Sponsor Name:	**Zealand Pharma**
Protocol Number and Title:	**ZP1848-17111** **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)**
Protocol Version and Date:	**Protocol Version 10.0** **27 January 2022**
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SAP Version:	**6.0**
SAP Version Date:	**25 August 2022**

SAP Version: 6.0 **Version Date: 25 August 2022**

I confirm that I have reviewed this document and agree with the content.

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1. PURPOSE

The purpose of this statistical analysis plan (SAP) is to ensure that the summary tables, patient data listings, and figures which will be produced, and the statistical methodologies that will be used, are complete and appropriate to allow valid conclusions regarding the trial objectives.

This SAP is based on protocol ZP1848-17111, Version 10.0, dated 27 January 2022.

1.1. RESPONSIBILITIES

Zealand Pharma will perform the statistical analyses and is responsible for the production and quality control of all tables, listings, and figures.

2. TRIAL OBJECTIVES

2.1. PRIMARY OBJECTIVE

- To confirm the efficacy of glepaglutide in reducing parenteral support (PS) volume in SBS patients

2.2. 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

2.3. ESTIMAND

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

1. The population is defined as patients with SBS randomized into the trial and having received treatment
2. The endpoint is the change 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

Primary estimand of primary efficacy endpoint

- *Difference between mean change 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.

Furthermore, a supplementary estimand of primary efficacy endpoint is defined where the intercurrent event of discontinuing treatment is handled under a 'hypothetical strategy'. This corresponds to the following estimand of interest

- *Difference between mean change from baseline in actual weekly PS volume at 24 weeks in the SBS population, as if treatment discontinuation never occurred.*

2.4. STUDY POPULATION

The study population will consist of SBS patients with a stable need for PS at least 3 days per week. The exclusion criteria will 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.

2.5. TRIAL DESCRIPTION

This is a multicenter, placebo-controlled, randomized, parallel-group, double-blind, fixed dose, Phase 3 trial to demonstrate the superiority of once weekly or 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. 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 output in the eDiary. PS volume 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 stable.

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. 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 output 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 output 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.

Main Trial Period:

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 twice weekly SC 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 daily 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 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.

Any changes to the content of PS are left to the discretion of the Investigator.

After completing the Treatment Phase (regardless of treatment adherence), patients (patients in all 3 treatment groups) will be eligible to enter an Extension Trial and receive glepaglutide. For patients not entering the Extension Trial, a Follow-up Visit will be conducted 4 weeks after completion of the Treatment Phase.

2.6. DETERMINATION OF SAMPLE SIZE

The sample size calculation for this trial is based on the effect achieved in the teduglutide Phase 3 trial and that the PS volume changes from baseline after 24 weeks of treatment (primary endpoint) are expected to be -4.5 L/week and -4.3 L/week with twice-weekly and once-weekly dosing, respectively, and -2.3 L/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 101-112 SBS patients are planned for inclusion, with 33-37 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 1 including the scenario where once-weekly and twice-weekly dosing are assumed to be slightly worse.

Table 1 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.

2.7. TREATMENT ASSIGNMENT & BLINDING

Randomization will be used to avoid bias in the assignment of patients to double-blind treatment (glepaglutide twice weekly, glepaglutide once weekly, or placebo) and to increase the likelihood that known and unknown patient characteristics will be evenly distributed between the treatment groups.

For each cohort, eligible patients will be randomly assigned on Day 1 after all visit procedures have been performed and eligibility for randomization confirmed. Patients will be randomized via an interactive web response system (IWRS) to receive trial drug (glepaglutide twice weekly, glepaglutide once weekly, or placebo) in a 1:1:1 ratio. Trial drug assigned to a patient may not be reused, even if the vial returned is unopened.

A designated randomization administrator from an external, independent vendor will maintain the randomization codes in accordance with standard operating procedures to ensure that the blind is properly maintained and only sponsor, CRO, and vendor personnel who require knowledge of treatment assignments will be unblinded during the trial.

Investigators are not to break the trial treatment blind except when information concerning the trial drug is necessary for the medical treatment of the patient. If a medical emergency requiring unblinding occurs, the investigator (or designated physician) is strongly encouraged to contact the medical or safety monitor to assess the necessity of breaking the trial drug blind. If unblinding is warranted, the investigator will obtain the treatment assignment information from the IWRS. Every effort is to be made to limit trial site personnel unblinding only to those individuals providing direct care to that patient. Any intentional or unintentional breaking of the blind is to be reported immediately to the sponsor.

If the blind is broken, the date, time, and reason must be recorded in the patient's eCRF, and any associated SAE report, if applicable.

2.8. ADMINISTRATION OF TRIAL MEDICATION

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 2 for a schematic overview.

Table 2 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

2.9. SCHEDULE OF ASSESSMENTS AND PROCEDURES

For the schedule of assessments and procedures, please refer to Section 11 of this SAP, or to the latest protocol version.

3. ENDPOINTS

3.1. PRIMARY EFFICACY ENDPOINT

The primary efficacy endpoint is the change in actual weekly PS volume from baseline to Week 24.

Baseline actual weekly PS volume is defined as the actual PS volume derived from a valid 7-day period prior to Visit 1 (Day 1), i.e. during the stabilization phase. The actual weekly PS volume at Weeks 1, 2, 4, 8, 12, 16, 20, and 24 will be derived as the actual weekly PS volume received during the valid 7-day period prior to the visit. The source for the derivation will be the PS volumes recorded by the patients in the eDiary; for details on the derivation of actual weekly PS volume see Appendix A. Supportive analyses will be performed to explore the robustness of the primary analysis with regard to the algorithm for deriving the primary endpoint, see 3.1.4.

Following the treatment policy strategy, for patients who prematurely discontinue treatment but complete the trial providing eDiary information up-to Week 24, the actual weekly PS volume derived at Week 24 after treatment discontinuation will be included in the primary analysis of the primary efficacy endpoint. For patients who prematurely discontinue treatment and did not complete the trial, having an end of treatment visit (Visit 10) prior to Week 24 - 14 days (169-14 days), the derived actual weekly PS volume at the end of treatment visit will not be carried forward to represent Week 24. Instead 1) the value at Week 24 will be set to missing and imputed with the multiple imputation method described below for the primary analysis of the primary endpoint and 2) the derived actual weekly PS volume at the end of treatment visit will be re-allocated to a previous visit and used in the MMRM model. If, when re-allocating the end of treatment visit there is already a visit in the same time window, then the value of the scheduled visit will be used in the analysis, not the re-allocated end of treatment value.

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 actual weekly PS volume at Week 24. The model will use actual weekly PS volume assessments at Weeks 1, 2, 4, 8, 12, 16, 20, and 24 as an independent variable, and will include the covariates of treatment group, baseline actual 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 comparisons are the contrasts (differences in least squares means) between the glepaglutide treatment groups and the placebo group at the Week 24 visit in this mixed-effects model for repeated measures (MMRM).

Missing values will be imputed using multiple imputation methods. For the primary analysis, a Copy Reference (CR) approach is used (main estimator), while for sensitivity analyses, a Jump to Reference (J2R) approach (sensitivity estimator 1) and a Copy Incremental from Reference (CIR) approach (sensitivity estimator 2) will be applied. Details are described in 5.3.1 below.

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 fully return 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 an additional sensitivity analysis (sensitivity estimator 3), the actual weekly PS volume will be derived using a valid 14-day period prior to the visit. For details on the derivation of actual weekly PS volume within a 14-day period, see Appendix A. This endpoint will be analyzed in the same manner as for the primary analysis for the primary endpoint.

Furthermore, a tipping point sensitivity analysis (sensitivity estimator 4) will be conducted to examine the impact of missing data on the primary efficacy endpoint analysis. The aim of this analysis is to explore the plausibility of missing data assumptions under which the conclusions change. The CR-imputed values will be varied independently in each treatment arm by adding an appropriate delta PS volume. The range will be determined such that it contains the tipping point, i.e. where conclusions start changing. Only clinical relevant scenarios will be included. The same primary analysis model will be applied. Conclusions from each analysis will be presented simultaneously in a heat map, with the varied delta PS volumes for imputed values in placebo treatment vs. glepaglutide TW/OW. Threshold for conclusions follow that of the testing strategy (3.3), i.e. each treatment comparison to placebo are evaluated in parallel at a two-sided 2.5% significance level. However one of the comparison may be evaluated at a two-sided 5% significance level instead, in case of alpha-recycling.

Lastly, a sensitivity analysis on the primary efficacy endpoint analysis (sensitivity estimator 5) will be conducted to examine the impact of using multiple imputation methods on missing data. This sensitivity analysis will apply the same primary analysis model on observed data only, regardless of treatment discontinuation.

A supplementary estimand will be included using a ‘hypothetical strategy’. This will evaluate the difference between mean change from baseline in actual weekly PS volume at 24 weeks in the SBS population assuming that the intercurrent event of treatment discontinuation would not have occurred (i.e. patients adhere to the randomized treatment until completion). Actual weekly PS volumes derived for visits prior to treatment discontinuation will be analyzed with an MMRM model like the one described for the primary analysis of the primary endpoint.

To get an overview of the different estimands and estimators for the primary efficacy endpoint, and how missing data will be handled, please see Table 3.

Table 3 Overview of estimands and handling of missing data for primary efficacy endpoint

Intercurrent event	**Data observed or missing**	**Primary estimand 'Treatment policy'**						**Supplementary estimand 'Hypothetical'**
		Main estimator	Sensitivity estimator 1	Sensitivity estimator 2	Sensitivity estimator 3 – PS vol derived based on 14-day period	Sensitivity estimator 4	Sensitivity estimator 5	Main estimator
Prematurely discontinuation of IMP	Observed	Value used as observed	Value used as observed	Value used as observed	Value used as observed	Value used as observed	Value used as observed	Values after treatment discontinuation treated as missing
	Missing	MI (CR Placebo)	MI (J2R Placebo)	MI (CIR Placebo)	MI (CR Placebo)	MI (CR Placebo) – tipping point	Missing	Missing
No prematurely discontinuation of IMP	Observed	Value used as observed	Value used as observed	Value used as observed	Value used as observed	Value used as observed	Value used as observed	Value used as observed
	Missing	MI (CR Placebo)	MI (J2R Placebo)	MI (CIR Placebo)	MI (CR Placebo)	MI (CR Placebo) – tipping point	Missing	Missing

Abbreviations: MI = multiple imputation, CR = copy reference, J2R = jump to reference, CIR = copy increments from reference
For subjects with missing PS volume at baseline: For analyses using multiple imputation, baseline will be imputed from a model using data from patients in FAS (not only patients on placebo). For other analyses, baseline will be derived from the mean of observed baseline data from patients in FAS.

3.1.1. Clinical meaningfulness

For all treatment groups combined, the empirical cumulative distribution function (eCDF) of the percent change in actual weekly PS volume from baseline to Week 12 and to Week 24 will be plotted. The eCDF will present the cumulative proportion of patients who achieved a PS volume percent change from baseline to Week 12 and to Week 24 at each observed change level or lower.

For the 7-point scaled patient reported outcome PGIC questionnaire, the eCDF and probability density function (PDF) curves will be produced for each response category ('Very much improved', 'Much improved', 'Minimally improved', 'No change', 'Minimally worse', 'Much worse' and 'Very much worse') and presented together (in separate figures at Week 12 and at Week 24). In addition, to improve interpretability of the analysis, the PGIC categories containing very few patients will be collapsed, resulting in a three-category version as well ('Very much improved'+'Much improved', 'Minimally improved' and 'No change'+'Minimally worse'+'Much worse'+'Very much worse'). Figures with both the un-collapsed and collapsed categories will be presented.

In addition, the PDF of the percent reduction in the weekly PS volume from baseline to Week 12 and to Week 24 will be plotted for all treatment groups combined. The PDFs will be made for all data and by PGIC response category (and collapsed PGIC categories) and approximated using kernel density estimation and overlaid onto one graph by week, as applicable.

The correlation between PGIC and percent change in PS volume from baseline will be evaluated graphically, and by Spearman rank-order and Kendall's tau-b correlation coefficients after 12 and 24 weeks of treatment.

3.1.2. Subgroup analysis

Subgroup analysis for the primary efficacy endpoint will be performed for the subgroups defined in Table 4. In general, if there are less than 5 patients per treatment group in a subgroup, the statistical analysis will not be conducted but summary statistics will be presented. However, for some patients, subgroups might be pooled to have sufficient patients per treatment group to conduct the analysis. The details of pooling subgroups will be documented prior to unblinding.

Table 4 Subgroups based on demographics and baseline characteristics

Subgroups	Categories	Order
Age group (years)	≥18 to <65	1
	≥65	2
Sex	Female	1
	Male	2
Race	American Indian or Alaska Native	1
	Asian	2
	Black or African American	3

Subgroups	Categories	Order
	Native Hawaiian or Other Pacific Islander	4
	White	5
	Other	6
Ethnicity	Hispanic or Latino	1
	Not Hispanic or Latino	2
Region	North America	1
	Europe	2
SBS anatomical classification	Group 1 (jejunostomy) [a]	1
	Group 2 (jejuno-colonic anastomosis)	2
	Group 3 (jejuno-ileo-colonic anastomosis)	3
	Other	4

Abbreviations: SBS: Short Bowel Syndrome
Notes: **a**: patients who do not have a colon-in-continuity as recorded in the CRF

Patients with their sex recorded as Unknown/Undifferentiated will be excluded from the sex subgroup analysis.

The analysis will be performed for patients in the FAS and conducted in a similar manner as for the primary analysis of the primary endpoint but including visit-by-treatment-by-subgroup interaction in the MMRM model. The treatment effect of OW and TW relative to placebo, will be estimated within the model, at Week 24 for each subgroup and presented with 95% CIs. No formal hypothesis testing will be performed.

If there are less than 5 patients per treatment group in a subgroup, the statistical analysis will not be conducted but summary statistics will be presented.

3.1.3. Impact of COVID-19

No sensitivity analyses are planned to assess the impact of COVID-19 on any of the confirmatory efficacy endpoints since actual PS volume is collected in a daily electronic diary which is not dependent upon attending clinical visits per the protocol Schedule of Assessments. Refer to Risk Assessment and Conclusion document dated 30-Nov-2020 entitled “Covid-19 pandemic contingency plan for glepaglutide phase 3 trials; ZP1848-17111 (EASE SBS 1) and ZP1848-17127 (EASE SBS 2).

3.1.4. Supportive analysis of primary endpoint derivation

The primary endpoint, actual weekly PS volume, will be derived as specified in Appendix A. The robustness of the primary analysis, with regard to the algorithm, will be explored through several supportive analyses, which each will address the impact of key algorithm elements. The analyses are considered ‘supportive’ as patients with missing PS entries continue in the trial, and no relevant intercurrent events are expected to trigger the missing entries.

Supportive estimator 1 is applying the derivation method specified in previous SAP versions (3.0 and 4.0) without any values carried forward. This is considered an extremely conservative estimate of the endpoint, as days without PS entries in the eDiary within a certain week will be imputed with the total weekly PS volume divided by the number of days of non-missing data.

Supportive estimator 2 and 3 assess the impact to the primary analysis by varying individual algorithm elements. That is by removing information collected through note-to-files, and increasing the minimum threshold necessary for calculating an endpoint with respect to percentage actual PS volume versus prescribed, respectively.

Supportive estimator 4 considers a continuum between main and supportive estimator 1, but without a threshold for setting the endpoint to missing. This is to explore the incremental impact of imputing missing PS entry days with either 0 L or the total weekly PS volume divided by the number of days of non-missing data.

To get an overview of the supportive estimators for the primary endpoint derivation, and each individual algorithm elements, please see Table 5.

Table 5 Overview of supportive analysis of primary endpoint derivation

	Main estimator	**Supportive estimator 1**	**Supportive estimator 2**	**Supportive estimator 3**	**Supportive estimator 4**
Algorithm element	*Appendix A algorithm*	*SAP v3.0/4.0 algorithm*	*Appendix A algorithm w/o NTFs*	*Appendix A algorithm with less deviation from prescribed amount allowed*	*Tipping individual PS entry days*
Threshold for setting endpoint missing	Yes, if actual PS entry days <7 and PS accounted for <90% vs prescribed PS	Yes, if actual PS entry days <4	Yes, if actual PS entry days <7 and PS accounted for <90% vs prescribed PS	Yes, if actual PS entry days <7 and PS accounted for <95% vs prescribed PS	No
If endpoint not set to missing and no. of PS entry days ≠ 7, missing PS entry days will be imputed with	0	Average of non-missing days	0	0	Tipping (from 0 to 1 by 0.1)*Average of non-missing days
Flexible window for endpoint assessment	7 consecutive days within the period between 1 and 14 days preceding visit date (30 days for baseline)	7 consecutive days preceding visit date	7 consecutive days within the period between 1 and 14 days preceding visit date (30 days for baseline)	7 consecutive days within the period between 1 and 14 days preceding visit date (30 days for baseline)	7 consecutive days preceding visit date
Allow window for endpoint assessment to cross previous visit	No (with exemption of Week 1 and 2)	Yes	No (with exemption of Week 1 and 2)	No (with exemption of Week 1 and 2)	Yes
Information from NTFs used	Yes	No	No	Yes	No

Abbreviations: PS: parenteral support, NTF = note-to-file

3.2. KEY SECONDARY EFFICACY ENDPOINTS

The following 4 key secondary efficacy endpoints will be analyzed to assess the treatment effect using the FAS. The key secondary endpoints will be derived based on the actual weekly PS volume or the number of days on actual PS a week, using the selected valid 7-day period at Week 12, 20, and 24, respectively. For details on the derivation see Appendix A.

First key secondary efficacy endpoint: Clinical response, defined as at least 20% reduction in actual weekly PS volume from baseline to both Weeks 20 and 24
The primary estimand will handle the intercurrent event of treatment discontinuation under a 'composite strategy', i.e. incorporating the intercurrent event in the response definition. For patients who discontinue treatment prematurely, a non-response will be imputed, indicating failure to randomized treatment. This assesses the treatment effect of

- *Difference in percentage of clinical response without treatment discontinuation between glepaglutide treatment group and placebo in the SBS population.*

Missing data for other reasons will be imputed with a non-response.

The difference in clinical response rates between each glepaglutide group and the placebo will be calculated using Mantel-Haenszel weighing and presented with 95% confidence intervals (using the Wald estimation method). Whether the difference equals zero will be tested using the Cochran-Mantel-Haenszel (CMH) adjusted for the stratification factor. In the presence of strata with zero counts in the crosstabulation between stratification factor and treatment, an unadjusted difference in clinical response rates between each glepaglutide group and the placebo will be calculated (i.e. without stratification), to avoid inappropriate weighing of the risk differences in each stratum. The null hypothesis of a difference equals zero will still be tested using the Cochran-Mantel-Haenszel (CMH), with a single contingency table.

As a sensitivity analysis of the primary estimand of first key secondary efficacy endpoint, the actual weekly PS volume will be derived using a valid 14-day period prior to the visit. For details on the derivation of actual weekly PS volume within a 14-day period, see Appendix A. Handling of missing data and analysis of this endpoint will follow that of the primary estimand of first key secondary efficacy endpoint.

In addition, a supplementary estimand will be included using a 'treatment policy strategy' assessing the treatment effect of

- *Difference in percentage of clinical response between glepaglutide treatment group and placebo in the SBS population, regardless of whether treatment is discontinued.*

Response status will be derived from the CR-imputed data sets generated from the multiple imputation approach described for the primary estimand (main estimator) of the primary efficacy endpoint. The difference will be analysed in the same way as the primary analysis of first key secondary efficacy endpoint. The test statistics based on the MI will be combined by applying Rubin's rule after Wilson-Hilferty transformation (Wilson et al 1931).

To get an overview of the different estimands and estimators for the binary key secondary efficacy endpoints, and how missing data will be handled, please see Table 6.

Second key secondary efficacy endpoint: Reduction in days on PS ≥ 1 day/week from baseline to Week 24
The same estimands (main estimators) and sensitivity estimator will be investigated, as for the first key secondary efficacy endpoint (see Table 6). The same analysis method will be applied as well.

Primary 'composite' estimand for the second key secondary efficacy endpoint

- *Difference in percentage of reduction in days on PS ≥ 1 day/week from baseline to Week 24 without treatment discontinuation between glepaglutide treatment group and placebo in the SBS population.*

Supplementary 'treatment policy' estimand for the second key secondary efficacy endpoint

- *Difference in percentage of reduction in days on PS ≥ 1 day/week from baseline to Week 24 between glepaglutide treatment group and placebo in the SBS population, regardless of whether treatment is discontinued.*

For the supplementary estimand, response status is derived using the same MI model as for primary analysis of primary efficacy endpoint with the number of PS days as dependent variable.

Third key secondary efficacy endpoint: Change in actual weekly PS volume from baseline to Week 12
The same estimands (main estimators) and sensitivity estimators will be investigated, as for the primary efficacy endpoint (see Table 3). The same analysis method will be applied as well (see 3.1).

Primary 'treatment policy' estimand for the third key secondary efficacy endpoint

- *Difference between mean change from baseline in actual weekly PS volume at 12 weeks in the SBS population regardless of whether treatment is discontinued.*

Supplementary 'hypothetical' estimand for the third key secondary efficacy endpoint

- *Difference between mean change from baseline in actual weekly PS volume at 12 weeks in the SBS population, as if treatment discontinuation never occurred.*

Fourth key secondary efficacy endpoint: Reduction in weekly PS volume of 100% (weaned off) at Week 24
The same estimands (main estimators) and sensitivity estimator will be investigated, as for the first key secondary efficacy endpoint (see Table 6). The same analysis method will be applied as well.

Primary 'composite' estimand for the fourth key secondary efficacy endpoint

- *Difference in percentage of reduction in weekly PS volume of 100% (weaned off) at Week 24 without treatment discontinuation between glepaglutide treatment group and placebo in the SBS population.*

Supplementary 'treatment policy' estimand for the fourth key secondary efficacy endpoint

- *Difference in percentage of reduction in weekly PS volume of 100% (weaned off) at Week 24 between glepaglutide treatment group and placebo in the SBS population, regardless of whether treatment is discontinued.*

Note, the main analyses of the primary estimands follow the treatment policy strategy for the primary and third key secondary endpoints and the composite strategy for the first, second and forth key secondary endpoint. This is according to the confirmatory testing strategy described in the protocol version 10.0.

Table 6 Overview of estimands and handling of missing data for binary key secondary efficacy endpoints

Intercurrent event	**Data observed or missing**	**Primary estimand 'Composite'**		**Supplementary estimand 'Treatment policy'**
		Main estimator	Sensitivity estimator – PS vol derived based on 14-day period	Main estimator
Prematurely discontinuation of IMP	Observed	Non-response	Non-response	Value used as observed
	Missing	Non-response	Non-response	Response status derived from MI (CR Placebo)*
No prematurely discontinuation of IMP	Observed	Value used as observed	Value used as observed	Value used as observed
	Missing	Non-response	Non-response	Response status derived from MI (CR Placebo)*

Abbreviations: MI = multiple imputation, CR = copy reference
*For 'Reduction in weekly PS volume of xx%' endpoints, response status is derived from the values generated by the MI model used for primary analysis of primary efficacy endpoint. For 'Reduction in days on PS ≥ 1 day/week from baseline to Week 24' endpoint, response status is derived using the same MI model as for primary analysis of primary efficacy endpoint with the number of PS days as dependent variable.

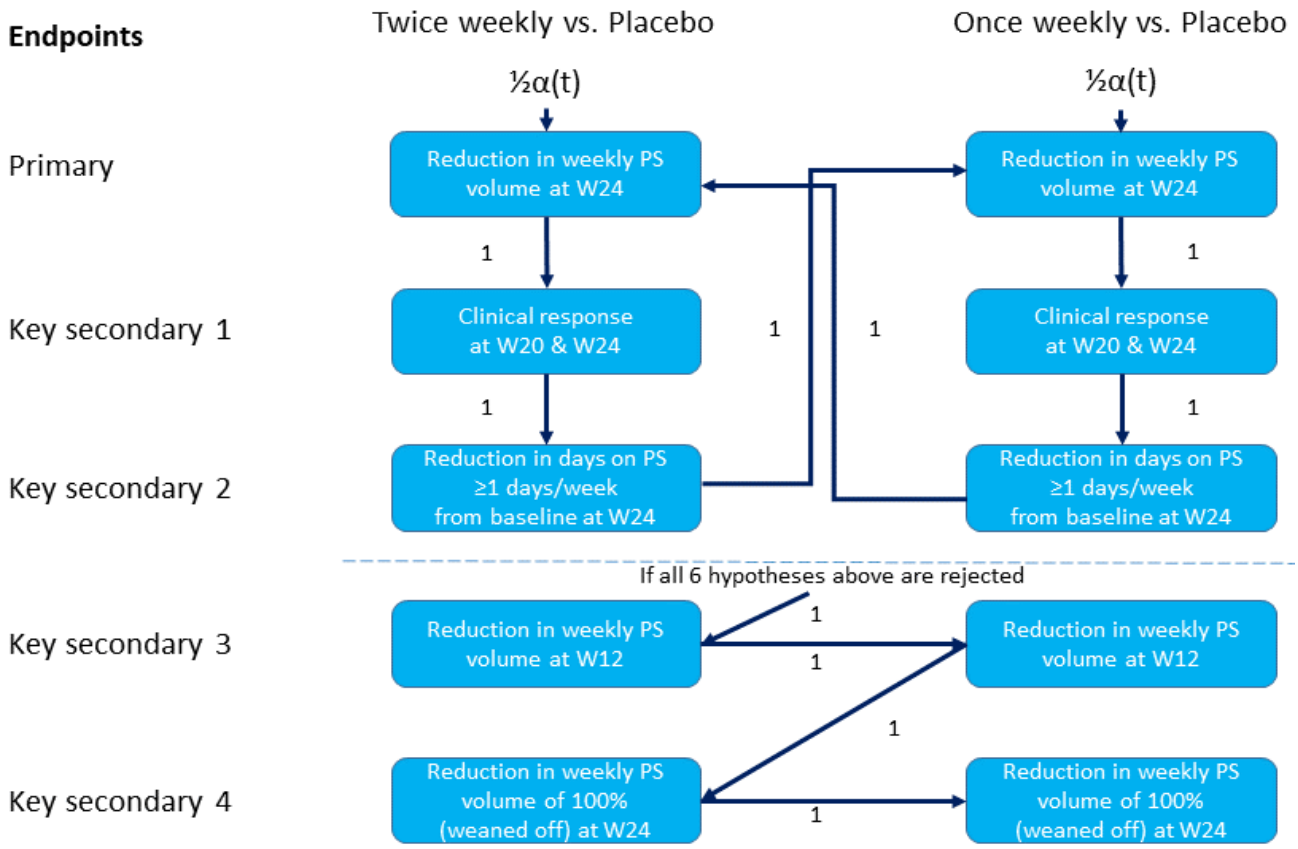
3.3. GATEKEEPING PROCEDURE

A parallel gatekeeping testing procedure will be used to protect the overall type I error rate of α (alpha) 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 at the appropriate step within the testing procedure.

The confirmatory testing strategy follows the approach by Bretz et al 2011. Loosely described, 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. If all of the three tests for the other regimen result in p-values less than or equal to α/2, the procedure continues where it was previously stopped, at α level for the first 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 testing 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 below in Figure 1.

Figure 1 Testing hierarchy



3.4. SECONDARY EFFICACY ENDPOINTS

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

Change of at least 20% in actual weekly 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 calculated energy content of parenteral macronutrients from baseline to Week 24

The calculated energy content provided by PS in kcal and in kcal/kg of body weight will be summarized descriptively at baseline and at Week 24 and include the number of non-missing observations, mean, SD, median, minimum, and maximum values.

For a given visit the calculated energy content will be derived as the sum of lipids, glucose and

amino acids recorded in the eDiary during the valid 7-day period used for deriving the actual weekly PS volume. The change in calculated energy content of parenteral macronutrients from baseline to Week 24 will be analyzed with an MMRM model similar to the one specified for the primary efficacy endpoint. The analysis will be conducted for the calculated energy content in kcal and kcal/kg, separately.

As supporting analyses the calculated energy content (in each unit separately), will be derived based on the records in the eDiary during the valid 14-day period used for the deriving the actual weekly PS volume endpoint on a 14-day period.

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

The difference in change in the number of days on PS per week from baseline to Week 24 between each glepaglutide treatment group relative to placebo will be analyzed with an MMRM model similar to the one described for the primary endpoint.

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

The difference in response in reduction of ≥ 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 adjusted for the stratification factor will be analyzed in a similar manner as the clinical response endpoint (first key secondary endpoint).

Reduction of at least 30% in actual weekly PS volume from baseline to both Weeks 20 and 24

The difference in response in reduction of ≥ 30% 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 adjusted for the stratification factor will be analyzed in a similar manner as the clinical response endpoint (first key secondary endpoint).

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

The difference in response in prescribed weekly PS volume of 100% (weaned off) at Week 24 between each glepaglutide treatment group comparison to placebo will be tested using the CMH test adjusted for the stratification factor will be analyzed in a similar manner as the weaned off endpoint based on actual PS volume (fourth key secondary endpoint).

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

PGIC improvement is defined as responding “Very Much Improved” or “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 by week, using the CMH test adjusted for the stratification factor. In similar fashion, improvement between each glepaglutide treatment regimen versus placebo will be tested using collapsed categories of Improvement, No Change, and Worsening, where Improvement is defined as a response of “Very Much Improved” or “Much Improved” or “Minimally Improved”, and No Change is defined as the response of “No Change”, and Worsening is defined as a response of “Minimally Worse” or “Much Worse” or “Very Much Worse”. Improvement using collapsed

categories between each glepaglutide treatment regimen compared to placebo will tested by week using a Mantel-Haenszel chi-squared test for ordered categories. No imputation for missing values will be performed.

Change in weight from baseline to Week 24

The change in body weight (kg) from baseline to Week 24 will be presented descriptively by baseline BMI subgroup. Descriptive statistics will include the number of non-missing observations, mean, SD, median, minimum, and maximum values. A shift table by BMI subgroup from baseline to Week 24 will be presented. The BMI subgroups are defined as: <18.5 kg/m2, ≥18.5 to <25 kg/m2, ≥25 to <30 kg/m2 and ≥30 kg/m2.

The change in body weight (kg) from baseline to Week 24 will also be presented by three groups: less than 5% change, 5% to 10% change, and greater than 10% change.

Prescribed weekly PS volume

The prescribed weekly PS volume at baseline, Weeks 1, 2, 4, 8, 12, 16, 20, and 24 will be defined as the prescribed weekly PS volume received during the 7-day period prior to these visits.

The source for the derivation will be the prescribed PS volume information recorded in the eCRF by the investigator; for details on the derivation see Appendix A.

The change in prescribed weekly PS volume from baseline to Week 24 will be analyzed with the same statistical methodology as for the primary analysis of the primary endpoint.

3.5. OTHER EFFICACY ENDPOINTS

The following other efficacy endpoints will be summarized to assess the treatment effect using the FAS.

The endpoints related to the number of days on actual PS a week will be derived using the selected valid 7-day period used for the primary endpoint. Additionally to summary statistics, these endpoints will be analyzed with the same methodology as for the primary analysis of the confirmatory binary endpoints.

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

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

placebo.

Change in duration of actual PS infusions per week from baseline

The change will be presented as descriptive statistics at Weeks 1, 2, 4, 8, 12, 16, 20, and 24.

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.

For a given visit the parental micronutrients content will be derived from the eDiary values recorded during in valid 7-day period used for deriving the actual weekly PS volume.

Actual PS intake of micronutrients will be used for the analysis involving PS content. Each of the 4 micronutrients will be summarized separately (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.

For SBS-I, the average ordinal change from baseline to Week 24 for each question will be calculated for all patients combined and by each SBS Anatomical Group (see Section 3.1 for the SBS anatomical subgroups definition). Differences in the average change between each glepaglutide treatment regimen compared to placebo will be tested using a t-test. The sum of scores for each question will be calculated and the difference in change from baseline to Week 24 between each glepaglutide treatment regimen compared to placebo will be assessed using a t-test.

For EQ-5D-5L, results will be presented in three ways.

1. Presenting results from the EQ-5D-5L descriptive system as a health profile at baseline and Week 24: The frequencies and percentages of each level of problem (No problems, Slight problems, Moderate problems, Severe problems, and Extreme problems/unable to do) will be presented at baseline and at Week 24 for each health dimension (Mobility, Self-care, Usual Activities, Pain/Discomfort, and Anxiety/Depression) by treatment group. A further frequency/percentage summary by demographic subgroup (e.g., age group, sex) and/or by SBS Anatomical Group may be presented if applicable.

2. Presenting results of the EQ VAS as a measure of overall self-rated health status: The EQ VAS will be presented descriptively for n, mean, standard deviation, median, minimum value, maximum value, 25th percentile, and 75th percentile by treatment group at baseline and at Week 24.

3. Presenting results from the EQ-5D-5L index value: The index value will be presented descriptively using n, mean, standard deviation, median, minimum value, maximum value, 25th percentile, and 75th percentile by treatment group at baseline and at Week 24. Differences in the mean values between each glepaglutide treatment regimen compared to placebo will be tested using a t-test. Subgroup tables by demographic and/or baseline characteristics (e.g., age group, sex, smoking status, etc.) and/or by SBS Anatomical Group may be presented if applicable.

<u>Change in bowel movements or stoma bag emptying from baseline to Week 24</u>

The number of bowel movements recorded in the eDiary during the 48-hour period prior to baseline and Week 24, and the change from baseline to Week 24 will be presented descriptively. The number of stoma bag emptyings recorded in the eDiary during the 48-hour period prior to baseline and Week 24 will be summarized in a similar manner, for the subset of patients in the FAS who have a stoma.

<u>Measurements of hydrational status</u>

To evaluate the trends on hydration status the urine volume (mL) and the oral fluid intake (mL) recorded by the patients in the eDiary during 48-hour periods prior to the nominal visits will be presented graphically together with the actual weekly PS volume (L/Week) as follows:

- The individual trajectories of 48-hour urine volume (mL), 48-hour oral fluid intake (mL) and weekly PS volume (L) recorded in the patients in the eDiary will be plotted by actual times (start of 48-hour period or visit). Profiles will be gathered in different panels by SBS anatomical classification and treatment group.
- Mean plots of urine volume (mL by 48-hours), oral fluid intake (mL by 48-hours) and actual weekly PS volume (L/Week). Plots by nominal visit and treatment group
    - y1-axis L/Week (for actual weekly PS volume)
    - y2-axis mL/48-hours (for urine volume and oral fluid intake)

3.6. SAFETY ASSESSMENTS

Safety assessments are:

- Adverse events
- Clinical evaluations:
  - Vital signs
  - ECG
- Laboratory assessments:
  - Hematology
  - Biochemistry
  - Urinalysis
  - Standard bone markers
- Immunogenicity assessments

All safety analyses will be conducted using the Safety Analysis Set. No inferential tests of safety data will be performed.

The association between immunogenicity assessments and PK, efficacy, and safety assessments will be summarized as appropriate. See Section 7.7 for immunogenicity analysis details.

Standard bone markers will be assessed as an exploratory endpoint.

See the description of Safety, Section 7 for more details about the analysis for the safety endpoints.

3.7. PHARMACOKINETICS

Blood sample will be collected for pharmacokinetic (PK) evaluation. A total of 9 or 10 samples will be collected from each subject: one sample on visit 1 prior to treatment initiation and one sample on visits 3, 4, 5, 6, 7, 8, 9, and 10 during treatment and one sample at the follow-up visit should the subject not enter the extension trial (see Section 9 – Schedule of Assessments).

Subjects are given scheduled visit days, however, there is an allowed visit window, e.g., visit 3 is ±2 days and visit 10 is ±7 days (Section 9). Given these allowed visit windows, the PK sampling times relative to the last drug administration are not pre-determined. Charles River Laboratory will perform the analysis of the PK samples.

When glepaglutide is injected into the subcutaneous compartment, two main metabolites are formed: M1 ($ZP1848_{1-35}$) and M2 ($ZP1848_{1-34}$). The PK samples will be analyzed by use of a liquid chromatography mass spectrometry method optimized for the parent compound, M1 and M2. The method is validated according to regulatory guidelines. Only PK samples from subjects that have been given an active treatment will be analyzed.

The PK concentrations will be presented as the conceptual concentration (glepaglutide = parent + M1 + M2), the parent compound and the two main metabolites, M1 and M2.

Pharmacokinetic endpoints will be assessed as exploratory endpoints.

4. ANALYSIS POPULATION

4.1. FULL ANALYSIS SET (FAS)

The FAS will consist of all randomized patients, who received at least one dose of trial drug (glepaglutide or placebo). All efficacy analysis will be based on the FAS. Patients will be included in the analyses as randomized.

4.2. SAFETY ANALYSIS SET

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. Patients will be included in the analyses as treated.

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

4.3. PROTOCOL DEVIATIONS

Protocol deviations are documented in Phar-Olam's Clinical Trial Management System (CTMS). The CTMS deviation classifications are:

- Informed Consent (e.g., missing dates and/or signatures, wrong version used, PI-ICF updated but new version not signed in due time, study procedures before IC obtained, trial procedures done after IC withdrawal)
- In-and Exclusion Criteria (e.g., patient randomized despite violations in eligibility criteria)
- Study Drug (e.g., administration errors, dispensing of expired drug or drug that has been stored outside the required storage conditions, continuation of IP despite fulfilling withdrawal criteria)
- Trial procedures (e.g., single missed assessment related to the endpoints, repeatedly missed assessments/study procedures such as ECG, vital signs, physical exam, PK or safety sample(s) not collected, out of order, collected in wrong tube, or error during shipment etc., missing lab reports, lab reports not timely signed off, incorrectly performed tests, use of prohibited prescription or non-prescription medications or prohibited activities)
- GCP Non-Compliance (e.g., protocol or protocol amendment implemented prior to approvals and / or PI signature, non-authorized / untrained site staff completing study procedures, lack of PI involvement/oversight, missing source documents, unreported SAE or un-timely reporting of SAE)
- Miscellaneous (any items deemed noteworthy by the CRA/On-site Monitor)

All deviations will be reviewed by medical personnel and classified as major or minor before database lock and unblinding. Protocol deviations will be presented by category (major vs. minor) and deviation category within each category and summarized in 2 tables as follows:

- With frequencies and percentages of patients with at least one deviation in each category. Patients with multiple deviations will only be counted once for a given major/minor deviation category and once for the specific protocol deviation category within the major/minor; and

- With all incidences of the protocol deviations counted separately in each category. The total count of protocol deviations will be used as the denominator for percentages in this table.

A listing of all protocol deviations by patient and deviation category will also be provided, indicating which are major, before unblinding.

5. GENERAL ASPECTS FOR STATISTICAL ANALYSIS

5.1. GENERAL METHODS

- All analyses and summaries will be produced using SAS version 9.4 (or higher).
- Continuous variables will be summarized using the number of patients with evaluable data, mean (or geometric mean), standard deviation (or coefficient of variation), median, minimum and maximum. The same number of decimal places as in the raw data will be presented when reporting minimum and maximum, 1 more decimal place than in the raw data will be presented when reporting the mean and median, and 2 more decimal places than in the raw data will be presented when reporting the SD.
- Categorical variables will be summarized using the number of observations (n), frequency and percentage of patients. All percentages will be presented as one-decimal point, unless otherwise specified. Percentages equal to 100 will be presented as 100% and percentages will not be presented for zero frequencies.
- Unless stated otherwise, the percentages will be based on the number of non-missing observations. Treatment group headers will still contain the number of patients in the treatment group. If applicable, a row for the number of non-missing observations in the table (at each time point) for each variable will be included as part of the descriptive statistics.
- All comparisons will be between each ZP1848-17111 treatment group and placebo.
- Any calculated p-values will be presented to 3 decimal places; p-values less than 0.001 will be presented as ‘p<0.001’ and p-values greater than 0.999 will be presented as ‘p>0.999’.
- All relevant patient data will be included in listings and sorted by treatment group, patient ID, and visit, as applicable, for all patients relevant for the listing.
- Unscheduled or repeat assessments will not be included in summary tables unless specified otherwise, but they will be included in the patient listings.
- All tables, listings and figures will include footers that identify the name of the program that created the item, together with the date and time on which it was created. Headers will include the total number of pages that the presentation contains and, for each page, the number of the page within the presentation.

5.2. KEY DEFINITIONS

5.2.1. Baseline Values

For efficacy analyses, baseline is defined as the last available value prior to or on Visit 1 (Day 1).

In particular, the baseline actual PS value (L/week) will be defined as the actual PS volume received during a valid 7-day period prior to Visit 1 (Day 1). See Appendix A for details on the actual weekly PS volume derivation.

For safety analyses, baseline is defined as the last available value prior to or on the date of first dose of investigational product.

5.3. MISSING DATA AND DATA HANDLING RULES

Every effort will be made to collect all data at specified time points, according to the schedule of trial events. Reasons for withdrawal from the trial will be recorded on the eCRF.

For actual weekly PS volume, a detailed algorithm is included in Appendix A. In brief, for each visit, the actual weekly PS volume will be derived by using a valid 7-day period closest to the visit where

1. the actual weekly PS volume accounted for ≥ 90% of prescribed weekly PS volume

or

2. there is at least one actual PS volume accounted for by the patient in the eDiary for each day in the valid 7-day period

For all post-baseline visits, the 7-day period is allowed to start up to 14 days before a visit, provided that it does not start before the previous visit. For baseline visits, the 7-day period is allowed to start up to 30 days before the baseline visit, provided that it does not start before the latest optimization visit. The Day 3 and Week 1 visits are not included in the calculations.

Note that, if the last PS prescription during the treatment period has been stopped more than 7 days prior to a visit, and there are no entries of actual PS volumes recorded by the patient in the eDiary within 7-days prior to this visit then the actual weekly PS volume will be set to zero.

The efficacy endpoints will be missing at visits, where no valid 7-days period can be found based on the above algorithm.

Any missing data during the trial prior to trial completion or discontinuation will remain as missing at that time point. However, several multiple imputation methods will be used to impute missing data for the primary efficacy endpoint as well as for the key secondary efficacy endpoints. See 5.3.1 for details of the multiple imputation method.

Imputation rules for the handling of missing data for PROs and health economic assessments will follow rules per each assessment manual.

5.3.1. Multiple Imputation

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) or also known as *Copy Placebo*, 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 weekly PS volume will be analyzed based on data derived while the patient remains on trial as well as data imputed using multiple imputation (MI) methodology for time points at which no value is derived. 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 dropout rate seen in the teduglutide Phase 3 trial.

Imputation of values in the placebo (control) arm will assume MAR. Imputation of values in the glepaglutide arms will be done as if the subject 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 derived 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, a burn-in of 1000, 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 weekly 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 100 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 are specified in Table 7 below.

Table 7 Random seeds and number of imputations

	Random seed for imputing monotone missingness (if applicable)	**Random seed for imputation of remaining data**	**Number of imputations**
Primary imputation	2125	590774	100

Each of the 100 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 CR 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* (CIR) 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.

5.3.2. Patient Reported Outcomes

The PGIC, SBS-I and EQ-5D-5L PROs will be used to investigate the effects of treatment on health-related quality of life. There are three questionnaires:

1. The Patient Global Impression of Change (PGIC) is a one item outcome measure on a 7-point scale ordered from 'Very much worse' to 'Very much improved'.
2. The SBS-I scale assesses the symptoms and impact of SBS on everyday life and comprises 8 items, where responses to the individual items are registered on a numerical rating scale from 0=Not at all to 10=worst possible. By design, only integer responses are allowed. However, for existing recordings (3 instances), scores which are recorded in half increment values and entered into the database as such will be rounded to the higher response value for analysis purposes.
3. The EQ-5D-5L is a standardized measure of health status developed by the EuroQol Group and comprises 5 items, each on a 5-point scale, plus a general health item using a VAS scale 0-100.

Questionnaires must be completed at site visits prior to any other trial related assessment. The PGIC must be completed first, followed by the SBS-I, and 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.

An overall summary table of the number and percentage of subjects who provided each type of questionnaire data will be presented by treatment group. Percentages presented in subsequent

tabular summaries for each type of questionnaire will be based on the number of subjects who provided respective questionnaire data.

All questionnaires will be analyzed per item descriptively by treatment group and by SBS Anatomy Group. Summaries will be presented by domain as defined within each questionnaire. Presentations will focus on the 12 and 24-week time points.

The association between PGIC and the percentage change from baseline in PS volume at 12 and 24 weeks will be evaluated. Association between percentage improvement in PS volume and PGIC data (categorical data) for all treatment groups combined will be assessed using the Spearman rank-order and Kendall's tau-b correlation coefficients. Graphical methods will be applied as applicable.

The empirical cumulative distribution function and probability density functions overall and by PGIC response category (and by collapsed response category, if applicable) will be displayed.

The analyses of the SBS-I will be considered only as exploratory, as the instrument has not yet been validated.

Additionally, exit interviews are conducted for a subset of patients. The Exit Interviews are qualitative interviews related to SBS and parental support will also include quantitative questions on a 7-point, 0-10 numerical rating scale and 5-point scale, respectively. The exit interviews will be conducted at sites located in the United States, United Kingdom, France, Germany, and Denmark. The analysis of the exit interviews will be described in a separate Data Analysis Plan and reported elsewhere than in the Clinical Study Report.

5.4. POOLING OF SITES

No pooling of sites is planned for the efficacy analyses.

6. DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Descriptive statistics will be presented for age, age group, race, ethnicity, gender, height (cm), weight (kg), BMI (kg/m^2), body temperature (^oC), weekly PS volume requirements (stratification factor), region and SBS anatomical group for the Full Analysis Set.

6.1. PATIENT DISPOSITION AND WITHDRAWALS

Summary statistics will tabulate the number and percentage of patients who are screened, screening failures, randomized, who completed the trial, who prematurely discontinued the trial prior to Week 24, who prematurely discontinued the trial during the follow-up period and who prematurely discontinued the trial overall, together with reasons for discontinuation by treatment group for all patients screened. The number and percentage of patients included in each of the analysis populations will be presented. No statistical testing will be performed on these data. The number of patients in the FAS for each treatment group will be used as the denominator for percentages.

6.2. MEDICAL HISTORY

The number and percentage of patients with medical history by system organ class (SOC) and preferred term (PT) will be produced for patients in the FAS by treatment group. The medical history tables will be separated as follows:

- Past medical history defined as any medical history with an end date before the date of the screening visit

- Concurrent medical history defined as any medical history commenced or ongoing at the date of the screening visit

Medical history tables will be sorted by descending frequency of SOC and PT based on the total patient column using the Medical Dictionary for Regulatory Activities (MedDRA) coding dictionary, V24.1. For the summary tables, a patient may appear more than once if that patient has more than one medical history finding coded under different SOCs or more than one medical history finding with a different PT under the same SOC.A by-patient listing with coded SOC and PT along with verbatim eCRF term will be also provided.

6.3. CONCOMITANT MEDICATION

Concomitant medications will be coded by using the Anatomical Therapeutic Chemical (ATC) classification codes and preferred drug name according to the World Health Organization (WHO) Drug Global Q3 2021.

The number and percentage of patients in the FAS using concomitant medications will be summarized by ATC1 category, ATC4 category and generic drug name and sorted by descending frequency descending frequency (%) in the following hierarchy: glepaglutide 10 mg

TW > glepaglutide 10 mg OW > placebo.

Separate concomitant medication tables and listings will be prepared as follows:

- Concomitant medication commenced or ongoing at the first dose date
- Concomitant medication commenced after the first dose date

For each patient, the medication will be counted only once within a given ATC level and only once within a given generic drug name level. A patient may appear more than once if he/she has more than one concomitant medication coded under different ATC categories.

7. SAFETY

All safety analyses will be conducted using the Safety Analysis Set, unless specified otherwise. Patients will be analyzed according to the actual treatment received, rather than that to which they were randomized.

7.1. EXPOSURE AND TREATMENT INTERRUPTIONS

It should be noted that according to the protocols, the investigator can decide to pause treatment with the investigational product if a patient experiences an AE that is considered related to investigational product and if the investigator judges that dose pausation is required. These treatment pauses are allowed to an accumulated maximum of 4 weeks. Due to the long half-life of glepaglutide and the potential, longer persistence of the PD effects, it is considered appropriate to treat all AEs with an onset after the first dose of investigational product as treatment-emergent, including AEs with an onset during a treatment pause. To ensure a consistent approach for the calculation of adverse event rates, treatment pauses are ignored both in the numerator (i.e. including AEs with an onset during treatment pauses) and in the denominator (i.e. including the entire observation period, regardless of any treatment pauses in-between). Hence, patient-years of observation (PYO) will be calculated as [end of trial date – date of first trial dose + 1] / 365.25, where the 1 day is added to account for the first day of exposure to investigational product.

Note: the end of trial date is the Date of Disposition Event from the CRF Trial Completion Status form.

Exposure will be summarized by number of patients exposed and patient-years of observation.

Treatment interruptions will be defined as either 1) missing entries in the patient's eDiary because patient forgot to administer drug or forgot to record the dosing data or 2) treatment pauses agreed with the investigator as per protocol and reported as such in the eDiary.

Treatment interruptions will be summarized by number of patients with at least one treatment interruption, patient years of treatment interruptions, due to treatment pauses or due to missing data entry (because patient forgot to administer drug or forgot to record the dosing data), average duration of treatment interruptions (weeks) and by categories of cumulative duration of treatment interruptions.

Treatment pauses will be summarized by number of patients with at least one treatment pause, patient years of treatment pauses, average duration of treatment pauses (weeks) and by categories of cumulative duration of treatment pauses.

7.2. ADVERSE EVENTS

Adverse events (AEs) will be coded using MedDRA V24.1. All treatment-emergent AEs (TEAEs) from the start of trial drug dosing will be collected.

Treatment-emergent AEs are defined as AEs with onset date on or after the first day of exposure to investigational product. Unless otherwise stated, all outputs will be presented for treatment-emergent AEs and, for simplicity, referred to as AEs.

Related AEs are defined as events classified as 'possibly related' or 'probably related' by the investigator.

Severity categories will include mild, moderate, and severe. Any missing severity will be imputed as severe prior to selecting the report that will contribute to the summary. As a result, a patient would be counted as severe due to a missing severity, even if the patient reported similar events at a lesser degree of severity. The same logic will be applied to the related AEs.

For patients who permanently discontinued treatment due to an AE and who also withdrawal trial prematurely, the AE leading to treatment discontinuation is counted as an AE leading to trial withdrawal (regardless of the cause marked as leading to trial withdrawal).

Common AEs are defined as events (preferred terms) occurring in ≥5% of patients in the glepaglutide 10 mg OW or glepaglutide 10 mg TW treatment groups.

For all AE tables summarized by SOC and PT, a patient contributes only once to the count for a given AE on the SOC level and on the PT level within SOC. AE incidence tables will be sorted by descending frequency counts in hierarchical order of glepaglutide 10 mg twice weekly, then by glepaglutide 10 mg once weekly, and then by placebo. PTs will be sorted in similar hierarchical fashion within the SOC.

If there are less than 5 AEs of a type (AEs leading to treatment discontinuation, AEs leading to trial withdrawal, AESIs or AEs in other safety areas of interest) in the entire observation period, then only a listing will be prepared.

The following AE summaries will be presented by treatment group and will present the following information:

- n: number of patients experiencing at least one AE
- %: percentage of patients experiencing at least one AE
- E: number of AEs
- R: event rate (calculated as the number of AEs divided by PYO multiplied by 100)

An overall summary table will include the number and percentage of

- All adverse events
- Serious adverse events

- Severity (Mild, Moderate, Severe, Missing)
- Relationship (Possibly, Probably, Unlikely, Not Related, Missing)
- AEs leading to trial withdrawal
- AEs leading to treatment discontinuation
- AEs leading to treatment pause
- Outcome (Recovered/Resolved, Recovering/Resolving, Recovered/Resolved with Sequelae, Not Recovered/Not Resolved, Fatal, Unknown, Missing)

Additional AE tables will include:

- AEs by System Organ Class (SOC) and Preferred Term (PT)
- AEs Occurring in >=5% of Patients Treated with Glepaglutide by SOC and PT
- Summary of Serious AEs
- Serious AEs by SOC and PT
- Related AEs by SOC and PT
- Severe AEs by SOC and PT
- Moderate AEs by SOC and PT
- Mild AEs by SOC and PT
- AEs of Special Interest (AESIs) by Type and PT
- Other safety areas of interest (see Table 8) by SOC and PT

Other safety areas of interest are defined based on:

- their relevance for the SBS population or known class effects with other glucagon-like peptide-2 (GLP-2) receptor agonists (RAs) (e.g. gastrointestinal stenosis and intestinal obstruction [including stoma complications]; fluid retention and volume overload; hepatic disorders)
- general applicability to injectable peptide drugs (e.g. injection site reactions; hypersensitivity reactions)
- general interest for any new drug administered long-term (e.g. rare events; adverse event by organ system or syndrome [e.g., cardiac, renal and hepatic events]).

For these other safety areas of interest, events will be captured by applying the MedDRA queries as defined in Table 8. Both overall summary tables and tables by PT will be presented by treatment group for each safety area of interest separately. The following plots will be presented for injection site reactions and abdominal pain:

- Survival probability function plots (one minus the Kaplan-Meier estimator) for the time to the first occurrence of the selected AEs will be presented. Time to the first AE will be calculated from the date of first dose of investigational product.

- Mean cumulative function plots to evaluate recurrent AEs occurring after the date of the first dose of investigational product, showing the mean number of events over time.

Patients that do not experience the 'event' will be censored at the patient end of trial date. The purpose of these outputs will be to provide a visual presentation of the occurrence over time of these AEs after the first dose of investigation product, rather than any inference or comparison across the treatment groups. AEs with missing (or partially missing) onset date will not be included in these type outputs with a clear statement in a footnote if applicable.

Point prevalence plots with the proportion of patients with events over time will be presented for abdominal pain. The denominator at a specific time point will be the number of patients at risk.

Table 8 Methodology for capture of adverse events relevant for other safety areas of interest

Safety area of interest	**Methodology**
Gastrointestinal stenosis and intestinal obstruction *(including stoma complications)*	SMQ 'Gastrointestinal obstruction' (narrow scope) combined with HLT 'Stoma complications' (all terms)
Gallbladder and biliary disease	SMQ 'Biliary tract disorders' (narrow scope) combined with HLT 'Cholecystitis and cholelithiasis' (all terms)
Fluid retention and fluid overload	SMQ 'Haemodynamic oedema, effusions and fluid overload' (narrow scope)
Abdominal pain	HLT 'Gastrointestinal and abdominal pains (excl oral and throat)' (all terms)
Hypersensitivity reactions	SMQ 'Hypersensitivity' (broad scope)
Injection site reactions	Tick mark for 'study treatment injection site reaction' in eCRF (AE form)
Cardiac adverse events	SMQ 'Cardiac arrhythmias' (narrow scope) combined with SMQ 'Cardiac failure' (narrow scope)
Renal adverse events	SMQ 'Acute renal failure' (broad scope)
Hepatic adverse events	SMQ 'Hepatic disorders' (broad scope)
Rare events	CMQ based on EMA Designated Medical Event list, 2020[1]

[1] https://www.ema.europa.eu/en/documents/other/designated-medical-event-dme-list_en.xlsx
Abbreviations: CMQ: customized MedDRA query; EMA: European Medicines Agency; HLT: higher level term; MedDRA: Medical Dictionary for Regulatory Activities; SMQ: standardized MedDRA query

The risk difference with 95% confidence intervals (using the Miettinen and Nurminen estimation method) will be estimated to quantify the uncertainty of the treatment comparison between placebo and glepaglutide 10 mg OW, placebo and glepaglutide 10 mg TW, and placebo and either of the glepaglutide groups (glepaglutide total); no formal hypothesis testing will be performed. This comparative analysis will be done for AEs leading to treatment discontinuation, AEs leading to trial withdrawal, SAEs, prespecified AESIs, other safety areas of interest and AEs occurring in >=5% of patients treated with glepaglutide (10 mg once weekly or 10 mg twice

weekly). The result will be presented graphically with a dot plot in the left panel illustrating the event rates by treatment group (e.g., glepaglutide 10 mg once weekly and placebo) and a forest plot in the right panel presenting the corresponding risk difference and confidence intervals.

As a separate table, Symptoms of Injection Site Reactions will be presented with patient counts, percentages, number of events, and adverse event rate for injection site reactions including spontaneous pain, pain on palpitation, itching, redness, oedema, induration/infiltration, and other. The above symptoms are captured for AEs with a Tick mark for 'study treatment injection site reaction' in the eCRF (AE form).

The following patient listings will be provided as tables:

- Deaths
- Serious AEs
- AEs Leading to Trial Withdrawal
- AEs Leading to Treatment Discontinuation
- AEs Leading to Treatment Pause
- AESIs
- AESIs – Details on Neoplasms
- Other Important Events

A listing of all TEAEs will be provided.

7.3. CLINICAL LABORATORY EVALUATIONS

Clinical laboratory parameters include:

Hematology:
Hemoglobin, Hematocrit, Red blood cell (RBC) count, White blood cell (WBC) count with differential (including absolute value and % value for neutrophils, lymphocytes, monocytes, basophils, and eosinophils), Platelet count, and International normalized ratio (INR)

Biochemistry:
Sodium, Potassium, Chloride, Bicarbonate, Blood urea nitrogen, Creatinine, Estimated creatinine clearance, Glucose, Calcium, Phosphorous, Magnesium, Alkaline Phosphatase, Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Gamma-glutamyl transferase (GGT), Lactic dehydrogenase, Conjugated bilirubin, Total bilirubin, Total protein, Triglycerides, Cholesterol, Albumin, Amylase, Uric acid, Zinc, and C-reactive protein (CRP)

Urine:
Blood, Glucose, Leukocytes, pH, Osmolality, Protein, Sodium, and Potassium

Hematology, biochemistry, and pregnancy laboratory tests performed by a central laboratory will be included in ADLB dataset.

Due to the COVID-19 public health emergency, some of the trial participants were not able to come to the investigational site for all protocol-specified visits. Instead, the visits could be conducted as phone visits, if considered safe by the investigator. The investigator could also refer the patient to a local laboratory, if deemed necessary.

In general (independent of the COVID-19 pandemic), blood samples for analysis at a local laboratory can be drawn at the investigator's discretion during the trials; these values will be included in narratives for SAEs and AESIs as appropriate.

Descriptive statistics of the laboratory parameters will be presented by treatment group for all nominal trial visits at which they were collected. The change from baseline in hematology and biochemistry values will also be summarized by treatment group. Summary tables will also include the end of trial value defined as is the last available value in the trial and also the highest and lowest post-baseline values including values from unscheduled visits.Box plots of actual values will be produced for all hematology and biochemistry parameters. For laboratory parameters that are log-transformed the geometric mean will be presented in the box plot.

Values of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (TB), gamma-glutamyl transferase (GGT), creatinine, blood urea nitrogen (BUN), estimated glomerular filtration rate (eGFR), and amylase will be log-transformed, and outputs will show the results back-transformed to the original scale. For these parameters, the geometric mean and coefficient of variation will be presented.

Hematology and biochemistry parameters will be presented as shifts from baseline (low, normal, high, missing) to each post-baseline visit where laboratory samples are scheduled for assessment.

Urinalysis parameters will be summarized as shifts from baseline (low, normal, high) to each visit assessed by treatment group.

For listings and plots of patient trajectories, all post-baseline central laboratory measurements, including unscheduled measurements will be included.

A listing of abnormal laboratory values will be presented.

Pregnancy laboratory tests will be listed only.

Separate figures by treatment group with individual patient trajectories over time of AST , will be presented for patients with at least one post-baseline AST value >3 times the upper limit of normal.. Similar plots will be presented for ALT, for patients with at least one post-baseline ALT value >3 times the upper limit of normal.

All central laboratory values will be included in patient data listings.

7.4. VITAL SIGNS

Vital signs data include measurements of heart rate (beats/minute), blood pressure (mmHg), and oral body temperature (Celsius). Descriptive statistics of the vital signs will be presented by treatment group for all nominal trial visits at which they were collected. The change from baseline will also be summarized by treatment group. Summary tables will also include the end of trial value defined as the last available value in the trial and also the highest and lowest post-baseline values including values from unscheduled visits.

Box plots of actual values should be produced for all vital sign parameters.

Shifts from baseline (Normal, Abnormal Not Clinically Significant, Abnormal Clinically Significant, Missing) to each post-baseline visit will be tabulated by treatment group for each vital sign parameter.

All vital signs data will be listed.

7.5. ECG

ECG parameters include heart rate (beats/min), PR interval (ms), PR interval Aggregate (ms), QRS duration aggregate (ms), QT interval aggregate (ms), QTcF interval aggregate (ms), and RR interval (ms), as well as the overall interpretation of each subject's ECG recorded as Normal, Abnormal Not Clinically Significant, or Abnormal Clinically Significant.

Descriptive statistics of ECG parameters will be presented for the actual values and change from baseline for each nominal trial visits by treatment group. Summary tables will also include the end of trial value defined as is the last available value in the trial and he highest and lowest post-baseline values including values from unscheduled visits.

Box plots of actual values should be produced for all ECG parameters.

Shifts from baseline in ECG overall interpretation to each scheduled post-baseline visit where ECG was assessed will be summarized by treatment group in tabular form.

Categorical summaries will present the number and percentage of patients with at least one post-baseline observation meeting the outlier criteria specified in Table 9. A listing with ECG parameter values will be provided for all patients with at least one post-baseline QTcF value >450 msec.

Table 9 Outlier thresholds for QTcF

Parameter	Threshold
QTcF[a]	>450 ms >480 ms >500 ms Increase from Baseline in QTcF >30 ms

Increase from Baseline in QTcF >60 ms

Notes: a: based on threshold definitions in ICH E14, 2005.
Abbreviations: QTcF: QT interval corrected using Fridericia's formula.

To support the outlier analyses for QTcF, AEs related to QT prolongation (defined by the MedDRA SMQ 'Torsade de Pointes/QT prolongation' [narrow scope]) will be summarized by preferred term.

All ECG data will be listed.

7.6. PHYSICAL EXAMINATION

A listing with physical examination findings will be provided.

7.7. IMMUNOGENICITY

7.7.1. Immunogenicity Assessment

A serum sample is defined as anti-drug antibody (ADA)-positive if results of the anti-glepaglutide antibody screening and confirmatory assays are positive. If positive, a titer (highest dilution factor that still yields a positive reading) will be reported. Patients without any ADA data (evaluable samples) will be excluded from the analysis.

1. Pre-existing ADA (Yes/No) is defined, at the patient level, by the ADA status at trial baseline (i.e. Day 1), according to the presence or absence of ADA. This endpoint is defined for any patient with a valid ADA assessment at Day 1.

2. A treatment-boosted ADA positive patient (Yes/No) is defined, at the patient level, as any occurrence during the trial of at least a 4 fold increase from trial baseline in ADA-titer for patients with pre-existing ADA (and a titer at baseline).

3. A treatment-induced ADA positive patient (Yes/No) is defined, at the patient level, as any occurrence of ADA during the trial for patients without pre-existing ADA (based on a negative baseline ADA measurement).

4. An ADA positive patient (Yes/No) is defined by being either a treatment-boosted or treatment-induced ADA positive during the trial (for patients with a baseline ADA measurement).

Confirmed positive ADA samples will be further evaluated in three different ADA characterization assays: for in *vitro* glepaglutide neutralizing potential (NAb), for reactivity to the predominant metabolite (M2) and for cross-reactivity towards GLP-2. In case of a positive result in the ADA characterization assays, a titer will be estimated.

For ADA negative samples, the missing assessments for in vitro glepaglutide neutralizing potential (NAb), for reactivity to the predominant metabolite (M2) and for cross-reactivity towards GLP-2 will be considered negative in all below analyses and presentations.

In case of positive results at study baseline (Day 1) in the ADA characterization assays ('Pre-existing NAb', 'pre-existing M2 reactivity', 'pre-existing GLP-2 cross-reactivity'), the definitions and calculations for pre-existing ADA and treatment boosted ADA will correspondingly apply.

In case of a negative assessment in either of the four assays (ADA, NAb, M2 reactivity and GLP-2 cross-reactivity) the titer value will be set to ¼ MRD (Minimal Required Dilution) for illustration purposes.

7.7.2. Immunogenicity Statistical Methodology

All analyses will be done for all four ADA assessments (ADA, NAb, M2 reactivity and GLP-2 cross-reactivity), unless otherwise specified.

The overall anti-glepaglutide antibody incidence (sum of treatment boosted and treatment induced) will be tabulated by treatment group and visit, and overall (incidence of patients ADA positive any time during trial). Similar tables will be made separately for treatment boosted and treatment induced ADAs, if both types are observed. In addition, the ADA incidences by visit will be presented graphically.

For all treatment induced ADA positive patients, the titer levels will be summarized by treatment group and visit using descriptive statistics including median, geometric mean, geometric CV%, inter-quartile range, minimum, maximum, and number of observations (positive samples only).

For all treatment boosted ADA positive patients (if applicable), the titer levels and fold increases from baseline of titers will be summarized by treatment group and visit using descriptive statistics including median, geometric mean, geometric CV%, inter-quartile range, minimum, maximum, and number of observations (positive samples only).

Spaghetti plots of individual trajectories over time of ADA titers (and fold increases of ADA titers, if applicable) will be presented for each treatment group. Plots of the geometric mean ADA titers by visit and treatment group will be presented.

All immunogenicity data will be listed, including corresponding PK concentrations.

Investigating immunogenicity impact on efficacy and safety

To assess the impact of ADA on efficacy and safety, the results for each of the ADA assessments (ADA, NAb, M2 reactivity and GLP-2 cross-reactivity) will be categorized into three levels (negative, low titer positive, and high titer positive).

The ADA results will be grouped as either 1) or 2):

(1) ADA status/titer, if more than 5 patients have positive outcome in the assessment:

a. Level 1: ADA negative

b. Level 2: ADA low titer positive (titers ≤ median titer at Week 24)

c. Level 3: ADA high titer positive (titers > median titer at Week 24)

(2) ADA status/titer, if 5 patients or less have positive outcome in the assessment:

d. Level 1: ADA negative

e. Level 2: ADA positive

The median titer at Week 24 will be calculated for the ADA positive samples only.

Immunogenicity association with PK

The analysis of ADA association with PK will be made for all analytes, i.e. for the conceptual concentration (glepaglutide), the parent compound (parent) and the two main metabolites (M1 and M2) and using the assessments ADA and M2-reactivity only.

In cases, where an analyte will only contribute to a particular analysis with concentrations <LLOQ, the analysis will be omitted for that analyte.

The potential association between ADA and PK will be investigated graphically, by plots of individual observed trough concentrations by treatment and visit (Day 1, W2, W4, W8, W12, W24), where observations with ADA measurements at the same visits are marked according to the observed ADA titer categories defined above. A concentration is considered a trough measurement if taken more than 60 hours after the preceding dose of glepaglutide. The plots will also be made where all observations for each subject are marked according to the ADA level of the patient at week 24.

Similar plots will be made for all observed concentrations, regardless of timing of assessment relative to last dose.

Immunogenicity association with efficacy

The analysis of ADA association with efficacy will be made for the primary endpoint, change from baseline in actual PS volume, and using all four ADA assessments (ADA, NAb, M2-reactivity, and GLP-2 cross-reactivity).

The potential association between ADA and efficacy will be investigated graphically, by plots of individual trajectories of change from baseline in actual PS volume (L/week) by treatment and visit (Day 1, W2, W4, W8, W12, W24), where observations with ADA measurements at the same visits are marked according to the observed ADA titer category defined above. The plots

will also be made where observations for each subject are marked according to the ADA titer category of the patient at week 24.

Plots by treatment of individual change from baseline in actual PS volume (L/week) at week 24 versus titer values at week 24, will also be presented.

Immunogenicity association safety

The potential association between ADA and safety will be investigated by summarizing selected types of treatment emergent adverse events (TEAEs) by severity and seriousness by ADA titer category at Week 24 as defined above.

AEs which could potentially be associated with ADA include injection site reactions and conditions which may reflect immune-mediated adverse events, such as hypersensitivity reactions, infusion reactions, and inflammatory responses secondary to immune complex or complement-mediated reactions.

In addition, the potential association between levels of ADA, neutralizing ADA, or ADA binding to M2, or cross-reacting with human GLP-2 (endogenous counterpart), and AEs related to lack of efficacy will be investigated.

The AEs of interest will be selected according to the following definitions and MedDRA SMQs:

(1) Lack of efficacy/decreased drug effect (using the assessments ADA, NAb activity, M2 reactivity, and GLP-2 cross-reactivity) (Extracted with the MedDRA SMQ: Lack of efficacy/effect (narrow scope))

(2) Injection site reactions (using the assessment ADA only) (captured using the CRF tick mark ‘study treatment injections site reaction’)

(3) Allergic reactions (using the assessment ADA only) (Extracted with the following MedDRA SMQ: Hypersensitivity (broad scope)

Further analyses of associations between ADA and PK, efficacy or safety will be made if suggested by the graphical evaluations and summary statistics.

8. CHANGES FROM PROTOCOL

Protocol Section	SAP Section	Summary of Change
12	3	• For the primary, the key secondary efficacy endpoints and the endpoints related to the macro- and micro-nutrients (all derived from actual PS), sensitivity analyses will be made based on a valid 14-days period prior the relevant visits.
12.3	3.0	• Efficacy endpoint descriptions have been updated using the word "change" instead of "reduction".
N/A	3.1	• Added a COVID-19 statement that no sensitivity analyses will be done to assess the impact of COVID-19, and cited reference document describing COVID-19 continency plans for glepaglutide phase 3 trials. This text does not appear in the protocol.
12.3.4	3.4	• For the secondary efficacy endpoint of *Change in weight from baseline to Week 24*, the BMI subgroup categories were updated to match the BMI subgroup categories used in the ISS. In addition, a shift table from baseline to Week 24 by BMI subgroup categories has been added.
12.3.4	3.5	• Graphical presentations of urine volume, fluid intake together with PS vol, have been added. The presentations replace the MMRM analyses of FCE described in the protocol.
12.4	3.7	• The nomenclature of the conceptual analyte glepaglutide (parent + M1 + M2) was changed compared to the protocol.
12.2	4.0	• The Per-protocol Analysis Set has been deleted as a trial analysis set and replaced with a supplementary estimand using a hypothetical strategy assuming missing data to be missing at random (MAR).
12.6	5.3	• The algorithm defining when and how weekly PS volume is calculated and when it is considered missing is included.
12.7	5.3.1	• For the multiple imputation method, the number of imputed datasets changed from 1000 to 100.

9. CHANGES FROM PREVIOUS SAPS

SAP version	SAP section	Summary of change	Rationale for change
5.0 → 6.0	General	• Editorial changes throughout e.g. document version, dates, table and figure references, addition/removal of sections, changes to header titles etc.	• To have a coherent document
5.0 → 6.0	1.1	• Responsibility of TFL generation transferred to Zealand Pharma	• To reflect internal strategic decision
5.0 → 6.0	2.3	• Editorial changes • Addition of supplementary estimand	• To make the specification of primary estimand of primary endpoint clear • To make the specification of supplementary estimand of primary endpoint clear

5.0 → 6.0	3.1	• Introduction of terminology main estimator and sensitivity estimators
		
		• Addition two sensitivity analyses
		  ○ Tipping point analysis
		  ○ Analysis on all observed data
		
		• Addition of Table 3
		
		• Addition of Week 1 to the primary analysis model
			• For ease of reference
			
			• To further explore robustness of primary analysis of primary endpoint in terms of missing data assumptions under which the conclusions change and impact of using multiple imputation methods, respectively
			
			• To provide a better overview of the various estimands and estimators, and how missing data is handled
			
			• To accurately determine the study drug's treatment effect in the early weeks

5.0 → 6.0	3.1.1	• New section divider added • Definition of collapsing PGIC groups redefined	• For ease of navigation • Collapsing of groups is now made based on current data distribution to

SAP version	SAP section	Summary of change	Rationale for change
			support interpretation in the best possible way
		• Spearman rank-order and Kendall's tau-b correlation coefficients added	• To further specify the type of correlation coefficients used. The coefficients are chosen based on their typical use in psychometric evaluation of assessment tools
5.0 → 6.0	3.1.2	• New section divider added	• For ease of navigation
5.0 → 6.0	3.1.3	• New section divider added	• For ease of navigation
5.0 → 6.0	3.1.4	• New section introduced with addition of supportive analyses for primary endpoint derivation (actual weekly PS volume)	• To explore robustness of the primary statistical analysis with regard to the primary endpoint derivation
5.0 → 6.0	3.2	• Primary and supplementary estimand has been defined for each key secondary efficacy endpoint	• To further clarify the clinical question of interest and have a precise description of the treatment difference estimated for each of the key secondary efficacy endpoints
		• Primary and sensitivity analysis for primary estimand, and primary analysis for supplementary estimand are now defined for each key secondary efficacy endpoint	• To avoid any misalignment with assessment of robustness of primary estimate for each of the key secondary efficacy endpoints
		• Addition of Table 6	• To provide a better overview of the various estimands and estimators, and how missing data is handled
		• For binary key secondary efficacy endpoints, the method for weighing and estimating CIs and type of test have been specified, along with a model	• The exact model specifications are needed before unblinding as these are confirmatory endpoints

SAP version	SAP section	Summary of change	Rationale for change
		simplication procedure in case of zero strata occurrence	
		• For binary key secondary efficacy endpoints, Wilson-Hilferty transformation has been specified for the sensitivity analysis (using CR-imputed values)	• To handle asymptotics of Cochran-Mantel-Haenszel test under the null hypothesis of no association and applying Rubin's rule
5.0 → 6.0	3.3	• New high-resolution figure added	• Text in figure was not readable before
5.0 → 6.0	3.4	• Addition of endpoint 'Reduction of at least 30% in actual weekly PS volume from baseline to both Weeks 20 and 24'	• To further explore different definitions for clinical response based on the outcome of the clinical meaningfulness analysis specified in 3.1.1
		• Addition of endpoint 'Reduction in prescribed weekly PS volume of 100% (weaned off) at Week 24'	• The endpoint is considered clinically relevant and thus should be pre-specified
5.0 → 6.0	5.3	• Algorithm updated	• To align with Appendix A
5.0 → 6.0	5.3.1	• Removal of tuning and thinning specifications for non-monotone MCMC procedures	• The impact of specifying these technical details in terms of convergence of the MCMC method is considered negligible, and hence not considered for the programming. Imputations of non-monotone missing data pattern will be performed using the MCMC statement in PROC MI
		• Addition of Week 1 to imputation model	• To accommodate inclusion of Week 1 in the primary statistical model
5.0 → 6.0	5.3.2	• Biserial correlation coefficient replaced with Spearman rank-order and	• To align with section 3.1.1. The biserial correlation coefficient is

SAP version	SAP section	Summary of change	Rationale for change
		Kendall's tau-b correlation coefficients	used when there is one dichotomous variable, which is not the case
5.0 → 6.0	7.2	• Definition of AEs leading to withdrawal added	• As AEs leading to trial withdrawal are not readily available in data as collected, a need to define a conservative rule to identify these events was deemed necessary
		• Definition of common AEs added	• For ease of reference at time of reporting
5.0 → 6.0	13	• Section 'Table of Contents for Tables, Listings, and Figures' deleted	• This is not considered mandatory for a SAP and is specified elsewhere
5.0 → 6.0	A	• New terminology introduced (e.g. *accounted for*) and further clarification text added, and minor typos corrected	• To ensure the algorithm reads well
		• Allowing to look further back in time for a valid period for baseline specifically	• To allow a window of identifying a valid period for baseline aligned with the CTP (stabilization period between 2-4 weeks), i.e. using data from a period considered clinically representable of baseline
		• Not allowing to use data points from the optimization period	• In this period the patients are not considered stable
		• An exemption to the step 2 rule of *a valid period cannot start before the previous visit for post-baseline visits*, has been implemented for Week 1 (in addition to Week 2)	• To accommodate inclusion of Week 1 in the primary statistical model
5.0 → 6.0	B	• Two additional references added	• References necessary after updates to the SAP

10. GLOSSARY OF ABBREVIATIONS

Abbreviation	Description
ADA	Anti-Drug Antibody
ADaM	Analysis Data Model
AE	Adverse Event
AESI	Adverse Event of Special Interest
ATC	Anatomical Therapeutic Chemical
BMI	Body Mass Index
CI	Confidence Interval
CIR	Copy Increments from Reference
CMH	Cochran-Mantel-Haenszel
CR	Copy Reference
CTMS	Clinical Trial Management System
eCDF	Empirical Cumulative Distribution Function
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FAS	Full Analysis Set
FDA	Food and Drug Administration
ICH	International Conference to Harmonisation
ITT	Intent-To-Treat
IWRS	Interactive Web Response System
J2R	Jump To Reference
kg	Kilogram
K-M	Kaplan-Meier
MedDRA	Medical Dictionary for Regulatory Affairs
mg	Milligram
MI	Multiple Imputation
mL	Milliliter
mm	Millimeter
mmHg	Millimeters of Mercury

Abbreviation	Description
MMRM	Mixed Model for Repeated Measurements
ms	milliseconds
PDF	Probability Density Function
PGIC	Patient Global Impression of Change scale
PRO	Patient Reported Outcome
PT	Preferred Term
RR	Relative Risk
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SBS	Short Bowel Syndrome
SBS-I	SBS-Impact Scale
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment Emergent Adverse Event
TLF	Table, Listing and Figure
WHO	World Health Organization

11. 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

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
PK[11]				X		X	X	X	X	X	X	X	X	X
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 (US, UK, French, Danish, and German 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	

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
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 study 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 (US, UK, French, Danish, and German 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.

12. QUALITY CONTROL

Validation of analysis datasets and tables are conducted through independent parallel programming of the statistical output according to the agreed upon specifications defined in the protocol, SAP, table shells, and dataset specifications. In this process, two programmers working independently (i.e., without input from one another), program the same output and compare results (via SAS PROC COMPARE). Any discrepancies are discussed and resolved, and the validation cycle is repeated until no further differences are noted between the two outputs. Once the validation cycle is complete, the output is subjected to senior review by the statistician. All programs are submitted in batch mode to document the results of the PROC COMPARE indicating no unequal observations. Additionally, tracking logs are maintained which document all quality control and validation findings and their resolution.

APPENDIX A. CALCULATION OF PRESCRIBED AND ACTUAL WEEKLY PS VOLUME

Prescribed weekly PS volume based on a 7-day period

The prescribed weekly PS volume (L/week) record per patient per nominal visit attended on or after Visit 1 (Day 1) will be calculated. The source is the prescribed PS volumes recorded in the eCRF by the investigator (SDTM.CM where CM.CMSCAT= 'PARENTERAL SUPPORT PRESCRIPTION').

A prescribed PS regimen consists of ≥ 1'bag identifier(s)' (SDTM.CM.CMSPID). For each prescribed 'bag identifier' the volume (SDTM.CM.CMDOSE) is recorded in L/week. The prescription of a 'bag identifier' is considered current from the start date (CM.CMSDTC) until the end date (CM.CMENDTC) or until the end of trial if the last prescription end date is not available, hereafter referred to as a prescription period.

A prescription period might cover several visits. A PS prescription can be updated at the scheduled visits, but also in between visits at the discretion of the investigator.

If the end date of a current prescription period is the same as the start date of the next prescription period, the end date of the current period is set to the end date minus one day to avoid overlap.

The prescribed volume (AVAL) is derived as the sum of the prescribed volumes during the 7-day period just prior to the visit divided by 7 (to account for the volumes being recorded in L/week), i.e. the period is defined from the visit date minus 1 to the visit date minus 7. Hereafter referred to as the *prescribed volume*.

Note that, if the last PS prescription during the treatment period has been stopped more than 7 days prior to a visit, the prescribed weekly PS volume will be set to zero at the relevant visit.

Actual weekly PS volume based on a valid 7-day period

An actual weekly PS volume (L/week) record per patient per nominal visit attended on or after Visit 1 (Day 1) will be derived, with the exception of visits Day 3 and Week 1 which will not be included in the ADaM dataset (see steps below).

The source is the actual PS volume records entered into the eDiary by the patient (SDTM.ZP), hereafter referred to as volume records.

The actual weekly PS volume (AVAL) is derived as the sum of the volume records (ZP.ZPSTRESN) in a valid 7-day period prior to the relevant visit. Hereafter referred to as the *actual volume*.

The weekly PS volume accounted for is derived as the actual volume in a valid 7-day period prior to the relevant visit plus prescribed volumes documented not taken (via notes to file) during the same period. Hereafter referred to as the *volume accounted for*.

The relevant prescribed volume is derived as the sum of the prescribed volumes during the valid 7-day period divided by 7 (to account for the volumes being recorded in L/week). Hereafter referred to as the *relevant prescribed volume*.

The steps to select a valid 7-day period and derive AVAL related to an attended visit are as follows:

1. The first period considered will be the 7-days period from the visit date minus 1 day to the visit date minus 7 days.

2. A valid period cannot start before the previous visit for post-baseline visits (before the last optimization visit for baseline visits). If the considered period starts before the previous visit date then, AVAL will be set to missing, ANLxxFL is set to ’ ’ and ANLxxREA = "Period starts before previous visit" and the loop stops.

   Note that, the exemption to this rule will be that a Week 1 valid period will be allowed to start prior to the date of visit Day 3 (which is not included in the dataset) or baseline visit, and Week 2 valid period will be allowed to start prior to the dates of visits Week 1 or Day 3 (which is not included in the dataset).

3. Else, if there is at least one volume accounted for at each day in the eDiary for the period, then the period is considered valid, AVAL is derived as specified above, and ANLxxFL is set to ’Y’ and the loop stops.

4. Else, if for the period, the volume accounted for is ≥ 90% of the relevant prescribed volume, then the period is considered valid, AVAL is derived as specified above, and ANLxxFL is set to ’Y’ and the loop stops.

If the loop is not stopped in steps 1, 2, 3 and 4 then a new 7-day period is defined from the visit date minus 2 days to visit date minus 8 days and the loop is re-started (steps 2, 3, and 4). For post-baseline visits, the loop can be re-started until the new 7-day period is from the visit date minus 8 days to the visit date minus 14 days. For baseline visits, the loop can be re-started until the new 7-day period is from the visit date minus 24 days to the visit date minus 30 days. If the loop reaches earliest allowed period without stopping then AVAL will be set to missing, ANLxxFL =’ ’ and ANLxxREA = "No valid period identified".

Note that, if there are 7 days without relevant prescribed PS prior to a visit, then the period will be considered valid, AVAL will be derived as the sum of available volumes found in the 7-day prior to the visit, and the ANLxxFL will be set to ’Y’. If and there are no PS volume entries in the eDiary, AVAL will be set to 0 (zero).

Note: that for calculating ≥ 90% volume accounted for, the relevant prescribed volume will be calculated using the same time-period as the actual volume, in contrast to the prescribed parameter described above, which strictly uses the values in the period visit date minus 1 day to visit date minus 7 days for the derivation.

Prescribed and actual weekly PS volume based on a 14-day period

The same rules as stipulated above apply when calculating the **actual** weekly PS volume based on a 14-day valid period, except that the loop can be re-started until the 14-day period is from the visit date minus 28 days to the visit date minus 15 days.

Note that, if the last prescription end date is more than 14 days prior to the visit, and there are no PS volume entries in the eDiary, the actual weekly PS volumes (AVAL) will be set to 0, and ANLxxFL will be set to 'Y'.

Note that, for the actual volumes the Day 3, Week 1 and Week 2 visits will not be included in the dataset, and hence the Week 4 valid 14-day period is allowed to start before the date of any of these visits.

The **prescribed** weekly PS volume will be derived as described above but based on the period from the visit date minus 1 to the visit date minus 14. Note that, if the last prescription end date is more than 14 days prior to the visit, the prescribed weekly PS volumes (AVAL) will be set to 0, and ANLxxFL will be set to 'Y'.

For all parameter values the summations of the records span 14 days, the values will be divided by 2 to reflect L/week.

APPENDIX B. REFERENCES

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