 1), 7 (Day 85/Week 12), and 10 (Day 169/Week 24) only.

11.3.6.3 Bone Markers

The standard bone markers collected are: 25OH vitamin D, parathyroid hormone (PTH), thyroid stimulating hormone (TSH; thyrotropin), P-CTx (collagen I, C-terminal telopeptide-fragments), a marker of bone resorption and P-PINP (Pro-collagen, N-terminal pro-peptide) a marker of bone

formation. Blood samples for bone markers will be obtained at Visit 1 (Day 1) and Visit 10 (Day 169/Week 24) only.

11.3.6.4 Urinalysis

Urinalysis will be measured at every trial visit (with the exception of Visit 2-phone assessment). Urinalysis will include: blood, glucose, leukocytes, pH, osmolality, protein, sodium and potassium.

11.3.6.5 Pregnancy Screen

A pregnancy test (serum beta-human chorionic gonadotropin [hCG]) will be completed for all females of childbearing potential at Screening, at Optimization Phase visit 1 (Op1), Stabilization Phase visit 1 (St1), at Visit 1 (Day 1), and from Visit 5 (Day 29/Week 4) to Visit 10 (Day 169/Week 24). Sterilized or infertile patients, i.e., patients who have undergone surgical sterilization (vasectomy/bilateral tubectomy, hysterectomy, and bilateral ovariectomy) or women who are postmenopausal, which is defined as > 52 years of age and 12 months or more with no menstruation prior to enrollment and/or as FSH level >30 mIU/mL (as measured at site) are not regarded as being of childbearing potential.

11.3.6.6 Immunogenicity

Serum samples will be obtained prior to dosing (if dosing occurs on the visit day) at Visit 1 (Day 1), 4 (Day 15/Week 2), 5 (Day 29/Week 4), 6 (Day 57/Week 8), 7 (Day 85/Week 12), 10 (Day 169/Week 24), and Follow-up Visit (Day 197/Week 28) (if applicable) to determine the presence of ADA and the ADA titer. Each ADA sample will contain 5 mL of blood, resulting in 30 mL (35 mL with Follow-up Visit) of blood drawn for ADA analysis.

The serum samples will be analyzed using a tiered approach (screening, confirmation, and titration of confirmed anti-glepaglutide-antibody positive samples), followed by characterization of ADA-positive samples for *in vitro* ZP1848-neutralizing potential and cross-reactivity to the major metabolite ($ZP1848_{1-34}$) and GLP-2. Results will not be revealed to the sites.

All ADA samples will be retained at the special lab (Syrinx Bioanalytics Oy, Finland) or long-term storage facility (MLM Medical Labs GmbH, Germany) until drug approval by the US FDA and/or EMA or 15 years after end of trial at maximum. The retained ADA samples may be used for further confirmation and characterization of detected ADAs if required by health authorities or for safety reasons.

11.3.6.7 Pharmacokinetics

A blood sample will be obtained prior to administration of trial drug (if dosing occurs on the visit day) at every trial visit during the Treatment Phase (except for Visit 2-phone assessment) and the Follow-up Visit (Day 197/Week 28). The PK sampling schedule is set in order to be able to correlate PK values to ADA data and to obtain sufficient information from the dosing regimens tested. Results will not be revealed to the sites.

Each PK sample will contain 6 mL of blood, resulting in 60 mL of blood drawn for PK analysis.

11.3.6.8 HIV, Hepatitis B, and Hepatitis C

A blood sample will be obtained at screening to confirm the HIV, hepatitis B and C status. If positivity is confirmed, the patient will be excluded from participation in the trial.

11.4 Adverse and Serious Adverse Events

Timely, accurate, and complete reporting and analysis of safety information from clinical trials are crucial for the protection of patients. Investigators and the Sponsor are mandated by regulatory agencies worldwide to report the safety information.

11.4.1 Definitions

11.4.1.1 Adverse Events

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

AEs include:

- A clinically significant worsening of a concomitant illness
- A clinical laboratory AE: a clinical abnormality which is clinically significant, i.e., any abnormality that suggests a disease and/or organ toxicity and is of a severity that requires active management. Active management includes active treatment or further investigations, e.g., change of dose or more frequent follow-up due to the abnormality.

The following should **not** be considered as AEs:

- Pre-existing conditions, including those found as a result of screening procedures (pre-existing conditions should be reported as medical history or concomitant illness).
- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial-related activity after the patient has signed the informed consent.

When assessing an AE, the following definitions will be used:

Severity:

- **Mild:** No or transient symptoms, no interference with the patient's daily activities
- **Moderate:** Marked symptoms, moderate interference with the patient's daily activities
- **Severe:** Considerable interference with the patient's daily activities, which the patient finds unacceptable. A severe reaction does not necessarily deem the AE as serious and an SAE are not always severe in nature.

Causality:

- **Probably:** Good reason and sufficient documentation to assume a causal relationship
- **Possibly:** A causal relationship is conceivable and cannot be dismissed
- **Unlikely:** The event is most likely related to etiology other than the product
- **Not related:** No relationship to product.

Outcome:

- **Recovered/resolved:** The patient has fully recovered, or by medical or surgical treatment, the condition has returned to the level observed at the first trial-related activity after the patient signed the informed consent
- **Recovering/resolving:** The condition is improving and the patient is expected to recover from the event. This term is only applicable if the patient has completed the trial or has died from another AE
- **Recovered/resolved with sequelae:** The patient has recovered from the condition, but with lasting effect due to a disease, injury, treatment, or procedure. If a sequela meets an SAE criterion, the AE must be reported as an SAE
- **Not recovered/not resolved**: The condition of the patient has not improved and the symptoms are unchanged, or the outcome is not known
- **Fatal:** This term is only applicable if the patient died from a condition related to the reported AE. Outcomes of other reported AEs in a patient before he/she died should be assessed as "recovered/resolved", "recovering/resolving", "recovered/resolved with sequelae", or "not recovered/not resolved". An AE with fatal outcome must be reported as an SAE
- **Unknown**: This term is only applicable if the patient is lost to follow-up.
-

11.4.1.2 Adverse Events of Special Interest (AESI)

In this trial the following events are to be regarded as AESI, should they occur: neoplasms (malignant and benign), suspicion of liver injury, pancreatitis, and cholecystitis.

If the event is reported as an SAE, the timelines for SAE reporting apply. If the event is reported as non-serious AE, it should be reported in the dedicated eCRF page within 2 working days (if at all possible) of Investigator's first knowledge.

The event-specific information that needs to be captured in addition to the standard AE information is presented below:

Neoplasms:

Information on histopathology (date of examination and results), imaging (if imaging is performed), TNM staging, history of cancer (the patient's and family), treatment received for this event, and an event narrative. Copies of images (if performed) should be stored at each site. A retrospective imaging review may be performed if deemed necessary.

Suspicion of liver injury:

Suspicion of liver injury* is defined as:

- ALT or AST increasing more than 3 times from baseline value, or
- ALT or AST > 5 × ULN and total bilirubin > 2 x ULN, or
- ALT or AST > 5 × ULN and INR > 1.5

* In that case, the tests should be repeated at 48-72 hours for evaluation of the event course/confirmation. Furthermore, additional information needs to be provided by the site,

e.g. physical examination, information on alcohol consumption, concomitant therapy (including herbals).

- Narrative of the event should include:
  - Clinical signs and symptoms and how they developed over time. Aspects like e.g. abdominal pain, nausea, vomiting, jaundice, fever, rash, abdominal tenderness, hepatomegaly, splenomegaly, blood pressure, peripheral edema, jugular venous distension, signs of ascites, recent weight gain should be considered and described.
  - Information on relevant medical history, alcohol consumption, and relevant concomitant therapy (including herbals).
  - Lab tests (at local lab, if needed): as a minimum ALT, AST, Bilirubin (direct and total) and INR. Other tests may be warranted as clinically indicated.
  - Imaging diagnostic (ultrasound/CT scan/MRI/Other imaging modality). Copies of images (if performed) should be stored at each site.
  - Biopsy results (if performed).

Pancreatitis:

- Narrative of the event should include
  - Clinical signs and symptoms and how they developed over time. Aspects like pain (character of the pain including the anatomical region (e.g. sudden and in center abdomen) +/- irradiating pain in the back), tenderness of the abdomen, diarrhea, indigestion, fever, jaundice) should be considered and described.
  - Information on relevant medical history, alcohol consumption, and relevant concomitant therapy (including herbals).
  - Lab tests (at local lab, if needed): pancreatic and liver function tests, including lipase, amylase, ALT, AST, bilirubin, and alkaline phosphatase. Other tests may be warranted as clinically indicated.
  - Imaging diagnostic (ultrasound/CT scan/MRI/Other imaging modality). Copies of images (if performed) should be stored at each site.
  - Biopsy results (if performed).

Cholecystitis:

- Narrative of the event should include
  - Clinical signs and symptoms and how they developed over time. Aspects like pain (character of the pain including the anatomical region (e.g. sudden, after a large meal, in upper right or center abdomen) +/- irradiating pain to right shoulder or back), tenderness of the abdomen, nausea, vomiting, and fever should be considered and described.
  - Information on relevant medical history, alcohol consumption, and relevant concomitant therapy (including herbals).

- Lab tests (at local lab, if needed): liver function tests, including ALT, AST, bilirubin, and alkaline phosphatase. Other tests may be warranted as clinically indicated.
- Imaging diagnostic (ultrasound/X-Ray/CT scan/MRI/Other imaging modality). Details like presence of gall bladder stones and common bile duct diameter should be reported, if available.

11.4.1.3 Serious Adverse Event

An SAE is any untoward medical experience that at any dose fulfills any of the following criteria:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is otherwise medically important, that may not result in death, be life threatening or require hospitalization may be considered an SAE when (based on appropriate medical judgement) it may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definition of SAE. Examples could be emergency room or home treatment of allergic bronchospasm or convulsion.

SAEs must be reported to the appropriate Sponsor contact person(s) within 24 hours after obtaining knowledge about the event, followed by a complete SAE form as soon as more information is available.

11.4.1.4 Other Important Adverse Events

The following events must always be reported to Sponsor according to SAE timelines, regardless of whether the event is non-serious or serious:

- Suspicion of transmission of infectious agents via the trial product
- Overdose of the trial product
- Medication error involving the trial product
- Inadvertent or accidental exposure to the trial product.

11.4.1.5 Non-serious Adverse Event

A non-serious AE is any AE which does not fulfill the definition of an SAE.

11.4.1.6 Suspected Unexpected Serious Adverse Reactions

An AE is considered a Suspected Unexpected Serious Adverse Reaction (SUSAR) if the nature or severity is not consistent with the applicable product Reference Safety Information (RSI). Zealand Pharma or designee will notify the Investigator of SUSARs in accordance with local requirements and ICH GCP.

11.4.2 Collection, Recording and Reporting of Adverse Events

All AEs, whether serious or non-serious, will be reported from the time a signed and dated Informed Consent Form (ICF) is obtained until the end of the post-treatment follow-up period (which may include contacts for follow-up of safety). In addition, patients will be observed for any signs or symptoms and asked about their condition by open questioning, such as "How have you been feeling since you were last asked?" at each contact with the trial site (visit or telephone). Patients will also be encouraged to spontaneously report AEs occurring at any other time during the trial.

All AEs, regardless of seriousness, severity, or presumed relationship to trial product, must be recorded and evaluated by the Investigator. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology. If no diagnosis can be made, the Investigator should record each sign and symptom as individual AEs. Investigators must record their opinion concerning the relationship of the AE to the trial product. All measures required for AE management must be recorded in the source document and reported according to Sponsor instructions.

- All AEs will be reported in the eCRF.
- AE information should as a minimum include the following:
  - Date and time of onset
  - Date and time of Investigators first information about the AE
  - Seriousness
  - Severity
  - Causal relationship with trial product
  - Measures taken due to AE
  - Interruption or discontinuation of treatment with trial product
  - Date and time of resolution and final outcome.

All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 21.0.

SAEs, including those spontaneously reported to the Investigator within 30 days after the last dose of trial product must be reported to the appropriate Sponsor contact person(s) within 24 hours after obtaining knowledge about the event, followed by a complete SAE form as soon as more information is available. For each SAE, a separate SAE form should be completed in the eCRF.

It is the responsibility of Sponsor (or delegate) to report all SUSARs that occur in this trial to the relevant Regulatory Authorities in participating countries and it is the responsibility of the designated CRO or Investigator to report to IRBs/IECs in accordance with the local requirements in force and ICH guideline for GCP.

Zealand Pharma has outsourced the processing of SAEs and AESIs from this trial to PharmaLex.

Contact information for SAE reporting:

Company:	PharmaLex A/S
Address:	Agern Allé 24, DK-2970 Hørsholm, Denmark
Ph.:	+45 74 44 19 36 (24-hours)
Email:	PV-nordic@pharmalex.com

11.4.3 Follow-up of Adverse Events

The Investigator must record follow-up information. Follow-up information is reported by eCRF in a similar manner as initial AE/SAEs. Follow-up questions to Investigators regarding SAEs are queried directly by safety CRO to the Investigator.

Follow-up information must be reported according to the following:

- **SAEs:** All SAEs must be followed until the outcome of the events is "recovered/resolved", "recovered/resolved with sequelae", or "fatal", and until all queries have been resolved. Cases of chronic conditions, cancer, or AEs ongoing at time of death (where death is due to another AE) may be closed with the outcome "recovering/resolving" or "not recovered/not resolved". Cases can be closed with the outcome of "recovering/resolving" when the patient has completed the follow-up period and is expected by the Investigator to recover.

The SAE follow-up information should only include new (e.g., corrections or additional) information and must be reported **within 24 hours** of the Investigator's first knowledge of the information. This is also the case for AEs initially reported as non-serious which subsequently become SAEs.

- **Non-serious AEs**: Non-serious AEs must be followed until the outcome of the event is "recovering/resolving", "recovered/resolved", or "recovered/resolved with sequelae" or until the end of the follow-up period stated in the protocol, whichever comes first, and until all queries related to these AEs have been resolved. Cases of chronic conditions, cancer, or AEs ongoing at time of death (where death is due to another AE) may be closed with the outcome of "recovering/resolving" or "not recovered/not resolved". Cases can be closed with the outcome of "recovering/resolving" when patient has completed the follow-up period and is expected by the Investigator to recover.

IMPORTANTLY: If a potential hypersensitivity reaction is observed, additional blood samples are required to further characterize the potential hypersensitivity reaction. If an anaphylactic shock is suspected, samples should be taken for the measurement of tryptase. In this case, a blood sample should be within 3 to 4 hours after the event and again approximately 2 to 4 weeks later to determine tryptase baseline levels. In addition, assessments for elevated histamine levels may be considered. Furthermore, a blood sample should be taken 2 to 4 weeks after the event for the determination of ADA and characterization of a potential ADA response in order to be able to relate this to the clinical picture.

For a comprehensive safety evaluation, the treatment-emergent adverse events are a key parameter.

For an adverse event with onset before the first administration of the trial drug, which worsens in severity/seriousness in the period after the trial drug initiation, the worsening should be recorded as a separate adverse event with the onset date stated as the date of the severity and/or seriousness upgrade.

For an adverse event with onset after the first administration of the trial drug, which worsens over time, the worsening must be recorded as follow-up information to the initial event (with re-assessment of severity and/or seriousness) with no change to the initial onset date. The Investigator must ensure that the worst-case severity/seriousness of the event is recorded and kept throughout the trial.

If an AE is resolved and recurs, then the later event needs to be reported as a new AE.

Queries or follow-up request must be responded within 14 calendar days from the date of receipt of the request, unless otherwise specified in the follow-up request.

11.4.4 Pregnancy

Pregnancy during the trial should be avoided and the patients must be instructed in highly effective contraception as per local guidelines and to use this throughout the trial through 4 weeks after receiving the last trial drug dose. Female patients must be instructed to notify the Investigator immediately if she becomes pregnant or if she suspects she might be pregnant during the trial. All initial reports of pregnancy in female patients must be reported to the Sponsor by the trial site personnel within 24 hours of knowledge of the event using the appropriate pregnancy form. Abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomalies, and ectopic pregnancy) are considered SAEs and must be reported using the SAE form. If a patient becomes pregnant during the trial, the patient should be discontinued from trial treatment (see Section 7.5).

Highly effective contraception is defined as:

a. Having a male partner who is sterile (vasectomized or orchiectomized) prior to the female patient's entry into the trial and is the sole sexual partner for that female patient
b. Use of intra-uterine devices
c. Use of intrauterine hormone-releasing system
d. Bilateral tubal occlusion
e. Use of hormonal contraceptives containing combined estrogen and progestogen associated with inhibition of ovulation (intravaginal, transdermal)
f. Use of progestogen-only hormonal contraception associated with inhibition of ovulation (injectable, implantable)
g. True abstinence: When this is in line with the preferred and usual lifestyle of the patient (period abstinence [e.g. calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception)

Male patients who are not sterilized or infertile must use condom throughout the trial through 4 weeks after receiving the last trial drug dose. If their partner is a female of childbearing potential,

she must use highly effective contraception (as detailed above) throughout the trial through 4 weeks after last dose.

Because the effect of the trial product on sperm is unknown, pregnancies in partners of male patients must be reported by the trial site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy form. Male patients must be instructed to notify the Investigator immediately if his partner becomes pregnant or suspects to be pregnant.

The Investigator must follow the pregnancy until the pregnancy outcome is known and the newborn infant is 1 month of age. The Investigator must report information about the pregnancy, pregnancy outcome, and health of the newborn infant(s), as well as AEs in connection with the pregnancy, and AEs in the fetus and newborn infant.

11.4.5 Precautions

Normal precautions taken for a human trial, including the provision of emergency equipment, will be taken during this trial. Qualified and well-trained physicians and medical staff will instruct the patients. During a patient's participation in the trial, the Investigator should ensure that adequate medical care is provided to the patients for any AEs, including clinically significant laboratory values related to the trial. The Investigator should inform the patient when medical care is needed for intercurrent illnesses of which the Investigator becomes aware.

For further information on safety precautions for glepaglutide, refer to the current version of the Investigator's Brochure.

11.4.6 Safety Committee

An internal Zealand Pharma Safety Committee is constituted to perform ongoing blinded safety surveillance of clinical trials with glepaglutide, including this trial.

If safety signals or concerns are observed, whether based on reported SAEs, review of all AEs and laboratory parameters reported or any other notification of significant findings, the Safety Committee will respond appropriately to protect the safety of the patients.

The Safety Committee meets quarterly and additionally on an ad hoc basis as needed and will call upon external expertise when judged needed.

12 Statistics

12.1 General Considerations for Data Analysis

This is a multicenter, placebo-controlled, randomized, parallel-group, double-blind, fixed-dose, Phase 3 trial to demonstrate the superiority of glepaglutide 10 mg given twice weekly and once weekly SC versus placebo in stable SBS patients.

A detailed Statistical Analysis Plan (SAP) will be written specifying all analyses that will be performed and completed prior to database lock and analysis. The SAP will contain any modifications to the analysis plan described below, and thus serves as the overriding document dictating the statistical analyses to be performed. Any changes from the SAP will be explained in the Clinical Trial Report (CTR).

In general, all data will be summarized with descriptive statistics for each treatment group. Unless otherwise specified, continuous data will be summarized by presenting the number of

non-missing observations, mean, standard deviation (SD), median, minimum, and maximum. Categorical data will be summarized by presenting the number of patients and percentage for each category.

Baseline is defined as Visit 1 (Day 1) for efficacy analyses, and defined as the last available value prior to or on Visit 1 (Day 1) for safety analyses.

Inferential statistical analyses of the primary and secondary efficacy endpoints will be performed as described below. All comparisons will be between each ZP1848-17111 treatment group and placebo. Listings of individual patient data will be presented.

12.2 Analysis Sets

The following analysis sets will be defined:

- The Full Analysis Set (FAS) will consist of all randomized patients, who received at least one dose of trial drug (glepaglutide or placebo). All efficacy analyses will be based on the FAS.
- The Per-protocol Analysis Set will consist of all FAS patients who do not experience any major protocol deviations. Specific criteria for exclusion from the Per-protocol Analysis Set will be listed in the SAP. Final judgments on exclusion from the Per-protocol Analysis Set will be made prior to database lock. The Per-protocol Analysis Set will be used for supplementary analyses of the primary and key secondary efficacy endpoints.
- The Safety Analysis Set will consist of all randomized patients who received at least 1 dose of trial drug (glepaglutide or placebo). This is the same definition as for the FAS, but the two can deviate in special circumstances. All safety analyses will be based on the Safety Analysis Set.

Because of the extensive PS optimization and stabilization phases conducted between enrollment and randomization, safety data will be summarized for the period after enrollment, but before treatment with trial drug, i.e. separate from the Safety Analysis Set.

12.3 Efficacy Analysis

The primary efficacy analysis will be conducted using the FAS. The twice weekly treatment group and once weekly treatment group comparisons to placebo will be tested by splitting α into 2 α/2 comparisons. A parallel gatekeeping testing procedure will be used to protect the overall type I error rate of α when testing the primary endpoint together with the key secondary endpoints between each glepaglutide treatment group versus placebo. No adjustments for multiplicity will be performed for testing secondary endpoints.

12.3.1 Primary Efficacy Endpoint

The primary efficacy endpoint is the actual reduction in weekly PS volume from baseline to Week 24. Baseline weekly PS volume is defined as the PS volume received during the 7-day period prior to Visit 1 (Day 1), i.e. during the stabilization phase. The Week 1, 2, 4, 8, 12, 16, 20, and 24 weekly PS volume is defined as the PS volume received during the 7-day period prior to these visits.

The primary analysis uses a restricted maximum likelihood (REML)–based repeated-measures approach to compare treatment groups with respect to the mean change from baseline in weekly

PS volume at Week 24. The model will use weekly PS volume assessments at Week 1, 2, 4, 8, 12, 16, 20, and 24, and will include the covariates for treatment group, baseline weekly PS volume, visit (categorical variable), stratification factor (weekly PS volume requirements <12 L/week versus >12 L/week), and visit-by-treatment group interaction. Variance estimation is based on an unstructured covariance matrix, which does not presume a particular correlation structure for repeated weekly PS volume measurements within patients over time. The primary comparison is the difference in the reduction in weekly PS volume from baseline to Week 24 between each glepaglutide treatment group versus placebo.

Following the *Treatment Policy* estimand strategy, missing values will be imputed using multiple imputation methods. For the primary analysis, a *Copy Reference (CR)* approach is used with placebo treatment as reference, while for sensitivity analyses, a *Jump to Reference (J2R)* and a *Copy Incremental from Reference (CIR)* approaches will be applied. Details are described in Section 12.6 and in the SAP.

The Copy Reference approach is justified as the trophic effects on the intestines mediated by glepaglutide would take weeks to abate after treatment withdrawal. A longitudinal growth of the remaining intestines could also have occurred after prolonged therapy and such modifications would not be expected to return fully to the baseline condition. Other effects of GLP-2 agonism, like reduced fluid secretion from the upper part of the GI tract and effects on reducing motility would however be expected to return to the baseline conditions when treatment is withdrawn.

As a supplementary analysis, the analysis will be repeated using the Per-protocol Analysis Set.

A parallel gatekeeping testing procedure will be used to protect the overall type I error rate of α when testing the primary endpoint together with the key secondary endpoints between each glepaglutide treatment group versus placebo. See Section 12.3.3 for details.

12.3.2 Key Secondary Endpoints

The following 4 key secondary efficacy endpoints will be analyzed to assess the treatment effect using the FAS.

Clinical response, defined as achieving at least 20% reduction in weekly PS volume from baseline to both Weeks 20 and 24

The difference in clinical response between each glepaglutide treated group and the placebo treated group will be tested using the Cochran-Mantel-Haenszel (CMH) test with stratification on the randomization stratification factor. Missing values for the clinical response will be imputed as no clinical response. As a sensitivity analysis, missing values will be derived from the CR-imputed data sets generated from the multiple imputation approach described for the primary efficacy endpoint.

Reduction in days on PS ≥ 1 day/week from baseline to Week 24

The difference in response in reducing days on PS ≥ 1-day/week from baseline to Week 24 between each glepaglutide treatment group versus placebo will be analyzed using a CMH test stratified on the randomization stratification factor. Missing values for this endpoint will be imputed as no response. As a sensitivity analysis, missing values will be derived from the CR-imputed data set generated from the multiple imputation approach described for the primary efficacy endpoint.

Reduction in weekly PS volume from baseline to Week 12

The reduction in weekly PS volume from baseline to Week 12 between each glepaglutide treatment group versus placebo will be analyzed in the same model as the primary efficacy analysis. The comparison is the difference in the reduction in weekly PS volume from baseline to Week 12 between each glepaglutide treatment group versus placebo in the mixed effects model for repeated measures (MMRM) described for the primary endpoint. A *Copy Reference (CR)* approach is used for this analysis, while for sensitivity analyses, a *Jump to Reference (J2R)* and a *Copy Incremental from Reference (CIR)* approaches will be applied. This follows the same multiple imputation approach described for the primary efficacy endpoint.

Reduction in weekly PS volume of 100% (weaned off) at Week 24

The difference in response in reduction in weekly PS volume of 100% (weaned off) at Week 24 between each glepaglutide treatment group comparison to placebo will be analyzed using a CMH test stratified on the randomization stratification factor. Missing values for this endpoint will be imputed as no response. As a sensitivity analysis, missing values will be derived from the CR-imputed data set generated from the multiple imputation approach described for the primary efficacy endpoint.

12.3.3 Gatekeeping Procedure

A parallel gatekeeping testing procedure will be used to protect the overall type I error rate of α when testing the primary endpoint together with the key secondary endpoints between each glepaglutide treatment group versus placebo. The twice weekly treatment group and once weekly treatment group comparisons to placebo will be tested by splitting α into 2 α/2 comparisons.

The gatekeeping procedure starts by identifying the smallest maximum p-value of the first three tests (primary efficacy endpoint, 1st key secondary endpoint, and 2nd key secondary endpoint), among the two dosing regimens, once- and twice-weekly. For this regimen, the primary efficacy endpoint, 1st key secondary endpoint, and 2nd key secondary endpoint are evaluated at an α/2 significance level sequentially, only continuing to the next evaluation if the preceding test is statistically significant. If all of the first three tests result in p-values less than or equal to α/2, the procedure continues at an α level for the other regimen. If, on the other hand, one of the first three tests result in a p-value greater than α/2, the procedure stops for this regimen, and the procedure continues at an α/2 level for the other regimen.

Depending upon the results for the first three endpoints in the regimen where the procedure begins, the significance level is specified for the other regimen at α/2 or at α. The sequential test procedure then continues for the first three endpoints in the other regimen. If all six hypotheses (three first endpoints from both regimens) are significant, the last two key secondary endpoints are evaluated sequentially at the α significance level, only continuing to the next evaluation if the preceding is statistically significant.

The parallel gatekeeping procedure is displayed in Figure 3 below.

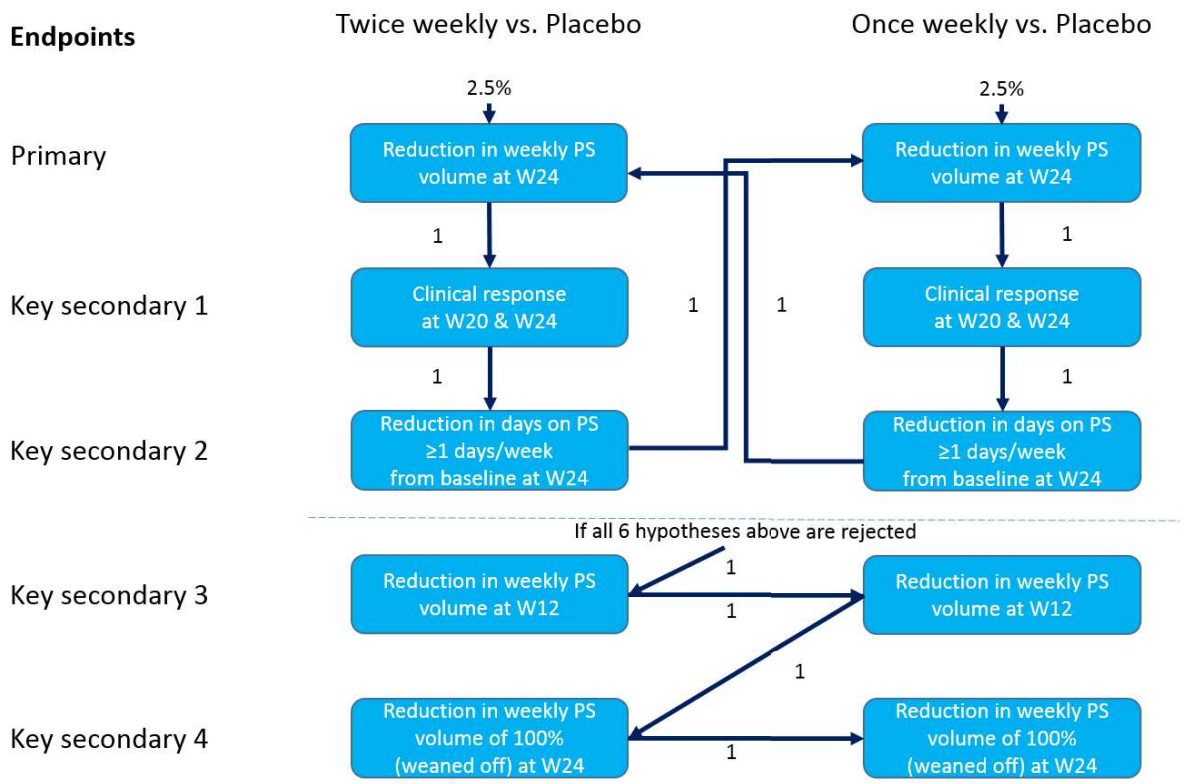

Figure 3: Testing Hierarchy

12.3.4 Secondary Efficacy Endpoints

The following **secondary efficacy endpoints** will additionally be analyzed to assess the treatment effect using the FAS. The tests will be non-hierarchical and type I error will not be adjusted for multiple testing:

Reduction of at least 20% in PS volume from baseline to both Weeks 12 and 24

The difference in response in reduction of ≥ 20% in PS volume from baseline to both Weeks 12 and 24 between each glepaglutide treatment group versus placebo will be analyzed in a similar manner as the first key secondary efficacy endpoint.

Change in FCE from baseline to Week 24

The absolute change of FCE[25], as defined by a reduction in PS volume (L/week), plus reduction in oral fluid intake volume (L/week), plus increase in urine volume (L/week) of the Stabilization Phase and at every trial visit (with the exception of Visit 2-phone assessment) will be analyzed. The FCE will be centrally calculated based on patients' eDiary information.

The difference in change in FCE from baseline to Week 24 between each glepaglutide treatment group versus placebo will be analyzed in a similar manner as the primary efficacy endpoint using a MMRM, but without multiple imputation.

Reduction in calculated energy content of parenteral macronutrients from baseline to Week 24

The difference in reduction in calculated energy content of parenteral macronutrients from baseline to Week 24 between each glepaglutide treatment group comparison to placebo will be analyzed in a similar manner as the primary efficacy endpoint using a MMRM, but without multiple imputation.

Reduction in number of days on PS per week from baseline to Week 24

The difference in reduction in number of days/week on PS from baseline to Week 24 between each glepaglutide treatment group comparison to placebo will be analyzed in a similar manner as the primary efficacy endpoint.

Reduction of at least 40% in PS volume from baseline to both Weeks 20 and 24

The difference in response in reduction of at least 40% in PS volume from baseline to both Weeks 20 and 24 between each glepaglutide treatment group comparison to placebo will be tested using the CMH test with stratification on the randomization stratification factor.

PGIC improvement at Weeks 4, 12, 20, and 24

PGIC improvement is defined as responding "Much improved" or "Very much improved" on a 7-point Likert Scale for each of the weeks 4, 12, 20, and 24. Improvement between each glepaglutide treatment regimen compared to placebo will be tested using the CMH test with stratification on the randomization stratification factor.

Change in weight from baseline to Week 24

The change in body weight (kg) from baseline to Week 24 will be presented descriptively in patients who are (1) below ideal weight at the start of the treatment period (BMI < 18.5 kg/m2 at baseline), and (2) maintaining an ideal/acceptable weight at the start of the treatment period (18 ≤ BMI ≤ 25 kg/m2 at baseline). The change in body weight (kg) from baseline to Week 24 will also be categorized into three groups of less than 5% change, 5% to 10% change, and greater than 10% change. In addition will the proportion of patients in each treatment group who are either underweight (BMI < 18.5 kg/m2), normal weight (18.5 ≤ BMI ≤ 25 kg/m2), or overweight (BMI > 25 kg/m2) will be presented descriptively in patients who are (1) below ideal weight at the start of the treatment period (BMI < 18.5 kg/m2 at baseline), and (2) maintaining an ideal/acceptable weight at the start of the treatment period (18 ≤ BMI ≤ 25 kg/m2 at baseline). Descriptive statistics will include the number of non-missing observations, mean, SD, median, minimum, and maximum values.

12.3.5 Other Efficacy Endpoints

The following **other efficacy endpoints** will be summarized to assess the treatment effect using the FAS. Only descriptive statistics will be presented for these other efficacy endpoints and no inferential statistics will be performed.

Reduction in days on PS ≥ 2 days per week from baseline to Week 24

The number and percent of patients who have a reduction in days on PS ≥ 2 days per week from baseline to Week 24 will be presented for each glepaglutide treatment group and placebo.

Reduction in days on PS ≥ 3 days per week from baseline to Week 24

The number and percent of patients who have a reduction in days on PS ≥ 3 days per week from baseline to Week 24 will be presented for each glepaglutide treatment group and placebo.

Reduction in duration of PS infusions per week from baseline.

The reduction will be presented descriptively and include the number of non-missing observations, mean, SD, median, minimum, and maximum values.

Concentration trough levels of glepaglutide and metabolites

Concentration trough levels of glepaglutide and metabolites will be summarized descriptively. The number of non-missing observations, mean, SD, median, minimum, and maximum values will be presented by visit.

Change in plasma citrulline levels from baseline to Week 24

The change in plasma citrulline levels from baseline to Week 24 will be presented descriptively and include the number of non-missing observations, mean, SD, median, minimum, and maximum values.

Change in weekly need for parenteral micronutrients (sodium, potassium, magnesium, and calcium) from baseline to Week 24

The change in weekly need for parenteral micronutrients from baseline to Week 24 will be summarized using number of non-missing observations, mean, SD, median, minimum, and maximum values for sodium, potassium, magnesium, and calcium.

Change in PROs (SBS-I and EQ-5D-5L) from baseline to Week 24

The change in patient-reported outcome scores (SBS-I and EQ-5D-5L) from baseline to Week 24 will be presented descriptively and include the number of non-missing observations, mean, SD, median, minimum, and maximum values.

Reduction in bowel movements or stoma bag emptying from baseline to Week 24

The reduction in bowel movements or stoma bag emptying will be presented descriptively by displaying the mean and the 95% confidence interval of the by treatment across the trial period.

12.4 Safety Analysis

Safety endpoints are:

- Incidence and type of AEs and SAEs
- Change in clinical evaluations:
  - Vital signs
  - ECG
- Change in laboratory assessments:
  - Hematology
  - Biochemistry
  - Urinalysis
  - Standard bone markers

- Immunogenicity

All safety analyses will be conducted using the Safety Analysis Set. No inferential tests of safety data will be performed. Descriptive summaries of safety data will be presented and for each AESI, a time to event analysis will be performed. This will be detailed in the SAP. Adverse events (treatment-emergent unless otherwise specified) will be presented by system organ class (SOC) and preferred term (PT) for each treatment group.

Treatment-emergent AEs are defined as AEs with onset date on or after the first day of exposure to randomized treatment up through the last day prior to the start day of the Extension Trial or to the Week 28 (for patients not entering the Extension Trial).

Immunogenicity endpoints will be associated with PK, efficacy, and safety endpoints as appropriate and described in detail in the SAP.

Standard bone markers will be assessed as an exploratory endpoint.

12.5 Demographic and Baseline Characteristics

The number and percentage of patients who were screened, randomized, discontinued (with reason for discontinuation), and completed the trial will be summarized descriptively for all screened patients.

Prior and concomitant medications as well as medical history will be summarized descriptively for the FAS.

12.6 Discontinuations and Data Handling Rules

The estimand of interest is the effectiveness of the assigned treatment in all randomized, treated participants, the treatment policy estimand (often called the intention-to-treat or de facto estimand). A placebo-based multiple imputation approach, Copy Reference (CR), will be used as the primary analysis to consider a missing-not-at-random (MNAR) mechanism for monotone missing data. Mean changes from baseline in actual PS volume will be analyzed based on data observed while the patient remains on trial as well as data imputed using multiple imputation (MI) methodology for time points at which no value is observed. The placebo arm is used as reference, as opposed to treatment-discontinuation patients, as it is expected that few patients on active treatment will discontinue treatment. This expectation is based on the drop-out rate seen in the teduglutide Phase 3 trial.

Imputation of values in the placebo (control) arm will assume missing-at-random (MAR). Imputation of values in the glepaglutide arms will be done as if the patient had been a member of the placebo arm. Imputed values in the glepaglutide arms will be sampled using the imputation model of the placebo arm, i.e., conditional on patient values observed at time points prior to discontinuation relative to the mean of the model for the placebo arm. This approach does not assume a sustained benefit of glepaglutide treatment after discontinuation and limits a post-discontinuation effect to that of placebo drug and trial effect as reflected in estimated correlations between time points in the placebo arm.

Intermittent (non-monotone) missing data will be imputed first based on the MAR assumption and a multivariate joint Gaussian imputation model using Markov chain Monte Carlo (MCMC) method within each treatment arm. The MCMC method will be used with a single chain,

2000 tuning units and a minimum number of 4 tuning cycles, a burn-in of 1000, and a thinning of 100 and non-informative priors for all parameters.

The remaining, monotone missing data for all patients who discontinue the trial prematurely will be imputed using sequential regression multiple imputation model estimated based on data from the placebo arm only. Each sequential regression model (i.e., for imputation of values at a given time point) will include explanatory variables (treatment group, visit (categorical variable), stratification factor (weekly PS volume requirements <12 L/week versus >12 L/week)), and all previous (Baseline weekly PS volume, Visit at Weeks 1, 2, 4, 8, 12, 16, 20, and 24) values of actual PS volume. Missing values at a given time point in placebo and glepaglutide arms will be imputed from the same imputation model, conditional on patient values observed or imputed at previous time points. No rounding or range restrictions will be applied to imputed continuous values.

Imputed data will consist of 1000 imputed datasets. A different and separate random seed number will be used for the partial imputation with the MCMC method, and for the sequential regression multiple imputation. Those random seed numbers will be specified in the SAP.

Each of the 1000 imputed datasets will be analyzed using the following analysis method. Change in actual PS volume from baseline to each post-baseline visit will be calculated based on observed and imputed data. Treatment group comparison at Week 24 will be based on the least squares mean (LSM) difference between glepaglutide groups and placebo in change from baseline in actual PS volume estimated by the analysis model in each of the imputed datasets. Results from analysis of each imputed dataset, i.e., LSM treatment differences and their standard errors, will be combined using Rubin's imputation rules to produce a pooled LSM estimate of treatment difference, its 95% confidence interval, and a pooled p-value for the test of null hypothesis of no treatment effect.

The Copy Reference (CR) and Jump to Reference (J2R) approaches multiply impute missing data using estimated means in the control group. This is justifiable scientifically under the assumption that patients who stop taking the therapy will no longer benefit from it in the future, and thus will tend to have outcomes similar to those in the control group. The difference in the two methods is that the Copy Reference approach presumes patients who withdraw from the active arm were on the control (rather than the active) treatment before dropout; the resulting positive residuals before withdrawal leads to imputed values that slowly (rather than quickly) trend toward the estimated mean on the control arm. The Copy Reference is used in the primary analysis and the Jump to Reference will be applied as a sensitivity analysis. As a second sensitivity analysis, the Copy Increments from Reference will be used. This scenario provides a contrast to the extreme effect of J2R by assuming that in the future a dropout continues from their established position, but the subsequent changes in mean profile follow that of the reference arm.

For all binary responder analyses, patients who discontinue for any reason will be considered as non-responders from the time of discontinuation onwards.

Imputation rules for the handling of missing data for PROs and health economic assessments will be described in the SAP.

No pooling of sites is planned for the efficacy analyses.

12.7 Sample Size and Power Considerations

The sample size calculation for this trial is based on the effect achieved in the teduglutide Phase 3 trial and that the PS vol, changes from baseline after 24 weeks of treatment (primary endpoint) are expected to be -4.5L/week and -4.3L/week with twice-weekly and once-weekly dosing, respectively, and -2.3L/week for placebo. The standard deviation of the treatment effect (once-weekly or twice-weekly versus placebo) is assumed to be 2.62.

A total of approximately 108 SBS patients are planned for inclusion, with 33-36 patients planned for each of the three treatment groups. The trial size will result in 93-95% power for detecting the assumed difference with either once-weekly or twice-weekly for the primary endpoint. The assumed effects include imputed effects for patients with missing data. The power calculations are shown in Table 5 including the scenario where once-weekly and twice-weekly dosing are assumed to be slightly worse.

Table 5: Power considerations

Power (%) to show superiority of either once-weekly or twice-weekly compared to placebo with respect to the primary endpoint						
Assumptions: ***PS vol, change from baseline (L/week)***			*Number of patients (total)*			
Twice-weekly	*One-weekly*	*Placebo*	99	108	117	129
-4.5	-4.3	-2.3	93	95	96	98
-4.3	-4.1	-2.3	88	91	93	95

The two comparisons, once-weekly vs placebo and twice-weekly vs placebo are tested two-sided in parallel at α=0.025 to control the overall type 1 error at 5% level.

13 Direct Access to Source Data/Documents

During the course of the trial, a Monitor will make site visits to review protocol compliance, compare eCRFs with individual patient's medical records, assess drug accountability, and ensure that the trial is being conducted according to pertinent regulatory requirements.

Checking of the eCRFs for completeness and clarity, and cross-checking with source documents, are required to monitor the progress of the trial. Moreover, Regulatory Authorities of certain countries, IRBs/IECs, and/or the Sponsor may wish to carry out such source data checks and/or on-site audits or inspections. Direct access to source data are required for these inspections and audits; they are to be carried out giving due consideration to data protection and medical confidentiality.

13.1 Trial Monitoring

Before an investigational site can begin enrolling patients into the trial, a representative of Zealand Pharma will visit the investigational trial site to:

- Determine the adequacy of the facilities

- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of Zealand Pharma or its representatives. This will be documented in a Clinical Trial Agreement between Zealand Pharma and the Investigator.

During the trial, a Monitor from Zealand Pharma or representative will have regular contact with the investigational site, for the following:

- Provide information and support to the Investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the eCRFs, and that investigational product accountability checks are being performed.
- Perform source data verification. This includes a comparison of the data in the case report forms with the patient's medical records at the hospital or practice, and other records relevant to the trial. This will require direct access to all original records for each patient (e.g., clinic charts).
- Record and report any protocol deviations to Zealand Pharma.
- Confirm AEs and SAEs have been properly documented on the eCRFs and confirm any SAEs have been forwarded to Zealand Pharma and those SAEs that met criteria for reporting have been forwarded to the IRB/IEC.

The Monitor will be available between visits if the Investigator(s) or other staff needs information or advice.

In addition to the above, medical monitors will be reviewing the clinically relevant data points to provide independent medical and safety oversight to protect the safety of the trial patients and the integrity of data, for more detailed procedures please refer to the Medical Monitoring Plan.

13.2 Audits and Inspections

Authorized representatives of Zealand Pharma or designee, a Regulatory Authority, IRB/ an IEC may visit the site to perform audits or inspections, including source data verification. The purpose of a Zealand Pharma audit or inspection is to systematically and independently examine all trial-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH GCP guidelines, and any applicable regulatory requirements. The Investigator should contact Zealand Pharma immediately if contacted by a Regulatory Agency about an inspection.

13.3 Institutional Review Board (IRB)/Independent Ethics Committee (IEC)

The Principal Investigator must obtain IRB/IEC approval for the investigation. Initial IRB/IEC approval, and all materials approved by the IRB/IEC for this trial including the patient's informed consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

14 Quality Control and Quality Assurance

To ensure compliance with GCP and all applicable regulatory requirements, Zealand Pharma or designee may conduct quality assurance audit(s).

14.1 Electronic Case Report Forms

Electronic case report forms will be used for data collection. Following training, trial staff will be given access to the CRF. Access to the database is restricted to staff participating in the trial and the extent of access will depend on the participants' user role in the trial.

The trial participants will be identified in the database by patient numbers. The Investigator or delegate will enter patient data into the eCRF within 2 working days (if at all possible). All data are to be entered in English. Data recorded in the eCRFs will be accessible to the trial staff throughout the trial.

After data entry, systematic data validation will be performed, and data entry discrepancies will be presented electronically directly to the site staff. Queries for discrepant data may be generated automatically by the software upon entry and/or generated manually by the monitor or the trial data manager. All queries, whether generated by the system or by trial staff, will be in electronic format.

All sections of the eCRF are to be electronically approved by the Investigator after the data have been entered and all queries have been resolved. Changes to any eCRF page subsequent to the approval require a new approval signature.

All queries and changes/corrections to the data are documented in the eCRF.

14.2 Patient Electronic Diaries (eDiaries)

Patients will use eDiaries to record PS volume, number of bowel movements/stoma bags emptying, 48-hour oral fluid intake according to drinking menu and urine volume, date, time and site of injection of trial drug. eDiaries will be in the patient's local language.

14.3 Data Processing

The trial is run as an EDC trial, i.e. all relevant data are entered by the site directly into the clinical database. The database and application are set up and managed by Pharm-Olam International. The eCRF is designed to capture all required information in compliance with GCP standards. Site staff will be provided with eCRF guidelines for this trial.

The General Data Protection Regulation 2016/679 applies for the processing of data regarding the trial patients.

15 Ethics

15.1 Ethics Review

The final trial protocol, including the final version of the ICF and patient facing material, must be approved or given a favorable opinion in writing by an IRB/IEC as appropriate. The Investigator must submit written approval from the IRB/IEC to Zealand Pharma or designee before he or she can enroll any patient into the trial.

Any changes to the protocol must be made in the form of an amendment that has the prior written approval of Zealand Pharma. The Investigator is responsible for informing the IRB/IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB/IEC must approve all advertising used to recruit patients for the trial. The protocol must be re-approved by the IRB/IEC upon receipt of amendments and annually, as local regulations require.

The Investigator is also responsible for providing the IRB/IEC with reports of any reportable serious adverse drug reactions from any other trial conducted with the investigational product. Zealand Pharma or designee will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB/IEC according to local regulations and guidelines.

15.2 Ethical Conduct of the Trial

The trial will be performed according to the current protocol and in accordance with the Declaration of Helsinki on Ethical Principles for Medical Research Involving Human Subjects, adopted by the General Assembly of the World Medical Association (1996)[26], ICH GCP[27] and other applicable regulatory requirements.

15.3 Written Informed Consent

The Investigator(s) at each center will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk and benefit of the trial. Patients must also be notified that they are free to discontinue from the trial at any time without any impact on their future care. The patient should be given the opportunity to ask questions and allowed time to consider the information provided.

The patient's signed and dated informed consent must be obtained before conducting any trial procedures.

The Investigator(s) must maintain the original, signed ICF. A copy of the signed ICF must be given to the patient.

15.4 Confidentiality

All trial findings and documents will be regarded as confidential. The Investigator and members of his/her research team must not disclose such information without prior written approval from the Sponsor.

The anonymity of participating patients must be maintained. Patients will be identified on eCRFs by their patient and/or randomization number. On the SAE reports and all other source documents, the patient will be identified via patient and/or randomization number and age. Documents that identify the patient (e.g., the signed informed consent) must be maintained confidentially by the Investigator.

15.5 Liability and Insurance

The Sponsor will take out reasonable third-party liability insurance coverage in accordance with all local legal requirements. The civil liability of the Investigator, the persons instructed by him or her and the hospital, practice, or institute in which they are employed and the liability of the

Sponsor with respect to financial loss due to personal injury and other damage that may arise as a result of carrying out this trial are governed by the applicable law.

The Sponsor will arrange for liability insurance if trial patients should be injured due to the participation in the trial and provided that Sponsor is legally liable for that.

Excluded from the insurance cover are injuries to health and deteriorations of illnesses already in existence which would have occurred or continued to exist even if the trial patient had not taken part in the clinical trial.

The insurance cover is jeopardized if the trial patient fails to report immediately to the Investigator or responsible physician any injury to health, which might have resulted from participation in the clinical trial, or if he/she undergoes any other medical treatment without their consent before the clinical trial has been completely finished insofar as the individual trial patient is concerned.

Any injury to health, which might have occurred as a result of participation in the clinical trial, must be reported by the trial patient to the Investigator without delay. The Investigator is obliged to make such a report in any case.

16 Data Handling and Record Keeping

16.1 Records

The Investigator or delegate will enter patient data into the eCRF within 2 working days of the trial visit (if at all possible).

16.2 Retention of Records

The Investigator must maintain all documentation relating to the trial for a period of 2 years after the last marketing application approval, or if not approved 2 years following the discontinuance of the test article for investigation. If it becomes necessary for Zealand Pharma or a Regulatory Authority to review any documentation relating to the trial, the Investigator must permit access to such records.

The Sponsor will maintain all documentation relating to the trial for a period of 25 years after completion or termination of the trial.

17 Clinical Trial Report and Publication Policy

17.1 Final Clinical Trial Report

Zealand will retain ownership of the data.

The final clinical trial report will be prepared and reviewed in cooperation with the coordinating Investigator. The coordinating Investigator will be appointed by Zealand Pharma to review and sign the clinical trial report on behalf of all participating Investigators. This report will include a summary of the trial results based on a statistical evaluation and clinical assessment of the protocol-defined endpoints.

17.2 Publication Policy

The information obtained during the conduct of this trial is considered confidential and may be used by Zealand Pharma for regulatory purposes and for the general development of the trial

product. No confidential information provided by Zealand Pharma in connection with the conduct of this trial shall be disclosed to others without prior written consent from Zealand Pharma. The Investigator will be provided with the randomization code once the clinical trial report has been finalized and signed.

At the end of the trial, 1 or more public disclosures are intended to be prepared collaboratively by the Investigators and Zealand Pharma. Results from the trial will be made publicly available irrespective of whether the outcome of the trial is positive, negative, or inconclusive. Zealand Pharma reserves the right to postpone publication and/or communication for up to 3 months after data base lock to protect intellectual property. Public disclosure includes publication of a paper in a scientific journal, abstract submission with a subsequent poster, or oral presentation at a scientific meeting, or disclosure by other means. All publications conform to the Vancouver guidelines for sound scientific publications. Additional publications may be proposed by the Investigators and prepared in collaboration with Zealand Pharma.

Zealand Pharma reserves the right to defer the release of data until specified milestones are reached, for example when the clinical trial report is available. All authors will be given the relevant statistical tables, figures, and reports needed to support the planned publication. The trial results will be reported in an objective, accurate, balanced, and complete manner, with a discussion of the strengths and limitations. In the event of any disagreement on the content of any publication, both the opinion of the Investigators and the opinion of Zealand Pharma will be fairly and sufficiently represented in the publication.

Authors of the primary publication must fulfil the criteria defined by the International Committee of Medical Journal Editors.

This clinical trial will also be registered on clinicaltrials.gov prior to initiation. It will also be available on the EMA Clinical Trial Register on https://www.clinicaltrialsregister.eu/, and other national or international web sites.

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19 Appendices

19.1 Clinical Classification of Patients based on PS Volume

On the basis of the energy and volume of the required PS, the patients are categorized into 16 combinations (adapted from Pironi *et al* (2016)[8]):

IV Energy Supplementation [b] (kcal/kg body weight)	**Volume of IV Supplementation [a] (L)**			
	≤ 1 [1]	**>1-2 [2]**	**>2-3 [3]**	**> 3 [4]**
0 (A)	A1	A2	A3	A4
1-10 (B)	B1	B2	B3	B4
11-20 (C)	C1	C2	C3	C4
> 20 (D)	D1	D2	D3	D4

IV=intravenous

a Calculated as daily mean of the total volume infused per week = (volume per day of infusion × number of infusions per week)/7.

b Calculated as daily mean of the total energy infused per week = (energy per day of infusion × number of infusions per week)/7/kg.