Data and Safety Monitoring Board

Statistical Analysis Plan

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Vitamin D and type 2 diabetes

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1 OVERVIEW & ADMINISTRATIVE INFORMATION

The present version 2 of the Statistical Analysis Plan (SAP) corresponds to version 1.9 of the protocol and associated Manual of Procedures.

The Statistical Analysis Plan (SAP) includes statistical hypotheses, outcome measures, methods for the primary outcome analysis and subgroup analyses. The SAP follows the outline and general principles outlined in the Data Analysis Plan section of the protocol; however, the SAP provides more detail compared to the protocol while previously proposed analyses that are of questionable value have been removed.

2 STUDY POPULATION & TREATMENT GROUPS

Defining the Study Population and Treatment Groups - The primary analysis is intention-to-treat (ITT), including all randomized participants in their assigned treatment arm and including *all diabetes events observed during the study*, irrespective of adherence to assigned treatment. For all participants, follow-up will be censored at the date of the last assessment for incident diabetes (see section 4).

Every effort has been made to establish eligibility prior to randomization; however, 13 participants (0.05% of the cohort) have been subsequently found not to meet all eligibility criteria. Some of these participants were ineligible because of a safety exclusion criterion and are not on study pills. Consistent with the ITT principle, participants who did not meet eligibility criteria will be included in all analyses. This proportion (0.05%) is considered small and, consistent with the data analysis plan in the protocol, does not warrant sensitivity analyses to assess the impact of excluding those individuals from the analyses.

3 OUTCOME VARIABLES

3.1 Primary outcome

The primary endpoint is time to incident diabetes, as described in the protocol and Manual of Procedures 18.

Cases of diabetes diagnosed outside of D2d (i.e., during routine clinical practice) among "inactive" participants that are discovered incidentally by D2d research staff will not count as diabetes cases and will not be included in the primary analysis.

3.2 Secondary outcomes

A number of secondary outcomes will be analyzed to clarify the interpretation of the results of the primary analysis. Some of these analyses (e.g., insulin secretion) will not be presented in the main manuscript but in separate publications.

3.2.1 Secondary outcomes – related to the primary outcome: continuous variables

- HbA1c (measured at baseline, semi-annually)
- FPG (baseline and semi-annually)
- 2hPG (baseline and annually)
- Insulin secretion, Insulin sensitivity and Disposition Index (indices from OGTT; baseline and annually)

3.2.2 Secondary outcomes – independent of the primary outcome: continuous variables

- Blood pressure (baseline and semi-annually)
- Cardiovascular risk factors: cholesterol profile, C-reactive protein, urine albumin excretion (baseline, M12 and M24)
- Atherosclerotic Cardiovascular Disease (ASSCVD) ACC/AHA composite risk score (baseline, M12, M24)

3.3 Explanatory outcome: continuous variable

- 25OHD concentration (baseline, M12 and M24)

3.4 Safety outcomes: binary variables

- Hypercalcemia, as defined in the protocol and Data Safety and Monitoring Plan
- Hypercalciuria, as defined in the protocol and Data Safety and Monitoring Plan
- Nephrolithiasis, as reported by clinical sites and Data Safety and Monitoring Plan

4 PRIMARY ANALYSIS & SUPPORTING ANALYSES

The *primary analysis* of D2d will assess whether, in participants with pre-diabetes, oral daily vitamin D_3 supplementation reduces the rate of progression from pre-diabetes to incident diabetes (=primary outcome).

All randomized participants are included in the analysis. Kaplan-Meier estimates of "time to confirmed diabetes" distributions will be calculated for each treatment group. The log-rank test will then be used to perform an unadjusted comparison of the time-to-event distributions in the two treatment groups. Follow-up of participants who do not complete the study (e.g. withdrew) will be "censored" on the date of their last follow-up encounter (see below). Cox proportional hazard modeling will be used to compare the risk of incident diabetes in the two groups.[1] The model will include an indicator for the randomized intervention as its main predictor variable. Clinical site at randomization will be a stratifying factor. The baseline variables (BMI, race, as entered in SPIRS) used to stratify the randomization will also be included as stratifying factors.

The p-value from the primary analysis will be based on the chi-square statistic from a likelihood ratio test obtained from proportional hazards models with and without the term for intervention arm. Comparisons of the intervention groups will be performed per the ITT principle, with all randomized participants grouped per their intervention assignment at randomization, regardless of adherence. Unless otherwise specified, p values will be two tailed, and values below 0.05 are considered statistically significant.

Definitions of follow-up time and censoring. Follow-up time for the primary outcome will be censored at the date of the *last assessment for diabetes with data in the electronic data capture system as of the cutoff date (as defined in the Manual of Procedures – section 20)*.

Censoring for participants *with diabetes* occurs at the date of the diabetes event, as shown in the *Diabetes Outcome* form in EDC. Specifically,

- If the diagnosis of diabetes is made within the study (i.e., using results by the Central Laboratory), the date of onset of diabetes is defined as the date of the first diagnostic glycemic value.
- If the diagnosis of diabetes is made outside of study (i.e., adjudicated), the date of onset of diabetes is determined during the adjudication process.

Censoring for participants *free of diabetes* occurs at one of the following dates:

- Death or active withdrawal.
- Last encounter *free of diabetes,* using the most recent of the following 3 options:
    - Scheduled encounter
        - Semi-annual visit (*or* non-visit contact)
        - Annual visit (*or* non-visit contact)
        - Interim phone call
    - Unscheduled visit to evaluate for diabetes in between semi-annual/annual visits [as described in Manual of Procedures – section 18].
    - Adjudicated event (that did not confirm diabetes).

Sensitivity analysis of the primary analysis

Competing event of diabetes medication use

The use of a diabetes medication (e.g., metformin for worsening pre-diabetes or for new diabetes diagnosed outside of D2d) is considered a "competing event." Specifically, if vitamin D supplementation is presumed to be effective at reducing diabetes risk, more participants in the placebo group will be placed on a diabetes medication (e.g., metformin) for worsening pre-diabetes or early stage diabetes. If these participants do not meet the D2d criteria (i.e., by central laboratory criteria or adjudication) when starting a diabetes medication, they continue to be followed for incident diabetes within D2d, per ITT analyses. However, *the use of a diabetes medication in these participants practically removes them from the potential pool of those likely to meet the incident diabetes endpoint as the diabetes medication will improve glycemia*. *Such competing events reduce the power of the trial by lowering diabetes risk.* Importantly, because starting on a diabetes medication is more likely to occur in the placebo group – if the underlying hypothesis holds – these events falsely change the relative risk reduction towards null. Cases in which a diabetes medication has been started without the participant meeting the D2d criteria for diabetes are not uncommon because of local clinical practice or laboratory variability between the central D2d laboratory and local laboratories.

To examine the potential effect of these "competing events" on study fidelity, *in sensitivity analyses,* the primary endpoint will be defined as *"time to incidence diabetes by D2d criteria or use of a diabetes-specific medication."* This analysis includes participants that started and remained for more than 31 days on a diabetes-specific medication (for worsening glycemia, for weight loss or for any other reason) but the D2d criterion of incident diabetes was not made (by central laboratory criteria or adjudication). In this analysis, follow-up of participants for the primary outcome is censored at the time when the diabetes-specific medication begun.

4.3 Heterogeneity of treatment effect (HTE) analyses in relation to the primary outcome

4.3.1 Subgroup analyses

Heterogeneity of treatment effect to vitamin D supplementation for the primary outcome will be assessed in a standard approach by **subgroups** defined by the following **baseline** characteristics (i.e., at the time of randomization) will be conducted. The analysis plan follows widely recommended best practices: pre-specification of subgroups (motivated by biologically plausible hypotheses), interaction tests for heterogeneity of effects adjusted for multiple comparisons (see below), and inference for subgroup-specific effects only in the context of a statistically significant interaction.[2-7] [8,9]

✓ In general, subgroup analyses - even when pre-specified - are considered exploratory, as the power to detect heterogeneity of effects is generally low. Nevertheless, to adjust for multiple comparisons, we will use the Hochberg Sequential procedure[10] when evaluating the significance (p-value for the interaction term) of each subgroup analysis. This procedure uses progressively more stringent statistical thresholds and is less rigid than the traditional Bonferroni correction method.

- Blood 25OHD concentration by a key Institute of Medicine cutoff (ng/mL) [justification: risk reduction will be larger among those with 25OHD < 20 ng/mL]
    b. < 20
    c. ≥ 20
- Race by self-reported definitions [justification: risk reduction will be larger among Blacks]
    a. White
    b. Black/African American
    c. Other
- Glycemic risk by pre-diabetes criteria [justification: risk reduction will be larger among those who meet all three criteria and are considered at higher risk for diabetes]
    a. Three criteria (FPG, A1c and 2hPG)
    b. Two criteria (all other participants: FPG/A1c, FPG/2hPG, A1c/2hPG)
- BMI by standard clinical categories based on BMI (km/m^2) [justification: risk reduction will be larger among those with higher BMI]
    a. Normal weight / Overweight < 30
    b. Obese ≥ 30
- Glycemic risk by meeting 2hPG criterion for pre-diabetes [justification: differences in the underlying pathophysiology of people diagnosed by 2hPG may be important for the effect of vitamin D]
    a. 2hPG (FGP/2hPG, A1c/2hPG, FPG/A1c/2hPG)
    b. FPG/A1c only
- Ethnicity
    a. Hispanic
    b. Non-Hispanic
- Sex
    a. Men
    b. Women
- Waist circumference
    a. < median value
    b. ≥ median value

- Age
    a. < median value
    b. ≥ median value
- Geographic location as a proxy for sun exposure
    a. above 37° latitude
    b. below 37° latitude
- Calcium intake [to test for potential interaction]
    a. < median value
    b. ≥ median value

5 SECONDARY ANALYSES

Effect of vitamin D supplementation on secondary outcomes (**as continuous variables**) will be based on a linear mixed model and account for within subject correlation in repeated measurements over time. The overall mean difference between vitamin D and placebo groups for these analyses will be tested using the nominal 0.05 type 1 error rate *without adjustment for multiple comparisons*.[11,12]

Some of these analyses (e.g., insulin secretion, cardiovascular risk factors) will not be presented in the main manuscript but in separate publications.

5.1 Related to the primary outcome (i.e., diabetes).

Change from baseline in:

- HbA1c, FPG and 2hPG
- Insulin secretion, sensitivity and disposition index. (indices derived from the OGTT).

5.2 Independent of the primary outcome

Change from baseline in:

- Blood pressure. A test for interaction by race (White, Black/African American vs. Other) will be done. [13,14]
- Cardiovascular risk factors:
    - Cholesterol profile
        - Total cholesterol
        - HDL
        - LDL
        - Triglycerides
    - C-reactive protein
    - Urine albumin excretion
- ASCVD risk score (ACC/AHA) calculated using clinical and laboratory criteria.
- 25OHD

NOTE: For certain variables (e.g., cardiovascular risk factors, 25OHD), the analyses will take place in a sub-cohort with available laboratory data at the BAS, M12 and M24 visit.

6 SAFETY ANALYSES

The safety analyses will examine the effect of vitamin D supplementation on the following safety outcomes.

- Hypercalcemia
- Hypercalciuria
- Nephrolithiasis

7 MISSING DATA

Missing data are inevitable in clinical research. Every effort has been made to minimize missing data in D2d. The primary analysis will assume that censoring for time-to-event variables is non-informative. However, we will perform sensitivity analyses to assess the degree to which the results are sensitive to the validity of the assumptions of non-informative censoring or non-informative missingness. For the secondary analyses using linear mixed effects models over time, results will be valid unless data are not missing at random.

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