Cover Page for Statistical Analysis Plan

Sponsor name:	Novo Nordisk A/S
NCT number	NCT05478252
Sponsor trial ID:	NN9535-4820
Official title of study:	Investigation of Clinical Comparability of Semaglutide Drug Products Based on the Proposed and the Approved Drug Substance Manufacturing Processes in Participants with Type 2 Diabetes
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Note: The date in the header from Page 2 is the date of compilation of the documents and not of an update to content.

9.1.9 Documentation of statistical methods

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

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Statistical Analysis Plan

Protocol Title: Investigation of Clinical Comparability of Semaglutide Drug Products Based on the Proposed and the Approved Drug Substance Manufacturing Processes in Participants with Type 2 Diabetes

Substance: semaglutide s.c.

[redacted]

Biostatistics GLP-1 Diabetes

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Version History

This Statistical Analysis Plan (SAP) for study NN9535-4820 is based on the protocol version 4.0 dated 20-Feb-2023.

SAP Version	Date	Change	Rationale
1.0	8-SEP-2023	Not Applicable	Original version

1 Introduction

This SAP covers statistical considerations and analyses for efficacy and safety data. Efficacy analyses will be based on data available at the time of partial (interim) database lock performed after the date of the last participant last treatment (LPLT) visit where Novo Nordisk will become unblinded. At this time complete efficacy data will be available, but safety data will only be partially complete. Safety analyses will therefore be based on all available data after the full database lock.

Changes to the protocol-planned statistical analyses are detailed in Section 4.8.

1.1 Objectives, Endpoints, and Estimands

The primary and secondary objectives and the primary and supportive secondary endpoints are presented in Table 1-1.

Table 1-1 Objectives and endpoints

Objectives	**Endpoints**		
Primary	Title	Time frame	Unit
To establish non-inferiority of semaglutide J s.c. against semaglutide B s.c. on change from baseline in HbA_{1c} at week 28 in participants with T2D as add-on to stable dose of metformin	Primary:		
	Change in HbA_{1c}	From baseline visit (visit 2; week 0) to end of treatment visit (visit 10; week 28)	%-point
Secondary	Title	Time frame	Unit
To compare the effect of semaglutide J s.c. and semaglutide B s.c. on change from baseline in body weight at week 28 in participants with T2D as add-on to stable dose of metformin	Supportive:		
	Change in body weight	From baseline visit (visit 2; week 0) to end of treatment visit (visit 10; week 28)	kg
To compare the number of treatment AEs between semaglutide J s.c. and semaglutide B s.c. in participants with T2D as add-on to stable dose of metformin	Supportive:		
	Number of treatment-emergent AEs	From the time of first dosing to end of study visit (visit 11; week 33)	Count
To compare the development of anti-semaglutide antibodies between semaglutide J s.c. and semaglutide B s.c. in participants with T2D as add-on to stable dose of metformin	Supportive:		

	Occurrence of anti-semaglutide antibodies (yes/no)
	• *In vitro* neutralising anti-semaglutide antibodies
	  • Anti-semaglutide binding antibodies cross-reacting with endogenous GLP-1
	  ○ *In vitro* neutralising cross-reacting antibodies to endogenous GLP-1
		From baseline visit (visit 2; week 0) to end of study visit (visit 11; week 33)	Count of participant

Objectives	Endpoints		
	Anti-semaglutide antibodies level	From baseline visit (visit 2; week 0) to end of study visit (visit 11; week 33)	%B/T and titre

Abbreviations: AEs = adverse events; GLP-1 = glucagon like peptide-1; HbA_{1c} = glycated haemoglobin; s.c. = subcutaneous, T2D = type 2 diabetes.

The population pharmacokinetics of semaglutide J s.c. and semaglutide B s.c. in participants with T2D as add-on to stable dose of metformin will be addressed based on a modelling analysis plan and all PK assessments will be presented in a comprehensive modelling report (See Section 4.6.1).

1.1.1 Primary estimand

The primary clinical question of interest for the primary objective is to evaluate whether semaglutide J s.c. is non-inferior to semaglutide B s.c. in its effect on change from baseline to week 28 in HbA_{1c} (%-points) with a threshold margin of 0.3%-points in patients with T2D irrespective of treatment discontinuation and use of rescue medication.

The primary estimand is defined by the following five attributes as defined in ICH E9(R1):[1]

- Population: Patients with T2D.
- Endpoint: Change in HbA_{1c} (%-points) from baseline to week 28.
- Intervention condition: Except for trial product, the treatment regimen evaluated is the same for both intervention groups and is defined as the trial product taken for up to 28 weeks with or without initiation of glycaemic rescue medications. The treatment regimen is add-on to background metformin including possible changes in dose.
- Remaining intercurrent events: No further events are identified. The three intercurrent events are addressed in the intervention condition attributes. Treatment discontinuation for any reason, initiation of rescue medication, and changes to metformin dose is handled by the treatment policy strategy.
- Population-level summary: Difference in mean changes between semaglutide J s.c. and semaglutide B s.c.

1.1.2 Additional estimand

An additional clinical question of interest is to evaluate whether semaglutide J s.c., if taken for the planned 28 weeks, is non-inferior to semaglutide B s.c. in its effect on change from baseline to week 28 in HbA_{1c} (%-points) with a threshold margin of 0.3%-points in patients with T2D had they not required glycaemic rescue medication.

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

- Intervention condition: Except for trial product, the treatment regimen evaluated is the same for both intervention groups and is defined as the trial product taken for 28 weeks without initiation of glycaemic rescue medications. The treatment regimen is add-on to background metformin including possible changes in dose.

- Remaining intercurrent events: No further events are identified. The three intercurrent events are addressed in the intervention condition attributes. Treatment discontinuation for any reason and initiation of rescue medication is handled with the hypothetical strategy. Changes to metformin dose is handled by the treatment policy strategy.

1.2 Study Design

See protocol Section 4.1.

2 Statistical Hypotheses

Non-inferiority will be declared if the following one-sided null hypothesis is rejected. Let the treatment difference of change from baseline to week 28 in HbA_{1c} between semaglutide J s.c. and semaglutide B s.c. be defined as μ = "change in HbA_{1c} for semaglutide J s.c." minus "change in HbA_{1c} for semaglutide B s.c.".

- H_0: $\mu \geq 0.3$%-points against H_a: $\mu < 0.3$%-points

The non-inferiority margin of 0.3%-points is justified in the protocol Section 4.2.

2.1 Multiplicity Adjustment

The type-1 error probability will be controlled at 2.5% under the aforementioned one-sided null-hypothesis, and as the study has only one primary analysis, and no confirmatory secondary analyses, adjustment for multiplicity is not applicable.

3 Analysis Sets

The following participant analysis sets are defined:

Participant analysis set (PAS)	Description
Full analysis set (FAS)	All randomised study participants. Participants will be included in the analyses according to the randomised intervention.
Safety analysis set	All participants who are exposed to study intervention. Participants will be included in the analyses according to the intervention they actually received for the majority of the period being treated.

The following data points sets are defined:

Defined data points set (DPS)	Description
DPS1 – in study	All observed data points from randomisation until the first date of: • end of study visit (visit 11) or • death or • withdrawal of informed consent or • 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 – in study or • the end of study visit (visit 11) or • last trial product administration +42 days.
DPS3 – on treatment without rescue	All observed data points from first drug date until the first date of: • initiation of rescue medication or • last trial product administration +7 days

The FAS and DPS1 are used to estimate the primary estimand (defined in Section 1.1.1).

The FAS and DPS3 are used to estimate the additional estimand (defined in Section 1.1.2).

The safety analysis set and DPS2 are used to present safety data with a long lag-time (AEs, hypoglycaemic episodes, anti-semaglutide antibodies and eye examination).

Safety analysis set and a modified DPS2 are used to present safety data with an acute onset (vital signs, laboratory assessments, physical examination). For these summaries, the DPS2 is modified by having an end date at date of last trial product administration +7 days or date of end of DPS1, whichever occurs first.

4 Statistical Analyses

4.1 General Considerations

A baseline assessment will be defined as the most recent measurement at randomisation. Missing baseline values will be imputed by the average of the non-missing baseline values.

The frequency and timing of intercurrent events as defined in Section 1.1.1 will be presented descriptively.

4.2 Primary Estimand Analysis

4.2.1 Main Analytical Approach

The primary estimand, presented in section 1.1.1, will be estimated based on the FAS and DPS1.

Missing end of treatment data will be imputed using multiple imputation (MI) assuming that missing data are missing at random (MAR). The imputation will be performed, under the non-inferiority null-hypothesis, by imputing missing end of treatment data separately within each to the four groups defined by randomised treatment and treatment status at end of treatment:

- semaglutide B s.c. and on-treatment at end of treatment,
- semaglutide B s.c. and off-treatment at end of treatment,
- semaglutide J s.c. and on-treatment at end of treatment,
- semaglutide J s.c. and off-treatment at end of treatment.

For each group an analysis of covariance (ANCOVA) with region as a factor and baseline HbA_{1c} as a covariate will be fitted to the observed end of treatment values. The estimated location and dispersion parameters will then be used to impute 500 values for each participant with missing end of treatment data. Furthermore, for participants randomised to semaglutide J s.c. a penalty of 0.3, corresponding to the non-inferiority margin, is added to the imputed values.

The 500 complete datasets will be analysed using an ANCOVA with randomised treatment and region as factors and baseline HbA_{1c} as a covariate. Rubin's rule[2] will then be applied to combine these estimates and draw inference.

In case of sparse data, defined as less than 5 participants with available end of treatment data, in some groups, the imputation model will be thinned by region. If this is not sufficient the imputation will be based on participants randomised to the same treatment regardless of treatment status using the imputation model region as a factor and baseline HbA_{1c} as a covariate. If this is still not sufficient, the imputation model may be thinned again in the aforementioned order.

4.2.1.1 Sensitivity Analysis

A two-way tipping point sensitivity analysis will be performed by repeating the ANCOVA described in Section 4.2.1. However, prior to analysis, penalties are added to the imputed end of treatment values in both intervention arms simultaneously. A range of penalties will be explored for

both treatment groups, and the impact on the conclusion will be assessed through a contour plot of the p-values. This sensitivity analysis evaluates the robustness of the non-inferiority conclusions to violations in missing data assumptions in both intervention arms.

4.2.2 Additional estimand Analysis

The additional estimand, presented in section 1.1.2 will be estimated based on the FAS and DPS3.

Missing end of treatment data will be imputed using multiple imputation (MI) assuming that missing data are missing at random (MAR). The imputation will be performed separately within each treatment group. First, intermittent missing values are imputed using a Markov Chain Monte Carlo (MCMC) 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. The imputation model will include region as a factor and baseline and post-baseline HbA_{1c} values observed prior to the visit in question as covariates.

The 500 complete datasets will be analysed using an ANCOVA with randomised treatment and region as factors and associated baseline HbA_{1c} as a covariate. Rubin's rule[2] will then be applied to combine the estimates and draw inference.

4.2.2.1 Sensitivity Analysis

A two-way tipping point sensitivity analysis as described in Section 4.2.1.1 will be performed.

4.3 Secondary Endpoints Analysis

4.3.1 Confirmatory Secondary Endpoints

Not applicable for this study.

4.3.2 Supportive Secondary Endpoints

4.3.2.1 Change in body weight

Primary estimand

A similar analysis as described in Section 4.2.1 will be performed, but with values of body weight instead of HbA_{1c}, and where no penalty (or rather a penalty of 0, corresponding to a superiority test) is added to imputed values for participants that are randomised to semaglutide J s.c.

Additional estimand

A similar analysis as described in Section 4.2.2 will be performed, but with values of body weight instead of HbA_{1c}.

4.4 Exploratory Endpoints Analyses

Not applicable for this study.

4.5 Other Safety Analysis

4.5.1 Adverse Events

The AEs will be summarised descriptively by treatment arm in terms of number of participants experiencing at least one event (N), proportion of participants in the safety analysis set experiencing at least one event (%), the number of events (E), and the event rate (R) per 100 PYE.

Number of treatment-emergent AEs

A treatment-emergent AE is defined as an AE with an onset date (or increase in severity) during the on-treatment observation period (see Section 3).

Treatment-emergent AEs will be summarised descriptively by treatment arm in terms of number of participants experiencing at least one event (N), proportion of participants in the safety analysis set experiencing at least one event (%), the number of events (E), and the event rate (R) per 100 person-years of exposure (PYE).

4.5.2 Anti-semaglutide antibodies

The occurrence of antibodies (positive/negative):

- *In vitro* neutralising anti-semaglutide antibodies
  - Anti-semaglutide binding antibodies cross-reacting with endogenous GLP-1
    - *In vitro* neutralising cross-reacting antibodies to endogenous GLP-1

during the time from baseline visit (visit 2; week 0) to end of study visit (visit 11; week 33) will be summarised descriptively by treatment and visit and positive at any time in terms of number of participants with a positive result (N), the proportion of participants in the safety analysis set with a positive result (%). Only visits with one or more positive results will be displayed. To visualise the impact of antibodies on HbA_{1c} (%), a spaghetti plot will be provided showing the change in HbA_{1c} (%) levels for each subject throughout the trial with all antibodies negative and positive subjects being in two different colours. Similarly, a spaghetti plot will be produced showing semaglutide pharmacokinetic concentration by occurrence of anti-semaglutide antibodies.

4.6 Other Analysis

4.6.1 Pharmacokinetic and/or pharmacodynamic modelling

The 8 PK samples for each participant will be used to fit a 1-compartment population PK model. In the visit schedule, 3 PK visit window types have been defined ensuring that PK sampling times relative to dosing times are distributed across the weekly dosing interval, allowing for estimation of the absorption rate, the volume of distribution, and the clearance rate for each participant in the population PK model, and from this C_{max}, C_{min} and C_{avg} will be estimated for each participant. Lastly, a comparison of C_{max}, C_{min} and C_{avg} between semaglutide J s.c. and semaglutide B s.c. will be made.

Additional population PK/PD exposure-response analyses may be included for exploratory pharmacodynamic analysis as needed.

The modelling will include data from all enrolled participants that were exposed to semaglutide s.c. in this study and might be performed as a meta-analysis. Actual dose, date, time and injection site of

all the administrations of trial product before PK sampling will be registered in the eCRF and used in the analysis, together with actual time point for PK sampling. The analysis will be further specified in a modelling analysis plan (MAP) that is to be prepared before unblinding at the partial (interim) database lock. The modelling analyses will be performed by Pharmacometrics at Novo Nordisk A/S and will be reported separately from the CSR.

4.7 Interim Analysis

This study will be subject to a partial (interim) database lock (DBL) at the end of the treatment period for all participants, i.e. after the date of the last participant last treatment (LPLT) visit. A full DBL will be performed, as per the usual procedures, after last participant last visit. The partial DBL is implemented to allow for an early submission to Agency and thereby securing the stable supply of the semaglutide molecule to meet the current high demand. Novo Nordisk employees not involved in data cleaning will become unblinded to potentially both participant level data and comparative results at the time of the partial database lock, whereas participants and investigators will remain blinded until after last participant last visit (LPLV). All efficacy analyses will be performed based on the data from the partial DBL and analysis of safety will primarily be based on the full DBL. No efficacy assessments are collected after LPLT and in turn the efficacy results cannot be biased by the early unblinding. The potential impact on safety is considered negligible, as most participants will have completed the follow-up visit at the time of the partial DBL.

4.8 Changes to Protocol-planned Analyses

- The event rate of AEs will be calculated per 100 PYE rather than PYE.

5 Sample size determination

See protocol Section 9.5.

6 Supporting Documentation

6.1 Appendix 1: List of abbreviations

AE adverse event

ANCOVA analysis of covariance

DBL database lock

DPS data point set

FAS *full Analysis set*

GLP-1 glucagon-like peptide-1

HbA_{1c} glycated haemoglobin

ICH International Council on Harmonization

LPLT last participant last treatment

MCMC Markov chain Monte Carlo

PAS participant analysis set

PD pharmacodynamic

PK pharmacokinetic

PYE person-years of exposure

s.c. subcutaneous

SAP statistical analysis plan

T2D type 2 diabetes

6.2 Appendix 2: Definition and calculation of endpoints, assessments and derivations

Type	**Title**	**Time frame**	**Unit**
Primary endpoint	Change in glycosylated haemoglobin (HbA_{1c})	From baseline (week 0) to week 28	%-points
Supportive secondary endpoint	Change in body weight	From baseline (week 0) to week 28	kg
Supportive secondary endpoint	Number of treatment emergent adverse events (AEs)	From the time of first dosing to week 33	count
Supportive secondary endpoint	Occurrence of anti-semaglutide antibodies	From baseline to week 33	count of participant

Type	Title	Time frame	Unit
Supportive secondary endpoint	Occurrence of anti-semaglutide binding antibodies cross-reacting with endogenous GLP-1	From baseline to week 33	count of participant
Supportive secondary endpoint	Occurrence of *in vitro* neutralising anti-semaglutide binding antibodies cross-reacting with endogenous GLP-1	From baseline to week 33	count of participant
Supportive secondary endpoint	Anti-semaglutide antibody level	From baseline to week 33	%B/T
Supportive secondary endpoint	Anti-semaglutide antibody level	From baseline to week 33	titre

7 References

1. European Medicines Agency. ICH E9 (R1) addendum on estimands and sensitivity analysis in clinical trials to the guideline on statistical principles for clinical trials. Step 5 EMA/CHMP/ICH/436221/2017). 2020.
2. Little RJA, Rubin DB. Statistical analysis with missing data. New York.: Wiley. 1987. xiv, 278 p.p.