Cover Page for Statistical Analysis Plan

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NCT number	NCT04865770
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Official title of study:	REMODEL - Renal mode of action of semaglutide in patients with type 2 diabetes and chronic kidney disease
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Statistical Analysis Plan

REMODEL - Renal mode of action of semaglutide in patients with type 2 diabetes and chronic kidney disease

Substance: **once-weekly semaglutide s.c.**

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Version history

This Statistical Analysis Plan (SAP) for Study NN9535-4662 is based on the protocol: *REMODEL - Renal mode of action of semaglutide in patients with type 2 diabetes and chronic kidney disease.* version 3.0 dated 21APR2022.

SAP Version	Approval Date	Change	Rationale
5.0	21-Mar-2025	Section 4	4.4 Exploratory endpoint analyses 6 Supporting documentations
4.0	29-JAN-2025	Appendix 6.2	Updated to accommodate changes in BAP
3.0	22-JAN-2025	Section 4.3.2, Section 4.4, Section6.2	4.3.2.2 Change in glomerular basement membrane width 4.3.2.4 Updated unit of natriuresis 4.4 Exploratory endpoint analyses 6.2 Bioinformatics Analysis Brief
2.0	31-JUL 2024	Section 3	To update the section 3 “Analysis sets” to align with the estimands
		Section 4	To update the endpoints and corresponding analysis in section 4.4 “Exploratory endpoint analyses”. Addition of analysis in section 4.6 “Other analyses”
1.0	22-APR-2021	Not Applicable	Original version

Overall rationale for preparing SAP, version 5.0

The overall rationale for the changes implemented in the amended SAP is to ensure the addition of information related to MRI and biopsy parameter and accommodate the changes in BAP

List of abbreviations

ADC	apparent diffusion coefficient
AE	adverse event
ANCOVA	analysis of covariance
BOLD MRI	blood oxygenation level dependent magnetic resonance imaging
CI	confidence interval
CKD	chronic kidney disease
CV	cardiovascular
FAS	full analysis set
GLP-1 RA	glucagon-like peptide-1 receptor agonist
IFTA	interstitial fibrosis tubular atrophy
IWRS	interactive web response system
MRI	magnetic resonance imaging
OW	once weekly
RNA	ribonucleic acid
SAP	statistical analysis plan
SAS	safety analysis set
SD	standard deviation
SGLT-2	sodium-glucose cotransporter-2
T2D	type 2 diabetes
TFL	tables, figures and listings

1 Introduction

This SAP covers specification of the statistical analyses for data on efficacy and safety, except for data related to the single nucleus RNA sequencing of cells from the kidney biopsies, which will be described in the separate 'bioinformatics analysis plan', prepared by the department of Development Data Science Omics, Novo Nordisk, and will be final prior to database lock.

Changes to the protocol-planned analyses are specified in Appendix 4.8

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

1.1 Objectives and endpoints

1.1.1 Primary objective

To investigate the effect of OW semaglutide s.c. versus placebo on renal inflammation and haemodynamics, as measured by MRI in participants with T2D and CKD.

1.1.2 Secondary objective

To investigate the effect of OW semaglutide s.c. versus placebo on renal oxidative stress, natriuresis, albumin excretion and kidney function in participants with T2D and CKD.

1.1.3 Exploratory objective

To explore the effect of OW semaglutide s.c. versus placebo on circulating and urinary biomarkers in participants with T2D and CKD.

1.1.4 Primary and secondary estimands

The estimands related to the primary and secondary objectives are summarised in the estimand overview Table 1-1.

Estimands related to the exploratory objective is detailed in Section 4.4.

For fold change in gene expression, no estimand is considered in this SAP. All details related to the analysis of gene-expression data will be described in the bioinformatics analysis plan prepared by the department of Development Data Science Omics, Novo Nordisk, and will be final prior to database lock.

Two strategies for handling intercurrent events are considered. The primary strategy: 'if all participants had adhered to the randomised treatment, without initiation of disallowed medication' is presented in Table 1-1 and detailed in 4.2.2 The supplementary strategy: 'while on treatment after week 26 if all participants had adhered to the randomised treatment at least until week 26, without initiation of disallowed medication' is detailed in Section 4.2.4.

Table 1-1 Estimand overview

Objective	Estimand category	Estimand				
		Treatment condition	*Variable(s)/Endpoint(s)*	*Population of interest*	*Intercurrent event strategy*	*Population-Level Summary Measure*
Primary Objective: To investigate the effect of OW semaglutide s.c. versus placebo on renal inflammation and haemodynamics, as measured by MRI in participants with T2D and CKD.	Primary*	Randomised treatment taken for up to 52 weeks as adjunct to the subject's pre-study SoC regimen including the required RAAS blocking agents and post-randomisation adjustments as per the investigator and subject decision	Change from baseline (week 0) to end of treatment (week 52) in • **kidney oxygenation (in s^{-1}) (cortex)**, BOLD MRI (R2*) • **kidney oxygenation (in s^{-1}) (medulla)**, BOLD MRI (R2*) • **global kidney perfusion (in mL/100g/min)** (MRI) • **kidney inflammation (in ms) (cortex)**, T1 mapping (MRI) • **kidney inflammation (in ms) (medulla)**, T1 mapping (MRI)	Participants with T2D and CKD as defined by the protocol inclusion/exclusion criteria disregarding the specific exclusion criteria for the kidney biopsy subgroup	**Permanent discontinuation of treatment:** • **Prior to week 39 visit (V10):** For this intercurrent event a hypothetical strategy is applied. Data will be imputed as described in Section 4.2.2. • **At or after week 39 visit (V10):** For this intercurrent event treatment policy strategy is applied. **Maintenance treatment at 0.5 mg or 1 mg semaglutide/placebo is not reached:** This is not allowed, and participants should be discontinued. • **For participants discontinued for this reason:** For this intercurrent event the same strategy as for permanent discontinuation will be applied. • **For participants that are not discontinued for this reason:** A treatment policy strategy is applied. **Initiation of GLP-1 RA, SGLT-2 inhibitor (only for participants not on SGLT-2 at baseline) and/or**	The geometric mean ratio between semaglutide and placebo

Objective	Estimand category	Estimand				
		Treatment condition	*Variable(s)/Endpoint(s)*	*Population of interest*	*Intercurrent event strategy*	*Population-Level Summary Measure*
					finerenone: Initiation of GLP-1 RAs, SGLT-2 inhibitors and/or finerenone is not allowed. **For usage >30 days**: Hypothetical strategy is applied. **For usage <=30 days**: Treatment policy strategy is applied. **Discontinuation or change of dose of SGLT-2 inhibitors (only for participants on SGLT-2 inhibitors at baseline):** A treatment policy strategy is applied.	
Secondary Objective(s): To investigate the effect of OW semaglutide s.c. versus placebo on renal oxidative stress, natriuresis, albumin excretion and kidney function in participants with T2D and CKD.	Secondary*	As for the primary estimand	Change from baseline (week 0) to end of treatment (week 52) in **glomerular basement membrane width (in nm)** (kidney biopsy)	As for the primary estimand, but including the specific exclusion criteria for the kidney biopsy subgroup (cf. protocol section 5.2.1)	As for the primary estimand	The mean difference between semaglutide and placebo
	Secondary*	As for the primary estimand	Change from baseline (week 0) to end of treatment (week 52) in • **ADC (in $mm^2 \times s^{-1} \times 10^{-3}$) (cortex)** (MRI) • **ADC (in $mm^2 \times s^{-1} \times 10^{-3}$) (medulla)** (MRI) • **mean RARI (unitless)** (MRI) • **mean arterial flow (mL/min)** (MRI)	As for the primary estimand	As for the primary estimand	As for the primary estimand

Objective	Estimand category	Estimand				
		Treatment condition	***Variable(s)/Endpoint(s)***	***Population of interest***	***Intercurrent event strategy***	***Population-Level Summary Measure***
	Secondary*	As for the primary estimand	Change from baseline (week 0) to end of treatment (week 52) in • **natriuresis (in mmol/day) (urinary sodium excretion)** (urinalysis) • **albumin excretion rate (in mg/24h)** (urinalysis) • **kidney function (creatinine clearance) (in mL/min/1.73m^2)** (urinalysis)	As for the primary estimand	As for the primary estimand	The mean difference between semaglutide and placebo

*Not related to any confirmatory hypothesis.

OW: once weekly; MRI: magnetic resonance imaging; T2D: type 2 diabetes; CKD: chronic kidney disease; RAAS: renin-angiotensin-aldosterone system; SoC: standard of care; BOLD: blood oxygenation-level dependent; RNA: ribonucleic acid; ADC: apparent diffusion coefficient; RARI: renal artery resistive index

1.2 Study design

This is a phase 3b/4, multi-centre, international, randomised, double-blinded, parallel-group, placebo-controlled study comparing OW semaglutide s.c. versus placebo, both added to standard-of-care treatment (including antidiabetic medication, CKD medication and CV medication) in participants with T2D and CKD.

The study will include a subpopulation (aiming for 45 participants or more) undergoing kidney biopsies at baseline and end of treatment. Participants will be invited to participate in the biopsy subpopulation at sites with the capacity to perform kidney biopsy.

The participants will be randomised 2:1 to semaglutide or placebo, respectively. The randomisation is stratified by:

- use of SGLT-2 inhibitors at baseline (yes/no),
- MRI field strength (1.5/3.0 T), and
- subject participation in the biopsy subpopulation (yes/no).

Stratification for biopsy subpopulation is done purely for administrative reasons, to balance the subpopulation treatment allocation and not because subpopulation membership is expected to be associated with the outcome. Accordingly, it will not be accounted for in the statistical analyses.[1] When the term 'stratification' factor is applied it refers to use of SGLT-2 inhibitors at baseline (yes/no) and MRI field strength (1.5/3.0 T).

A schematic illustration of the study design is shown in Figure 1-1.

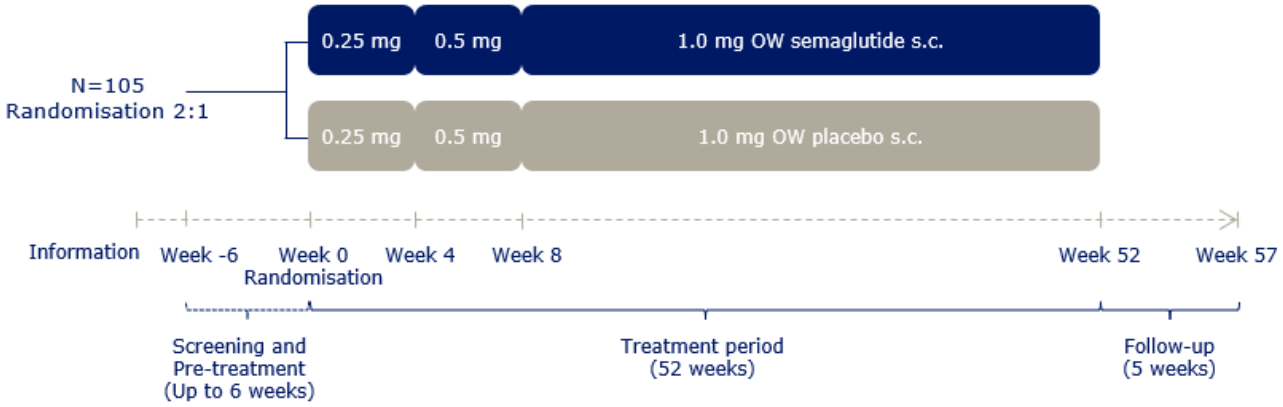

Figure 1-1 Study design

2 Statistical hypotheses

This study is explorative, and no confirmatory hypothesis testing is planned. Analyses and p-values from statistical models are considered descriptive in nature. No adjustment for multiple testing will be performed, except separately in connection with analyses of gene expression data where methods for controlling false discovery rates will be applied as needed (the details will be given in the bioinformatics analysis plan).

3 Analysis sets

For the purposes of analysis, the following analysis sets are defined:

Subject Analysis Set	**Description**
Full Analysis Set (FAS)	All participants randomly assigned to study treatment and who take at least 1 dose of study product. Exclusion of data from analyses should be used restrictively, and normally no data should be excluded from the FAS. Participants will be analysed according to the treatment they actually received.
Safety Analysis Set (SAS)	All participants randomly assigned to study treatment and who take at least 1 dose of study product. Participants are analysed according to the treatment they actually received.

Defined Analysis Data Sets	**Description**
DPS1- In-study	The in-study observation period for a subject is defined as the period from date of randomisation (as registered in IWRS) to the first of (both inclusive): • date of follow-up visit • date when subject withdrew consent • date of the last subject-investigator/site contact as defined by investigator for participants who are lost to follow up • date of death for participants who die before any of the above
DPS 2- On-treatment	The on-treatment period is defined as the period from the date of first administration of study product to the first of (both inclusive): • date of last study product administration +42 days (5 weeks follow-up + 7 days visit window of follow-up) • end-date of the in-study observation period.
DPS 3 - Primary Estimand	All observed data from DPS1 for which participants are considered exposed to randomised treatment and excluding data observed after the first date of any of the following: • The date of last study product administration for participants who have permanently discontinued treatment prior to week 39. • the date of initiation +30 days of GLP-1 RA, SGLT-2 inhibitor (only for participants not on SGLT-2 at baseline) and/or finerenone for usage more than 30 days.
DPS 4 - Additional Estimand	All observed data from DPS1 for which participants are considered exposed to randomised treatment and excluding data observed after the first date of any of the following: • The date of last study product administration for participants who have permanently discontinued treatment prior to week 26. • the date of initiation +30 days of GLP-1 RA, SGLT-2 inhibitor (only for participants not on SGLT-2 at baseline) and/or finerenone for usage more than 30 days.

Full analysis set (FAS) and DPS3 are used to estimate the primary estimand for the primary, secondary and exploratory endpoints (only for MRI and Biopsy parameters).

Full analysis set (FAS) and modified DPS3 are used to estimate the primary estimand for the non-MRI and non-Biopsy parameters. DPS3 is modified by having an end date as last study product administration +7 days (for efficacy parameters) for participants who have permanently discontinued treatment prior to week 39.

Full analysis set (FAS) and DPS4 are used to estimate the additional estimand for the primary, secondary and exploratory endpoints (only for MRI and Biopsy parameters).

Full analysis set (FAS) and modified DPS4 are used to estimate the additional estimand for the non-MRI and non-Biopsy parameters. DPS4 is modified by having an end date as last study product administration +7 days (for efficacy parameters) for participants who have permanently discontinued treatment prior to week 26.

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

Safety analysis set and a modified DPS2 are used to present safety data with an acute onset (vital signs, safety laboratory assessments, physical examination). The DPS2 is modified by having an end date as date of last study product administration +7 days (due to the dosing interval of semaglutide s.c.) or date of end of DPS1, whichever occurs first.

4 Statistical analyses

4.1 General considerations

The resulting comparisons of the statistical analyses will, unless otherwise stated, be presented as point estimates, two-sided 95% confidence intervals, and the associated two-sided p-values for semaglutide versus placebo. A significance level of 5% is chosen and as stated in Section 2 there will be no multiplicity adjustment, except in connection to analyses of gene expression data (detailed in the bioinformatics analysis plan).

As described in Section 1.2 stratification factor refers only to use of SGLT-2 inhibitors at baseline (yes/no) and MRI field strength (1.5/3.0 T).

Unless otherwise specified, a baseline assessment is defined as the most recent measurement at the randomisation visit (V3). Participants with missing baseline values will not contribute to any analysis that adjust for the given baseline. For eGFR the baseline assessment is defined as the mean of the two assessments from the randomisation visit (V3) and the screening visit (V1). If only one of the assessments is available, this will be used as the baseline assessment. For UACR, the baseline assessment is defined as the geometric mean of two assessments provided at the randomisation visit (V3). If only one assessment is available, this will be used as the baseline assessment.

The amount of missing data and data for which no statistical analysis is specified will be presented descriptively.

MRI measurements planned for V6 (week 4 visit) can be measured up until V8 (week 12 visit). V6 associated MRI measurements that are collected at or after V8 will not be used in the analysis. Subjects who discontinue between V5 and V6 (both inclusive) should have the V6 MRI scan performed.

4.2 Primary endpoints analyses

4.2.1 Definition of endpoints

The primary endpoints are derived from MRI scans. They are defined as the ratio from baseline (V3, at week 0) to end of treatment (V11, at week 52) in:

- kidney oxygenation (cortex), BOLD MRI (R2*) (s^{-1})
- kidney oxygenation (medulla), BOLD MRI (R2*) (s^{-1})
- global kidney perfusion (mL/100g/min)
- kidney inflammation (cortex), T1 mapping (ms)
- kidney inflammation (medulla), T1 mapping (ms)

Change from baseline analyses will, for each of these endpoints, be done on log scale. The log treatment differences will then be back transformed in order to obtain treatment ratios for reporting.

4.2.2 Main analytical approach

The primary estimands, presented in Table 1-1, will be estimated based on the FAS using data collected before or at the end of treatment visit (V11, at 52 weeks) and corresponding data point sets mentioned in Section 3, within the in-study observation period.

Primary analysis, if all subjects had adhered to the randomised treatment, without initiation of disallowed medication

The aim of the analysis is for each of the endpoints to estimate the treatment ratio between semaglutide and placebo after 52 weeks of treatment if all subjects had adhered to the randomised treatment of semaglutide/placebo, without initiation of disallowed medication.

The following intercurrent events are considered:

Permanent discontinuations of treatment prior to the week 39 visit: When subjects discontinue treatment, the end of treatment visit (V11) will be scheduled as soon as possible thereafter. Unless subjects have discontinued treatment prior to the week 26 visit (V9), the MRI scan and biopsy will be performed. However, data collected for subjects who discontinue treatment prior to the week 39 visit (V10) will not be applied in the analysis. For this intercurrent event a hypothetical strategy is applied, and data will be imputed as described below.

Permanent discontinuation of treatment at or after the week 39 visit: Data collected after this intercurrent event will be used in the analysis as week 52 assessments.

Maintenance treatment at 0.5 mg or 1 mg Semaglutide/placebo is not reached: A continued maintenance treatment at the 0.25 mg semaglutide/placebo is not allowed. Subjects who cannot tolerate a dose of at least 0.5 mg as a maintenance dose should discontinue treatment and data will be handled as described above.

- **For participants discontinued for this reason:** For this intercurrent event the same strategy as for permanent discontinuation will be applied.
- **For participants that are not discontinued for this reason:** A treatment policy strategy is applied.

Initiation of GLP-1 RA, SGLT-2 inhibitor (only for subjects not on SGLT-2 at baseline) and/or finerenone: Initiation of GLP-1 RAs, SGLT-2 inhibitors and/or finerenone is not allowed. Data collected after these intercurrent events from subjects that fulfil discontinuation criteria 6, 7, or 8 will not be used in the analysis, regardless of continued study participation.

- **For usage >30 days:** Hypothetical strategy is applied.
- **For usage <=30 days:** Treatment policy strategy is applied.

Discontinuation or change of dose of SGLT-2 inhibitors (only for subjects on SGLT-2 inhibitors at baseline): Discontinuation of SGLT-2 inhibitors is discouraged unless due to safety reasons. Change of dose of SGLT-2 inhibitors is allowed during the study, because this is expected to have little effect on the MRI and biopsy endpoints. Therefore, data collected after these intercurrent events will be applied in the analysis, using treatment policy strategy.

Imputation of missing data (either truly missing or omitted from analysis due to the hypothetical strategy) will be handled by multiple imputations assuming that the missing data are missing at random (MAR). Missing data will be imputed using observed data within the same actual treatment group. It is thereby assumed that the likely values of what the missing data would have been if available are best described by information from participants who receive the same actual treatment.

A sequential regression approach for imputing missing values at planned visits will be implemented for each endpoint starting with V6 and continuing to the planned end of treatment visit at week 52 (V11). For each actual treatment group an analysis of covariance (ANCOVA) model will be used to impute missing values at the planned post-baseline visits. The model will include region and stratification factors as categorical effects and baseline and (when applicable) the post-baseline value prior to the visit in question as covariates. A total of 500 copies of the dataset will be generated.

An ANCOVA with actual treatment, region and the stratification factors as categorical effects and the endpoint baseline value as a covariate will for each endpoint be used to estimate the treatment difference on the log scale at week 52 for each of the 500 complete data sets generated as part of the imputation of missing values. Rubin's rule[6] will be used to combine the estimates, which will then be back transformed in order to draw inference on the relative scale.

The estimated treatment ratio between semaglutide and placebo will be presented together with the associated two-sided 95% confidence interval and a two-sided p-value. Due to the explorative nature of the trial, no adjustment for multiplicity is made.

4.2.3 Sensitivity analysis, delta-adjustment

The aim of this analysis is to assess the sensitivity of results to the missing at random assumption. The analysis address if discontinued subject had adhered to the treatment regimen but would have responded less favourably than adherent participants.

In this analysis, participants from the semaglutide group with missing observations will be given a penalty (a delta value), i.e., it is assumed that participants with missing observations who are treated with semaglutide will have less favourable outcomes than participants with observed values who are treated with semaglutide.

The 500 complete datasets created for the primary analysis will be re-used for the delta adjustment analysis. For each of these datasets, a penalty is added to the imputed log-change from baseline to week 52 for participants with actual treatment semaglutide, followed by performing the ANCOVA applied for the primary analysis. A range of penalties will be applied to assess the impact on the results.

4.2.3.1 Sensitivity analyses on data

A sensitivity analysis will be performed for mean arterial flow and different kidney volumes based on correction for standardized body surface area (BSA) for to gauge the influence of BSA.

A sensitivity analysis for the primary or secondary endpoints maybe performed, if necessary, excluding or including data that is used in primary analysis, based on the primary estimand, to check the sensitivity of data points. The analytical approach is identical to the main analytical approach.

4.2.4 Supplementary analyses

4.2.4.1 Additional estimand

This supplementary analysis of the primary endpoints is performed under an additional strategy for handling intercurrent events. The aim of the analysis is to estimate the treatment ratio between semaglutide and placebo while on treatment after week 26 if all subjects had adhered to the randomised treatment at least until week 26, without initiation of disallowed medication. The estimand elements are as described in Table 1-1.

We refer to this analysis as 'while on treatment after week 26 if all participants had adhered to the randomised treatment at least until week 26, without initiation of disallowed medication'. The analyses will be based on the FAS using data collected before or at the end of treatment visit (V11, at 52 weeks) and corresponding data point sets mentioned in Section 3, within in-study observation period.

The following intercurrent events are considered:

- **Permanent discontinuation of treatment:**
    - **Prior to week 26 visit (V9):** For subjects who discontinue treatment prior to the week 26 data, the final MRI scan and biopsy will not be performed. For this intercurrent event a hypothetical strategy is applied. Data will be imputed as described before.
    - **At or after week 26 visit (V9):** Data collected after this intercurrent event will be used in the analysis. For this intercurrent event a 'while on treatment' strategy is applied.
- **Maintenance treatment at 0.5 mg or 1 mg semaglutide/placebo is not reached:** A continued maintenance treatment at the 0.25 mg semaglutide/placebo is not allowed. Subjects who cannot tolerate a dose of at least 0.5 mg as a maintenance dose should discontinue treatment and data will be handled as described above
    - **For participants discontinued for this reason:** For this intercurrent event the same strategy as for permanent discontinuation will be applied.
    - For participants that are not discontinued for this reason: A treatment policy strategy is applied.
- **Initiation of GLP-1 RA, SGLT-2 inhibitor (only for participants not on SGLT-2 at baseline) and/or finerenone:** Initiation of GLP-1 RAs, SGLT-2 inhibitors and/or finerenone is not allowed. Data collected after these intercurrent events from participants that fulfil discontinuation criteria 6, 7, or 8 will not be used in the analysis, regardless of continued study participation.
    - **For >30 days**: Hypothetical strategy will be applied
    - **For <=30 days**: Treatment policy strategy will be applied

- **Discontinuation or change of dose of SGLT-2 inhibitors (only for participants on SGLT-2 inhibitors at baseline):** As for the primary analysis, data collected after these intercurrent events will be applied in the analysis. A treatment policy strategy is applied.

The analytical approach is identical to the main analytical approach described in Section 4.2.2 except for while on treatment strategy which will be handled in dataset level.

4.2.4.2 Acute change from baseline

This supplementary analysis for the primary endpoints is performed to assess change from baseline (V3) to week 4 (V6), to assess the acute effect of treatment. The analyses will be based on the FAS and DPS1.

In order to impute missing values at week 4 (V6), we assume that missing data are missing at random (MAR). All missing values at week 4 (V6) will be imputed using multiple imputation (MI). The imputation will be performed separately within each actual treatment group.

For laboratory parameters, the 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 week 4 (V6). The imputation model will include region and stratification factors as categorical effects and baseline and the post-baseline value prior to the visit in question as covariates.

For MRI parameters, conditional linear regression model will be used to impute missing week 4 (V6) data, as there are no intermediate values between baseline and week 4. 500 copies of the dataset will be generated. The imputation model will include region and stratification factors as categorical effects and baseline value as covariates.

An ANCOVA with actual treatment, region and the stratification factors as categorical effects and the endpoint baseline value as a covariate for each endpoint will be used to estimate the treatment difference on the log scale at week 4 (V6) for each of the 500 complete datasets. Rubin's rule[6] will be used to combine the estimates, which will then be back transformed in order to draw inference on the relative scale.

A complete case sensitivity analysis will be done for the acute change from baseline analyses in order to check the robustness of missing data assumptions. I.e., the same analytical approach will be used without imputing missing values.

4.3 Secondary endpoint analyses

4.3.1 Confirmatory Secondary Analyses

Not applicable

4.3.2 Supportive secondary endpoints

The secondary estimands, presented in Table 1-1 (i.e. not including change in gene expressions), will be estimated based on the FAS using data collected before or at the end of treatment visit (V11, at 52 weeks) and corresponding data point sets mentioned in Section 3, within in-study observation period.

4.3.2.1 Fold change in gene expression

A secondary endpoint is the fold change values derived from the changes in the gene expression within relevant cell types comparing different conditions. In order to achieve this, first, a dimensionality reduction and clustering of the abundance data will be performed. Annotation of clusters will be done manually or via a state-of-the-art classification tool. In order to accurately annotate cell types and ensure comparability with existing studies, available data sets will be compared (or integrated) with the REMODEL data. After successful annotation of cell types, differences in cell type composition between placebo and semaglutide can be explored using differential abundance testing.

For all cell types of interest, changes in gene expression and underlying pathways within that cell type but between Semaglutide and placebo can be explored. To test for significance, state-of-the-art methods accounting for sample variation (pseudo-bulk, linear mixed models) will be used. All analyses of the single nucleus RNA sequencing of cells from the kidney biopsies will be described in detail in the bioinformatics analysis plan.

4.3.2.2 Change in glomerular basement membrane width

This kidney biopsy derived, supportive secondary endpoint is defined as change from baseline (V3, at week 0) to end of treatment (V11, at week 52) in:

- glomerular basement membrane width(/thickness) (in nm)

This analysis will be based on the subgroup of participants in the kidney biopsy strata. The response variable is not log transformed prior to analysis. Both primary and secondary estimand will be used.

The analytical approach is identical to the main analytical approach described in section 4.2.2, except for the missing data imputation. Missing data will not be imputed (either truly missing or omitted from analysis due to the hypothetical strategy) due to the subjective nature and low sample size of the endpoint in the biopsy domain.

The estimated treatment difference between semaglutide and placebo will be presented together with the associated two-sided 95% confidence interval and a two-sided p-value.

4.3.2.3 Change in ADC, mean RARI, and mean arterial flow

These MRI derived, supportive secondary endpoints are defined (analogously to the primary endpoints) as the ratio from baseline (V3, at week 0) to end of treatment (V11, at week 52) in:

- ADC (cortex) ($mm^2 \times s^{-1} \times 10^{-3}$)
- ADC (medulla) ($mm^2 \times s^{-1} \times 10^{-3}$)
- mean RARI (unitless)
- mean arterial flow (mL/min)

An analysis will be performed in accordance to the main analytical approach described in Section 4.2.2. Both primary and secondary estimand will be used. An additional analysis on these assessments identical to the supplementary analysis: ‘Acute change from baseline’ will also be performed.

4.3.2.4 Change in natriuresis, albumin excretion rate, and kidney function

These 24-hour urine derived, supportive secondary endpoints are defined as change from baseline (V3, at week 0) to end of treatment (V11, at week 52) in:

- natriuresis (urinary sodium excretion) (mmol/day)
- albumin excretion rate (mg/24h)
- kidney function (creatinine clearance) (mL/min/1.73m^2)

The analytical approach is identical to the main analytical approach described in Section 4.2.2, except the response variable is not log transformed prior to analysis. Only primary estimand will be used. An additional analysis on these assessments identical to the supplementary analysis: 'Acute change from baseline' will also be performed.

4.4 Exploratory endpoints analyses

The exploratory endpoints analyses will be based on the FAS using data collected before or at the end of treatment visit (V11, at 52 weeks) and corresponding data point sets mentioned in Section 3, within in-study observation period, unless otherwise stated. Only the primary estimand will be used for the exploratory endpoint analysis. If the number of sample is insufficient for model fitting, only descriptive statistics will be provided for exploratory biopsy parameters.

Values less than 0 for Proton Density Fat Fraction (PDFF), will be set to a small number 10^-3 as the parameter cannot be negative values. Values which are equal to zero will be replaced with a small value 10^-3 to ensure log transformation of the data.

Change in clinical parameters, biomarkers, MRI, and morphometric endpoints

The following exploratory endpoints are defined as change from baseline (V3, at week 0) to end of treatment (V11, at week 52) in:

- eGFR (ml/min/1.73m^2)
- Diastolic blood pressure (mmHg)
- Systolic blood pressure (mmHg)
- Body weight (kg)
- HbA1c (%-point)
- Sodium and fractional sodium excretion
- Cystatin C (Serum/Plasma)
- 24 hr urine volume
- Fractional sodium excretion from 24 h urine

The following exploratory endpoint is defined as the ratio from baseline (V3, at week 0) to end of treatment (V11, at week 52) in:

- UACR (mg/mmol and/or mg/g)
- hsCRP (mg/L)
- IL-6 (pg/mL)
- sTNFR1 (pg/mL)
- sTNFR2 (ng/mL)
- TNF-alpha (pg/mLor ng/L)

- uric acid (mg/dL conventional unit, mmol/L SI unit)
- fibrinogen (mg/dL conventional unit, g/L SI unit)
- MCP-1 (pg/mL)
- KIM-1 (ng/mL)
- NGAL (ng/mL)
- F2-isoprostanes per unit creatine (mg/kg)
- 8-oxo-2'-deoxyguanosine (8-OHdG) (pg/mL)
- Cholesterol
- HDL cholesterol
- LDL cholesterol
- Triglycerides
- Creatinine (Serum/Plasma)
- Peak Systolic Velocity (cm/s)
- End Diastolic Velocity (cm/s)
- Proton Density Fat Fraction (%)
- Cortex Volume (mL)
- Volume of Perirenal Fat (mL)
- Volume of Sinus Fat (mL)
- Filtration fraction (%)

Given the ambiguity surrounding the distribution of the parameters outlined below each parameter will undergo normality testing using QQ plots with QQ lines and density plots.

For parameters exhibiting normal distribution, changes from baseline will be assessed. Conversely, for parameters demonstrating log-normal distribution, ratio to baseline will be evaluated.

- Fractional interstitial area (ratio; no unit)
- Glomerular tuft area (μm^2)
- Glomerular volume – Wiggins ($10^6 \times \mu m^3$)
- Glomerular nuclear count (count)
- Mesangial matrix area (μm^2)
- Mesangial index (%)
- Mesangial volume – Wiggins ($10^6 \times \mu m^3$)
- Mesangial nuclear count (count)

Mean media of vessel and most diseased vessel

- Total area of the media of the vessels (μm^2) (Arterioles/ Interlobular Arteries)
- Total area of the intima of the vessels (μm^2) (Arterioles/ Interlobular Arteries)
- Total area of the lumina of the vessels (μm^2) (Arterioles/ Interlobular Arteries)
- Fractional area of the media of the vessels (ratio; no unit) (Arterioles/ Interlobular Arteries)
- Fractional area of the intima of the vessels (ratio; no unit) (Arterioles/ Interlobular Arteries)
- Fractional area of the lumina of the vessels (ratio; no unit) (Arterioles/ Interlobular Arteries)

- Interstitial Fibrosis and Tubular Atrophy (IFTA) (%)

- Glomerulosclerosis (%)
- Glomerular classification of Diabetic Nephropathy
- Arteriosclerosis score
- Arteriolohyalinosis score
- Inflammation score
- urine pH

Change from baseline analyses will, for each of the above MRI endpoints, be done accordance with the main analytical approach described in Section 4.2.2. Change from baseline analyses will, for each of the above biopsy endpoints will be done in accordance to the analytical approach described in section 4.3.2.2. For the categorical variables, descriptives will be presented.

For the parameters except MRI and Biopsy, the analytical approach is as follows.

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 actual 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 and stratification factors as categorical effects and baseline and post-baseline values observed prior to the visit in question as covariates.

The 500 complete datasets will be analysed using an ANCOVA with actual treatment, region and the stratification factors as categorical effects and the endpoint baseline value as a covariate. Rubin's rule[06] will then be applied to combine the estimates and draw inference.

Annual rate of change in eGFR

These exploratory endpoints are defined as the annual rate of change (slope) in

- eGFR (total eGFR slope) (ml/min/1.73m2/year) (based on creatinine, CKD-EPI creatinine-cystatin c 2021)
- eGFR (chronic eGFR slope) (ml/min/1.73m2/year) (based on creatinine, CKD-EPI creatinine-cystatin c 2021)

Annual rate of change analyses will, for each of these endpoints, be performed using a linear mixed-effects regression model with actual treatment, region and the stratification factors, time (as a continuous variable) and treatment by time interaction as fixed effects and including subject as a random intercept and time as a random slope (moreover, each subject will have a unique slope for eGFR over time). The random intercept and slope are assumed to be bivariate normal distributed with mean zero and an unstructured covariance matrix. The independent error term is assumed to be identical univariate normal distributed with mean zero. For total eGFR slope the model is fitted to observed and scheduled data from baseline and post-baseline. For chronic eGFR slope the model will be fitted to data collected at and after week 12. The parameter of interest is the regression coefficient for the treatment and time interaction term, which measures the slope difference between semaglutide and placebo.

4.5 Safety analyses

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

4.5.1 Extent of Exposure

Duration of exposure will be summarized categorically by treatment group for all observed data points from first date of study product until permanent discontinuation of treatment. In addition, time on study product and treatment pause will be summarized descriptively just like the other continuous endpoints.

4.5.2 Adverse Events

Adverse events (AEs) will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) coding. A treatment-emergent AE is defined as an AE with onset in all observed data points from first date of study product until permanent discontinuation of treatment.

The following assessments will be done and summarized by means of the number of patients with at least one event (N), the proportion of patients with at least one event (%), the number of events (E) and the event rate (R) per 100 patient years of exposure time for all observed data points from first date of study product until permanent discontinuation of treatment (resp. per 100 patient years of observation time for all observed data points from first date of study product until permanent discontinuation of treatment):

- Serious adverse events (SAEs)
- Adverse events (AEs) leading to discontinuation of randomized treatment
- Severe hypoglycaemic episodes
- Selected type of AE’s (SAE’s and non-serious adverse events) requiring additional data collection (Medication errors, misuse, and abuse)

Summaries will be presented as overall, by relationship to study product, by severity, by seriousness, by outcome, by treatment discontinuation status and by action taken to study product.

Total exposure time and observation time, as explained above, in years will be presented in the summary as well.

In addition, treatment emergent SAEs and AEs leading to discontinuation of randomized treatment will be summarized by system organ class and preferred term (All observed data points from first date of study product until permanent discontinuation of treatment).

The development over time in AEs leading to discontinuation will be presented graphically. Data on pregnancies in female patients and pregnancy outcome (until age 1 month) and AEs in the fetus or newborn infant will be presented in a listing.

Additional data collected regarding medication errors, misuse and abuse, and Neoplasm (Malignant and Non- malignant) of randomized treatment will be presented in listings.

4.5.3 Additional Safety Assessments

Not Applicable

4.6 Other analyses

The primary endpoints may be affected by differences in blood glucose. Therefore, the association between the pre-scan blood glucose levels will be plotted against the primary endpoints along with an associated linear regression line separately by actual treatment, visit and MRI field strength.

The site-effect on the primary and secondary endpoints maybe explored if deemed necessary.

4.6.1 Other variables

Not applicable.

4.6.2 Subgroup Analyses

Not applicable

4.7 Interim analyses

Not applicable.

4.8 Changes to Protocol-planned Analyses

For the supportive secondary endpoint “natriuresis (urinary sodium excretion)” unit has been revised from mmol/L to mmol/day.

5 Sample Size Determination

Please refer to protocol

6 Supporting documentation

6.1 Appendix 1: Definition of endpoints, assessments and associated analyses

Table 6-1 List of endpoints and analyses

Endpoint	Time frame	Metric (Unit)	Estimand	Model/method	Summary measure	Details
Primary						
Relative change in kidney oxygenation (cortex)	From baseline (V3) to end of treatment*	Ratio (s^{-1})	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	BOLD MRI (R2*). Analysed on log-scale.
Relative change in kidney oxygenation (medulla)	From baseline (V3) to end of treatment*	Ratio (s^{-1})	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	BOL MRI (R2*). Analysed on log-scale.
Relative change in global kidney perfusion	From baseline (V3) to end of treatment*	Ratio (mL/100g/min)	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Relative change in kidney inflammation (cortex)	From baseline (V3) to end of treatment*	Ratio (ms)	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Relative change in kidney inflammation (medulla)	From baseline (V3) to end of treatment*	Ratio (ms)	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Supportive secondary						
Change in gene expression	-	-	-	-	-	Kidney biopsy. Analyses are detailed in the bioinformatics analyses plan.
Change in glomerular basement membrane width	From baseline (V3) to end of treatment*	nm	Primary + Secondary	ANCOVA w/ MI	Mean difference	Kidney biopsy.
Relative change in ADC (cortex)	From baseline (V3) to end of treatment*	Ratio ($mm^2 \times s^{-1} \times 10^{-3}$)	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Relative change in ADC (medulla)	From baseline (V3) to end of treatment*	Ratio ($mm^2 \times s^{-1} \times 10^{-3}$)	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Relative change in mean RARI	From baseline (V3) to end of treatment*	Ratio (unitless)	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.

Relative change in arterial flow	From baseline (V3) to end of treatment*	Ratio (mL/min)	Primary + Secondary	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Change in natriuresis	From baseline (V3) to end of treatment*	mmol/day	Primary	ANCOVA w/ MI	Mean difference	urinalysis
Change in albumin excretion rate	From baseline (V3) to end of treatment*	mg/24h	Primary	ANCOVA w/ MI	Mean difference	urinalysis
Change in kidney function (creatinine clearance)	From baseline (V3) to end of treatment*	mL/min/1.73m^2	Primary	ANCOVA w/ MI	Mean difference	urinalysis
Exploratory						
Annual rate of change creatinine based eGFR (total eGFR slope)	From baseline (V3) to end of study (V12)	mL/min/1.73m^2 /year	Primary	Linear mixed effects regression	Mean slope difference	Renal function: Serum/Plasma
Annual rate of change CKD-EPI creatinine-cystatin c 2021based eGFR (total eGFR slope)	From baseline (V3) to end of study (V12)	mL/min/1.73m^2 /year	Primary	Linear mixed effects regression	Mean slope difference	Renal function: Serum/Plasma
Annual rate of change creatinine based eGFR (chronic eGFR slope)	From week 12 (V8) to end of study (V12)	mL/min/1.73m^2 /year	Primary	Linear mixed effects regression	Mean slope difference	Renal function: Serum/Plasma
Annual rate of change CKD-EPI creatinine-cystatin c 2021 based eGFR (chronic eGFR slope)	From week 12 (V8) to end of study (V12)	mL/min/1.73m^2 /year	Primary	Linear mixed effects regression	Mean slope difference	Renal function: Serum/Plasma
Change in eGFR	From baseline (V3) to week 12 (V8)	mL/min/1.73m^2	Primary	ANCOVA w/ MI	Mean difference	Renal function: Serum/Plasma
Change in diastolic blood pressure	From baseline (V3) to end of treatment (V11)	mmHg	Primary	ANCOVA w/ MI	Mean difference	Vital signs
Change in systolic blood pressure	From baseline (V3) to end of treatment (V11)	mmHg	Primary	ANCOVA w/ MI	Mean difference	Vital signs

Relative change in UACR	From baseline (V3) to end of treatment (V11)	Ratio (mg/g)	Primary	ANCOVA w/ MI	geom mean ratio	Renal function: First morning void spot urine Analysed on log-scale.
Change in body weight	From baseline (V3) to end of treatment (V11)	kg	Primary	ANCOVA w/ MI	Mean difference	
Change in HbA_{1c}	From baseline (V3) to end of treatment (V11)	%-point	Primary	ANCOVA w/ MI	Mean difference	Glucose metabolism
Sodium and fractional sodium excretion	From baseline (V3) to end of treatment (V11)		Primary	ANCOVA w/ MI	Mean difference	Renal function: On site random spot urine
Relative change in hsCRP	From baseline (V3) to end of treatment (V11)	mg/L and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Serum. Analysed on log-scale.
Change in IL-6	From baseline (V3) to end of treatment (V11)	pg/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Serum. Analysed on log-scale.
Change in sTNFR1	From baseline (V3) to end of treatment (V11)	pg/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Serum. Analysed on log-scale.
Change in sTNFR2	From baseline (V3) to end of treatment (V11)	ng/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Serum. Analysed on log-scale.
Change in TNF-alpha	From baseline (V3) to end of treatment (V11)	pg/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Serum. Analysed on log-scale.
Change in uric acid	From baseline (V3) to end of treatment (V11)	mg/dL (conventional) and mmol/L (SI) and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Serum. Analysed on log-scale.

Change in fibrinogen	From baseline (V3) to end of treatment (V11)	mg/dL (conventional), g/L (SI) and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Whole blood. Analysed on log-scale.
Change in MCP-1	From baseline (V3) to end of treatment (V11)	pg/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Urine. Analysed on log-scale.
Change in KIM-1	From baseline (V3) to end of treatment (V11)	ng/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Urine. Analysed on log-scale.
Change in NGAL	From baseline (V3) to end of treatment (V11)	ng/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Urine. Analysed on log-scale.
Change in F2-isoprostanes per unit creatine	From baseline (V3) to end of treatment (V11)	pg/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Urine. Analysed on log-scale.
8-oxo-2'-deoxyguanosine (8-OHdG)	From baseline (V3) to end of treatment (V11)	pg/mL and ratio	Primary	ANCOVA w/ MI	Geom mean ratio	Urine. Analysed on log-scale.
24 hr urine volume	From baseline (V3) to end of treatment (V11)		Primary	ANCOVA w/ MI	Mean difference	Renal function: *Statistical distribution to be analysed*
Urine pH	From baseline (V3) to end of treatment (V11)		Primary	ANCOVA w/ MI		Renal function: *Statistical distribution to be analysed*
Cystatin C	From baseline (V3) to end of treatment (V11)		Primary	ANCOVA w/ MI	Geom mean ratio	Renal function:
Lipids	From baseline (V3) to end of treatment (V11)		Primary	ANCOVA w/ MI	Geom mean ratio	

Fractional interstitial area	From baseline (V3) to end of treatment (V11)	Ratio (unitless)	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Glomerular tuft area	From baseline (V3) to end of treatment (V11)	μm^2	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Glomerular volume Wiggins	From baseline (V3) to end of treatment (V11)	$10^6 \times \mu m^3$	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Glomerular nuclear count	From baseline (V3) to end of treatment (V11)	count	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Mesangial matrix area	From baseline (V3) to end of treatment (V11)	μm^2	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Mesangial index	From baseline (V3) to end of treatment (V11)	%	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Mesangial volume – Wiggins	From baseline (V3) to end of treatment (V11)	$10^6 \times \mu m^3$	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Mesangial nuclear count	From baseline (V3) to end of treatment (V11)	count	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Total area of the media of the vessels	From baseline (V3) to end of treatment (V11)	μm^2	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Total area of the intima of the vessels	From baseline (V3) to end of treatment (V11)	μm^2	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Total area of the lumina of the vessels	From baseline (V3) to end of treatment (V11)	μm^2	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.

Fractional area of the media of the vessels	From baseline (V3) to end of treatment (V11)	Ratio (unitless)	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Fractional area of the intima of the vessels	From baseline (V3) to end of treatment (V11)	Ratio (unitless)	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Fractional area of the lumina of the vessels	From baseline (V3) to end of treatment (V11)	Ratio (unitless)	Primary	ANCOVA	*Statistical distribution to be analysed*	Kidney biopsy.
Peak Systolic Velocity	From baseline (V3) to end of treatment (V11)	cm/s	Primary	ANCOVA w/ MI	Geom mean ratio	MRI
End Diastolic Velocity	From baseline (V3) to end of treatment (V11)	cm/s	Primary	ANCOVA w/ MI	Geom mean ratio	MRI
Proton Density Fat Fraction	From baseline (V3) to end of treatment (V11)	%	Primary	ANCOVA w/ MI	Geom mean ratio	MRI
Cortex Volume	From baseline (V3) to end of treatment (V11)	mL	Primary	ANCOVA w/ MI	Geom mean ratio	MRI
Volume of Perirenal Fat	From baseline (V3) to end of treatment (V11)	mL	Primary	ANCOVA w/ MI	Geom mean ratio	MRI
Volume of Sinus Fat	From baseline (V3) to end of treatment (V11)	mL	Primary	ANCOVA w/ MI	Geom mean ratio	MRI
Filtration fraction	From baseline (V3) to end of treatment (V11)	%	Primary	ANCOVA w/ MI	Geom mean ratio	MRI
Interstitial Fibrosis and Tubular Atrophy (IFTA)	From baseline (V3) to end of treatment (V11)	%	Primary	ANCOVA w/ MI	*Statistical distribution to be analysed*	Biopsy

Glomerulosclerosis	From baseline (V3) to end of treatment (V11)	%	Primary	ANCOVA w/ MI	*Statistical distribution to be analysed*	Biopsy
Glomerular classification of Diabetic Nephropathy	At baseline (V3) and end of treatment (V11)	score	Descriptive	___		Biopsy
Arteriosclerosis score	At baseline (V3) and end of treatment (V11)	score	Descriptive	___		Biopsy
Arteriolohyalinosis score	At baseline (V3) and end of treatment (V11)	score	Descriptive	___		Biopsy
Inflammation score	At baseline (V3) and end of treatment (V11)	score	Descriptive	___		Biopsy
Fractional sodium excretion from 24 h urine	From baseline (V3) to end of treatment (V11)		Primary	ANCOVA w/ MI	Mean difference	Urine
Acute change from baseline in kidney oxygenation (cortex)	From baseline (V3) to week 4 (V6)	Ratio (s^{-1})	-	ANCOVA w/ MI	geom mean ratio	BOLD MRI (R2*). Analysed on log-scale.
Acute change from baseline in kidney oxygenation (medulla)	From baseline (V3) to week 4 (V6)	Ratio (s^{-1})	-	ANCOVA w/ MI	geom mean ratio	BOL MRI (R2*). Analysed on log-scale.
Acute change from baseline in global kidney perfusion	From baseline (V3) to week 4 (V6)	Ratio (mL/100g/min)	-	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Acute change from baseline in kidney inflammation (cortex)	From baseline (V3) to week 4 (V6)	Ratio (ms)	-	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Acute change from baseline in kidney inflammation (medulla)	From baseline (V3) to week 4 (V6)	Ratio (ms)	-	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.

Acute change from baseline in ADC (cortex)	From baseline (V3) to week 4 (V6)	Ratio ($mm^2 \times s^{-1} \times 10^{-3}$)	-	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Acute change from baseline in ADC (medulla)	From baseline (V3) to week 4 (V6)	Ratio ($mm^2 \times s^{-1} \times 10^{-3}$)	-	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Acute change from baseline in mean RARI	From baseline (V3) to week 4 (V6)	Ratio (unitless)	-	ANCOVA w/ MI	geom mean ratio MRI	
Acute change from baseline in arterial flow	From baseline (V3) to week 4 (V6)	Ratio (mL/min)	-	ANCOVA w/ MI	geom mean ratio	MRI. Analysed on log-scale.
Acute change from baseline in natriuresis	From baseline (V3) to week 4 (V6)	mmol/day	-	ANCOVA w/ MI	Mean difference	urinalysis
Acute change from baseline in albumin excretion rate	From baseline (V3) to week 4 (V6)	mg/24h	-	ANCOVA w/ MI	Mean difference	urinalysis
Acute change from baseline in kidney function (creatinine clearance)	From baseline (V3) to week 4 (V6)	mL/min/1.73m^2	-	ANCOVA w/ MI	Mean difference	urinalysis

ANCOVA w/ MI: analysis of covariance with multiple imputation; geom mean ratio: geometric mean ratio

Primary estimand refers to the 'if all participants had adhered to the randomised treatment, without initiation of disallowed medication' while the secondary estimand refers to the 'while on treatment after week 26 if all participants had adhered to the randomised treatment at least until week 26, without initiation of disallowed medication'

*For the hypothetical estimand the end of treatment assessment refers to the assessment obtained at V11. For the while-on-treatment estimand the end of treatment assessment refers to the last assessment obtained after week 26.

6.2 Bioinformatics Analysis Brief

Please refer to the bioinformatics analysis plan (BAP) prepared by the department of Development Data Science Omics, Novo Nordisk, for more information.

BAP covers the analyses of gene expression data (single nucleus and spatial transcriptomics) from the kidney biopsies for pre-specified mechanisms implicated in patients with T2D and CKD. For differential expression analysis, multiple testing corrections will be applied as needed.

Analysis and reporting will be done after the study intervention period.

snRNA-seq Computational Data Analysis

The following steps will be performed for data processing and analysis of snRNA-seq data

1. **From fastq to count matrix:**

- Cell Ranger with the version compatible with the applied library construction kit as specified by 10x Genomics will be used to process raw snRNA-seq data from fastq to count matrix. This is available via nf-core/scrnaseq (version 3.0.0)
- Count matrix will be generated by alignment to the human reference GRCh38 Ensembl release 110(version Homo_sapiens.GRCh38.dna.primary_assembly.fa.gz)

2. **Steps for Quality control (QC) and data processing**

- The count matrices files will be further processed using Seurat V5, as well as SoupX (version 1.6.2) to remove ambient RNA.
- Seurat V5, [1] will be applied for genes/cell filtering to minimize mitochondrial and ribosomal read contamination and relevant QC metrics, and plots will be generated representing the sample level cell count and gene counts (i.e. feature counts).
- During QC step to remove technical variation originating from snRNASeq library preparation assay will be selected using relevant function in Seurat V5. This will further allow inspection of cell cycle scoring, identification of highly variable features and scaling.
- Visualize the expression level of sex-linked genes (e.g., XIST, DDX3Y, TTTY14). Those samples with inconsistency between expression of sex-linked genes and reported sex will be deleted before downstream analysis.

A metadata matrix (SeuratObject) will be generated using Seurat V5, which includes the normalized and QC'ed data, followed by dimensionality reduction including optimal principal component identification, integration and batch correct using RPCA (version 1.2.0), and cell clustering (using Louvain algorithm), as well as generation of embedding and visualization of the high-dimensional non-linear data in 2-D space (UMAP) with default parameters as per best practices.

- Cell Type annotation step will be performed using the Reference Markers available from Kidney Precision Medicine Project marker list and nomenclature of cell type labels and gene sets available from the consortium and corresponding publication

3. Differential State Analysis:

- DEGs (log2 fold change) and pathway enrichment analysis (e.g. gene-set enrichment analysis) across cell type will be defined as change from baseline (V2, at week 0) to end of treatment (V11, at week 52) in that are reflective of below mechanisms majorly implicated in T2D and CKD to investigate effect of treatment:
- Table 6-2 reflects the mechanisms in which genes corresponding pathways will be checked after cell type specific differential gene expression analysis using Pseudo bulk (and/or linear mixed effect) approach to avoid the highly inflated p-value directly.

The kidney biopsy snRNASeq data will be represented in forms of volcano plots, heatmap, dotplot and relevant state-of-the-art graphics to depict and demonstrate the impact of OW semaglutide s.c. versus placebo on the above pre-specified mechanisms in patients with T2D and CKD

Table 6-2 Mechanisms for Pathway-Specific Gene Analysis

Mechanisms	Cell Type	Endpoint Category	Summary Measure	Pathway enrichment measure
Inflammation	All kidney cell types	Supportive secondary	Log2Fold changes	p-adjusted
Renal injury and function	PT (proximal tubule epithelial cells), TAL (thick ascending limb), LoH (loop of henle cells), DCT (distal	Supportive secondary	Log2Fold changes	p-adjusted

	convoluted tubule cells), Collecting duct cells (CD), endothelial cells (EC), podocytes			
Fibrosis	Fibroblasts, Myofibroblasts, EC, Epithelial cells, podocytes, Mesangial cells, Macrophages	Supportive secondary	Log2Fold changes	p-adjusted
Glucose and lipid metabolism	PT, Podocytes, TAL	Supportive secondary	Log2 Fold changes	p-adjusted
Oxidative stress	Major kidney cell tyoes	Supportive secondary	Log2 Fold changes	p-adjusted
Endothelial function	ECs; for cross talk, mesangial cells, podocytes, PT	Supportive secondary	Log2 Fold changes	p-adjusted
Metabolic reprogramming	PT, TAL	Supportive secondary	Log2Fold changes	p-adjusted
Sugar transport(ers)	PT, Vascular Smooth Muscle cells, Mesangial cells	Supportive secondary	Log2Fold changes	p-adjusted

4. **Analysis of exploratory Biomarkers**: The following exploratory biomarkers will be assessed for their relative change in normalized expression and cell count distribution from baseline (V2, at week 0) to end of treatment (V11, at week 52) in snRNASeq kidney biopsies and displayed as a heatmap in relevant cell types per donor:

   - IL-6
   - KIM-1
   - MCP-1
   - NGAL

- sTNFR1
- sTNFR2
- TNF-alpha
- PLA2G7

6.3 Spatial transcriptomic computational and statistical analysis

The following steps will be performed for data processing and analysis of spatial transcriptomics (sp Tx) data. Below steps outline the analytical steps with sp Tx data:

1. **Preprocessing steps from tissue block to count matrix:** The Xenium instrument takes FFPE tissue blocks sliced into 5um thick sections, performs the chemistry to obtain fluorescent signals, decodes the signals into specific RNA transcripts, performs cell segmentation, and outputs count matrices of 5000 base + 100 customized RNA transcripts based on the hg38 human genome for downstream analyses. Each transcript within the count matrix is also associated with an x,y spatial coordinate to indicate where it lies on the tissue. Pre/post samples from the same individual will be run on the same slide/within the same batch of Xenium analysis to minimize batch effects. Only samples passing snRNASeq QC will be considered for spatial transcriptomic analysis

2. **Steps for Quality control (QC) and data processing**: The count matrices files will be further processed using Seurat V5. Seurat V5, will be applied for genes/cell filtering to minimize off target probe and empty cell contamination and relevant QC metrics, and plots will be generated representing the sample level cell count and gene counts (i.e. feature counts).

    - As a QC step, to remove technical variation originating from batch effects across different Xenium runs, standard Seurat V5 processing will be applied. This will entail normalization, scaling, and dimensionality reduction.
    - For each sample, a metadata matrix (SeuratObject) will be generated using Seurat V5, which includes the normalized and QC'ed data, followed by dimensionality reduction including optimal principal component identification. Any sample with a high proportion (>33%) of cells which do not pass quality control will not be included in downstream celltype validation as the sample is of too low quality to reliably analyze. As we will be using labels transferred directly from the snRNASeq data, we will not perform celltype clustering or umap dimension reduction on the spatial transcriptomics datasets.

- To validate celltype identification from snRNASeq, Seurat V5 FindTransferAnchors and TransferData will be used to transfer labels from the final integrated snRNASeq count matrix to the spatial transcriptomics datasets using the genes identified as highly variable from within the spatial transcriptomics data. As a QC step, cells with low transfer probabilities (<25%) will be discarded from further analyses.

3. **snRNASeq Cell type Validation:** Labelled cells which pass QC filtering will be used to verify the validity of celltype assignment from within the snRNASeq data. Since cells within snRNASeq are dissociated from the tissue, spatial transcriptomics allows for the mathematical transfer of celltype assignment from disassociated snRNASeq cells to in situ spatial transcriptomics cells. This allows for direct visualization and inspection of snRNASeq celltype assignment within the in-situ context of the tissue. First, state-of-the-art graphics such as heat maps and dot plots will be used to molecularly validate the label transfer of the snRNASeq celltype assignments to the spatial transcriptomics data. To do this, expression of KPMP marker genes within each predicted cell type will be verified. Next, cell types will be visualized on the tissue section to confirm that the predicted cell type lies within a reasonable structure within the tissue (i.e. podocytes in glomeruli, and thick ascending limb cells concentrated in medullary tubular areas). Similarly, key cell type marker transcripts will be visualized on the tissue sections and with other top of the line techniques such as dot plots to confirm the validity of the predicted cell types from the label transfer process.

Differential state analysis: Findings from the differential state analysis comparing changes in normalized gene expression from baseline (V2, at week 0) to end of treatment (V11, at week 52) that are reflecting mechanisms majorly implicated in T2D and CKD to investigate effect of treatment will be validated within the spatial transcriptomics data provided the implicated transcripts are available within the subset of 5000 base + 100 custom transcripts from the spatial transcriptomics data. Specifically, the normalized counts of the exploratory biomarkers (listed in protocol) and genes which are differentially expressed within the snRNASeq data belonging to Table 1 will be assessed for corresponding changes from baseline (V2, at week 0) to end of treatment (V11, at week 52) to confirm the treatment effect.

7 References

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