Cover Page for Statistical Analysis Plan

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Statistical Analysis Plan

Investigation of once-weekly semaglutide s.c. dose-response in patients with type 2 diabetes and overweight – a participant- and investigator-blinded and sponsor open-label study

Substance: Semaglutide

Redacted statistical analysis plan
Includes redaction of personal identifiable information only.

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CDS-GBS Biostatistics

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Version History

This Statistical Analysis Plan (SAP) for study NN9535-4984 is based on the protocol version 2.0 dated 16JUN2022.

SAP Version	Date	Change	Rationale
1.0	04 August 2022	Not Applicable	Original version

List of abbreviations

AE	adverse event
ANCOVA	analysis of covariance
BMI	body mass index
CI	confidence interval
DMC	data monitoring committee
EMA	European Medicines Agency
FDA	US Food and Drug Administration
ICH	International Council on Harmonization
LLoQ	lower limit of quantification
MedDRA	medical dictionary for regulatory activities
SAP	Statistical Analysis Plan
SMQs	standardized MedDRA queries
TFL	tables, figures and listings

1 Introduction

There are no changes to the analyses described in the protocol.

Specifications of tables, figures and listings (TFL) and other specifications not included in this SAP will be described in the mock TFL.

1.1 Objectives, Endpoints, and Estimands

Table 1 Objectives and Endpoints

Objectives	Endpoints		
Primary	Title	Time Frame	Unit
To characterise the dose-response curve of once-weekly semaglutide s.c. for change in HbA1c from baseline to week 40 in patients with T2D and BMI ≥ 27 kg/m2 as an add-on to a stable dose of metformin	Change in HbA1c	From baseline (week 0) to end of treatment (week 40)	%-point
Secondary	Title	Time Frame	Unit
To characterise the dose-response curve of once-weekly semaglutide s.c. for change in body weight from baseline to week 40 in patients with T2D and BMI ≥ 27 kg/m2 as an add-on to a stable dose of metformin	Confirmatory		
	Change in body weight	From baseline (week 0) to end of treatment (week 40)	kg
To characterise the dose-response curve of once-weekly semaglutide s.c. for safety and tolerability from baseline to week 49 in patients with T2D and BMI ≥ 27 kg/m2 as an add-on to	Supportive		
	Number of treatment-emergent adverse events (TEAEs)	From baseline (week 0) to end of study (week 49)	Count of events
	Number of treatment emergent severe hypoglycaemic episodes	From baseline (week 0) to end of study (week 49)	Count of events

a stable dose of metformin			

1.1.1 Estimands

The estimands and their rationale are described in detail for each of the two primary and secondary objectives. Two intercurrent events were identified:

- Premature discontinuation of randomised treatment
- Initiation of rescue medication (refer protocol Section 6.8.1)

1.1.2 Addressing the primary objective

1.1.2.1 Primary estimand

The primary estimand addresses the main question of interest: What is the dose-response curve of once-weekly semaglutide s.c/pooled placebo for change in HbA1c (%-point) from baseline to week 40 in patients with T2D and BMI ≥ 27 kg/m2, as an add-on to a stable dose of metformin regardless of premature discontinuation of randomised treatment and initiation of recuse medication? Notably, in the treatment regimen evaluated, participants who cannot tolerate the received dose will have treatment discontinued.

The primary estimand is defined by the following five attributes:

- Population: Patients with T2D and BMI ≥ 27 kg/m2.
- Endpoint: Change from baseline to week 40 in HbA1c (%-point).
- Treatment condition: Randomised treatment as add-on to a stable dose of metformin, with or without premature discontinuation of treatment and initiation of rescue medication.
- Remaining intercurrent events: No further intercurrent events are identified. The two intercurrent events described as part of the treatment condition will all be handled by a treatment policy strategy.
- Population-level summary: Difference in mean changes from baseline.

Rationale for estimand: This estimand quantifies the difference in treatment effects between the different treatment regimens that can be expected in clinical practice. It reflects the clinical practice, under which the treatment regimens are to be applied.

1.1.2.2 Additional estimand

The additional estimand addresses an additional question of interest: What is the dose-response curve of once-weekly semaglutide s.c/pooled placebo for change in HbA1c (%-point) from baseline to week 40 in patients with T2D and BMI ≥ 27 kg/m2, as an add-on to a stable dose of metformin, if all participants had remained on randomised treatment without initiation of rescue medication? Notably, in the treatment regimen evaluated, participants who cannot tolerate the received dose will have treatment discontinued.

The estimand attributes: population, endpoint, and population-level summary are the same as for the primary estimand. The remaining attributes are:

- Treatment condition: Randomised treatment as add-on to a stable dose of metformin, without premature discontinuation of treatment and initiation of rescue medication.
- Remaining intercurrent events: No further intercurrent events are identified. The two intercurrent events described as part of the treatment condition will all be handled by a hypothetical strategy.

Rationale for estimand: This estimand quantifies the achievable difference in treatment effects between the different treatment regimens. It reflects the drug efficacy.

1.1.3 Addressing the secondary objective

The relevant endpoint is the secondary confirmatory endpoint "change from baseline to week 40 in body weight (kg)".

Change in body weight - primary estimand

The main clinical question of interest for this secondary confirmatory objective is similar to the primary estimand for the primary objective.

The estimand attributes: population, treatment condition, remaining intercurrent events, and population-level summary are the same as for the primary estimand for the primary objective. The remaining estimand attribute is:

- Endpoint: Change from baseline to week 40 in body weight (kg).

Rationale for estimand: The rationale is the same as for the primary estimand for the primary objective.

Change in body weight – additional estimand

The additional clinical question of interest for this secondary confirmatory objective is similar to the additional estimand for the primary objective.

The estimand attributes: population, treatment condition, remaining intercurrent events, and population-level summary are the same as for the additional estimand for the primary objective. The remaining estimand attribute is:

- Endpoint: Change from baseline to week 40 in body weight (kg).

Rationale for estimand: The rationale is the same as for the additional estimand for the primary objective.

1.2 Study Design

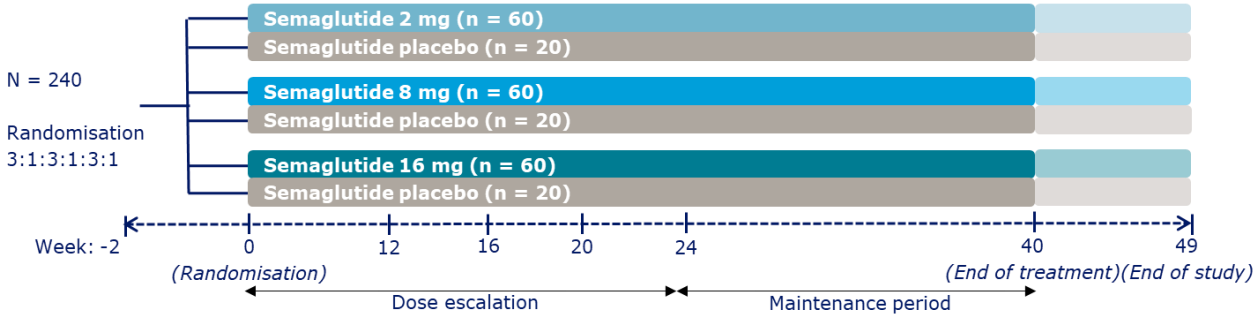
This is an interventional, 40-week, multi-centre, parallel-group, randomised, placebo-controlled, participant and investigator-blinded within dose level, sponsor open-label, dose-response study of

once-weekly subcutaneously administered semaglutide in patients with T2D and overweight with a baseline HbA1c of 7.0–10.5% and as an add-on to a stable dose of metformin (Figure 1).

A total of 240 participants will be randomised in a 3:1:3:1:3:1 ratio to receive semaglutide s.c. (2 mg, 8 mg, 16 mg) or matching placebo. All participants are to escalate to the randomised target maintenance dose according to the dose escalation outlined in Table 6-2.

Randomisation will be stratified according to HbA1c at screening (<8.5%/≥8.5%).

Figure 1 Study design



The study consists of:

- an up to 3-week screening period
- a dose escalation period of 12-24 weeks (see Table 6-2)
- a maintenance period of 16-28 weeks (see Table 6-2)
- a 9-week follow-up period

For each participant, the maximum intervention and study duration is 40 weeks and 52 weeks, respectively.

For further study design information, refer the protocol section 4.1.

2 Statistical Hypothesis

Not applicable

2.1 Multiplicity Adjustment

Not applicable

3 Analysis Sets

Two participant analysis sets are defined, see Table 2:

Table 2 Participant analysis sets

Participant analysis set	Description
Full analysis set	All randomised participants. Participants will be included in the analyses according to the planned randomised treatment.
Safety analysis set	All participants who are exposed to randomised treatment. Participants will be included in the analyses according to the treatment they actually received.

Four data points sets are defined, see Table 3:

Table 3 Data points sets

Data points set (DPS)	Description
DPS1 – in-study	All observed data points from randomisation until the first date of: • end of study visit (V17) • death • withdrawal of informed consent • last contact as defined by investigator for participants that are lost to follow up
DPS2 – on-treatment	All observed data points from first drug date until the first date of: • end of DPS1 • last administration of randomised treatment +63 days
DPS2 - modified	All observed data points with acute onset (laboratory assessments, and physical examination) from first drug date until the first date of: • end of DPS1 • last administration of randomised treatment +7 days
DPS3 – on-treatment without rescue	All observed data points from first drug date until the first date of: • initiation of rescue medication • last administration of randomised treatment +7 days

The full analysis set (FAS) will be used when analysing efficacy endpoints and assessments, and the safety analysis set (SAS) will be used when analysing safety endpoints and assessments.

The FAS and DPS1 are used to estimate the primary estimand for the primary endpoint and the primary estimand for the confirmatory secondary endpoint.

The FAS and DPS3 are used to estimate the additional estimands for the primary and confirmatory secondary endpoint.

The SAS and DPS2 are used as the primary data points set to evaluate safety data (AEs, eye examination, vital signs and hypoglycaemic episodes). The primary evaluation of antibodies will be based on the safety analysis set and DPS2.

The SAS and a modified DPS2 are used to present safety data with an acute onset (laboratory assessments, and physical examination).

4 Statistical Analyses

4.1 Dose-response modelling

To characterise the dose-response relationship for change in HbA1c (%-point) and change in body weight (kg) at week 40 for the primary and additional estimand, the following dose-response candidate models in Table 4 will be fit separately. The variable d is the nominal dose level, and the other variables are model parameters to be estimated.

Table 4 Dose-response candidate models

Model	Functional form f(d,θ)
Linear log-dose	$E_0 + \beta log(d+1)$
E_{max}	$E_0 + E_{max}\frac{d}{ED_{50}+d}$
Sigmoidal E_{max}	$E_0 + E_{max}\frac{d^{\lambda}}{{ED_{50}}^{\lambda}+d^{\lambda}}$
Linear + E_{max}	$E_0 + \beta d + E_{max}\frac{d}{ED_{50}+d}$

Emax:

The Emax model, describes the concentration-effect relationship in terms of a baseline effect when no drug is present (E_0), the maximum possible effect (E_{max}), and the drug concentration producing half-maximal effect (ED_{50}) and the nominal dose level (d).

Sigmoidal Emax:

The Emax model including slope parameter (λ) is referred to as the sigmoidal Emax model.

Linear log-dose:

The linear log-dose model, describes the concentration-effect relationship in terms of a baseline effect when no drug is present (E_0), with location-scale model which is expressed as $log(d + c)$ where $c = 1$ and nominal dose level (d) and β is slope associated with $log(d + c)$.

Linear + Emax:

The combination of linear and Emax model, where in the linear model describes the concentration-effect relationship in terms of a baseline effect when no drug is present (E_0), slope parameter (β), the nominal dose level (d). Emax has been described as above.

The candidate models will be fit to the mean estimated endpoint changes at week 40 for the evaluated semaglutide s.c. doses and pooled placebo derived from the analyses models described above according to the estimand evaluated. When fitting the models, the mean estimated endpoint change will be weighted by their inverse estimated variances.

The model ultimately used to evaluate dose-response will be selected among the candidate models based on the best fit to data. The best fit will be evaluated based on convergence and Akaike information criterion (AIC) value.

4.2 General Considerations

From the statistical analyses the point estimate, two-sided 95% CIs, and the associated two-sided p-values will be presented for the following comparison:

- Semaglutide 2 mg vs pooled placebo
- Semaglutide 8 mg vs pooled placebo
- Semaglutide 16 mg vs pooled placebo
- Semaglutide 8 mg vs Semaglutide 2 mg
- Semaglutide 16 mg vs Semaglutide 8 mg
- Semaglutide 16 mg vs Semaglutide 2 mg

For all analyses and reporting, the three placebo arms will be pooled into one placebo group to have an unbiased analysis due to the differentiation of response to semaglutide s.c. from response due to other factors such as study participation and intensified medical attention.

Furthermore, to investigate the pooling of placebo descriptive statistics will be provided of change in HbA_{1c} at week 40 and change in body weight (kg) at week 40 will be done without pooling the placebo.

If an assessment has been made both at screening and randomisation, the value from the randomisation visit will be used as the baseline value. If the value measured at the randomisation visit is missing, and the assessment was made at screening, the screening value will be used as the baseline value. The participants with missing baseline values will not contribute to any analysis that adjust for the given baseline.

The stratification factor in the statistical analysis will be based on observed baseline value of $HbA_{1c} \geq 8.5\%$ (Y/N).

4.3 Primary Endpoint Analysis

4.3.1 Definition of Endpoint

Definition of primary endpoint: Change in HbA_{1c} (%-point)

Change from baseline (week 0) to week 40 in HbA_{1c} is defined as:

$$HbA_{1c} \text{ change} = HbA_{1c} \text{ at week } 40 - HbA_{1c} \text{ at baseline}$$

4.3.2 Main Analytical Approach

4.3.2.1 Analysis addressing the primary estimand

The primary estimand, presented in Section 1.1.1, will be estimated based on the FAS using the in-study data points set (DPS1).

The primary analysis for this estimand is an analysis of covariance (ANCOVA) with randomised treatment and stratification factor as fixed effects and baseline HbA1c as a covariate.

Handling of missing data

Jump to reference: Missing week 40 data will be imputed using multiple imputation assuming that missing data are missing not at random. The imputation will be performed by sampling among all available assessments at week 40 in the pooled placebo group. A pattern mixture model approach is applied where withdrawn participants without a follow-up visit are assumed to respond as if treated with placebo for the entire study. This approach is also known as "jump to reference" and makes the assumption that participants instantly after discontinuation lose any effect of randomised treatment beyond what can be expected from the pooled placebo group.

Multiple copies (500 copies) of the full dataset will be generated by imputing missing values based on estimated parameters for the pooled placebo group. This will be done as follows:

- 500 copies of the dataset will be generated.
- An enriched ANCOVA model with stratification factor and sex as fixed effects and baseline HbA1c as covariate will be fitted to the change from baseline to week 40 in HbA1c for the completers in the pooled placebo group only.
- For each of the 500 copies of the dataset, the estimated parameters and their variances from this model will be used to impute missing week 40 values for participants in all treatment arms, based on their factor level and the values of the covariate.
- For each of the 500 complete datasets, the change from baseline to week 40 in HbA1c will be analysed repeating the ANCOVA described above. The estimates and standard deviations (SDs) for the 500 data sets are pooled to one estimate and associated SD using Rubin's rule.

4.3.3 Supplementary Analysis

Additional estimand analysis

The additional estimand, presented in section 1.1.2.2, will be estimated based on the FAS using post-baseline data collected up to and including week 40 from the 'on-treatment without rescue' observation period (DPS3).

Imputation of missing data will be handled by MI assuming that missing data are MAR. The imputation will be performed separately within each treatment group defined by randomised treatment. First, intermittent missing values are imputed using a Markov Chain Monte Carlo method to obtain a monotone missing data pattern, generating 500 complete data sets. Secondly, a

sequential conditional linear regression approach for imputing monotone missing values will be implemented starting with the first visit after baseline and sequentially continuing to the last planned visit at week 40. The model used for imputation will include stratification factor and sex as fixed effects and baseline and post-baseline HbA1c values observed prior to the visit in question as covariates.

The 500 complete datasets will be analysed using an ANCOVA with stratification factor and randomised treatment as fixed effects and baseline HbA1c as a covariate. Rubin's rule will be applied to combine the estimates and draw inference.

4.4 Secondary Endpoint Analysis

4.4.1.1 Primary estimand

For the endpoint "change from baseline to week 40 in body weight (kg)" a similar analysis as described in Section 4.3.2.1 will be performed, but with values of body weight instead of HbA1c.

4.4.1.2 Supplementary analysis

Additional estimand

A similar analysis as described in Section 4.3.3 will be performed, but with values of body weight instead of HbA1c.

4.5 Exploratory Endpoints Analysis

Not applicable

4.6 Safety Analysis

All safety analyses will be made on the safety analysis set. The standard safety assessments (AEs, laboratory parameters, vital signs, etc.) will be reported descriptively based on the DPS2 and modified DPS2; including any notable changes of clinical interest in laboratory parameters.

The primary evaluation of antibodies will be based on the safety analysis set and DPS2.

4.6.1 Adverse Events

Summaries of TEAEs are presented as an overview including all AEs, serious AEs, AEs leading to withdrawal, AEs by severity, AEs by relation to treatment and outcome of AEs. Furthermore, summary tables based on system organ class and preferred term is made for:

- All TEAEs
- Serious TEAEs
- AE Leading to premature treatment discontinuation

4.6.2 Additional Safety Assessments

Not applicable

4.7 Other Analyses

Pharmacokinetic and/or pharmacodynamic modelling

Population PK and exposure-response analyses will be used to characterize the exposure response curve of semaglutide s.c., and to support selection of a dose for potential future investigation in patients with T2D.

The population PK model will be implemented identical to that reported for NN9535-4506. A single pre-specified exposure-response analysis will be applied to investigate trends towards higher effect at higher exposure levels, based on the average semaglutide concentration estimated at the planned maintenance dose. These analyses will be based on the active arms (excluding placebo) conducted for change in HbA_{1c} and change in body weight at end of treatment (Change.EOT), assessing exposure-response as if subjects were randomized at week 16.

Change.EOT = E0+Slope*log(Cavg)+p*Change.Week.16

Where E0, Slope and p, are parameters in the model.

Additional exposure-response modelling will be considered exploratory and potentially conducted together with other semaglutide trials. Modelling results will be reported separately from the CSR.

Anti-Semaglutide antibodies

Anti-Semaglutide antibodies will be summarised in terms of the number and percentage of subjects per visit that are antibody-positive, antibody-negative, cross-reacting and in vitro neutralising, respectively.

4.7.1 Other Parameters

All collected continuous or categorical data that were not defined as endpoints will be summarised by descriptive statistics or by count and percentage respectively using FAS.

Pulse

For the endpoint “change from baseline to week 40 in pulse” a similar analysis as described in Section 4.3.2.1will be performed, but with values of pulse instead of HbA1c.

Responder for HbA_{1c}

- HbA_{1c} <7.0% at week 40 (yes/no) ,
- ≤6.5% at week 40 (yes/no) and
- <5.7% at week 40 (yes/no)

The endpoints will be analysed using the imputed data from primary endpoint as described in Section 4.3.2.1

The statistical model for the responder endpoints relating to HbA1c will be analysed using logistic regression with factor and covariates used as for primary endpoint with baseline HbA1c.

Responder for Body weight

- Weight loss ≥ 5% at week 40 (yes/no),
- Weight loss ≥ 10% at week 40 (yes/no).

The endpoints will be analysed using the imputed data from primary endpoint as described in Section 4.4.1.1

The statistical model for the responder endpoints relating to body weight will be analysed using logistic regression with factor and covariates used as for primary endpoint with baseline body weight.

4.7.2 Subgroup Analysis

Not applicable.

4.8 Interim Analysis

Not applicable.

4.9 Changes to Protocol-planned Analysis

- Linear model has been removed from "Dose response modelling" instead Linear +Emax has been included and the order of the model to be used has been changed in Table 4.

- The following text in protocol:

"The FAS and DPS1 are used to estimate the primary estimand for the primary endpoint and the secondary estimand for the confirmatory secondary endpoint."

is replaced to:

"The FAS and DPS1 are used to estimate the primary estimand for the primary endpoint and the primary estimand for the confirmatory secondary endpoint."

- The following text in protocol:

"The best fit will be evaluated based on convergence, model complexity, Akaike information criterion (AIC) value, and visual evaluation. "

is replaced to:

"The best fit will be evaluated based on convergence and Akaike information criterion (AIC) value."

- The following text in protocol:

"A modelling analysis plan will be prepared before database lock outlining details of the analyses. The modelling will be performed by Quantitative Clinical Pharmacology at Novo Nordisk and will be reported separately from the CSR."

is replaced to:

"The population PK model will be implemented identical to that reported for NN9535-4506. A single pre-specified exposure-response analysis will be applied to investigate trends towards higher effect at higher exposure levels, based on the average semaglutide concentration estimated at the planned maintenance dose. These analyses will be based on the active arms (excluding placebo) conducted for change in HbA_{1c} and change in body weight at end of treatment (Change.EOT), assessing exposure-response as if subjects were randomized at week 16.

Change.EOT = E0+Slope*log(Cavg)+p*Change.Week.16

Where E0, Slope and p, are parameters in the model.

Additional exposure-response modelling will be considered exploratory and potentially conducted together with other semaglutide trials. Modelling results will be reported separately from the CSR."

5 Sample size determination

See the protocol section 9.5.

6 Supporting Documentation

Not applicable

7 References

Not applicable