Statistical Analysis Plan

A randomised, double blind, placebo controlled, phase II, multi-centre study to investigate the effects of vitamin D2 or D3 supplementation on metabolic parameters in people at risk of type 2 diabetes

(short title: Vitamin D supplementation in people at risk of type 2 diabetes)

EudraCT 2009-011264-11

Collaborative project between MRC Epidemiology Unit, Cambridge, and Barts & The London and QMUL, London

Final version agreed: 13th September 2012

1 Introduction

This is the plan for the analyses of the efficacy and safety endpoints, and acceptability/feasibility measures from a randomised, double blind, placebo controlled, phase II, multi-centre pilot study to investigate the effects of vitamin D2 or D3 supplementation on metabolic parameters in people at risk of type 2 diabetes.

Other endpoints, including quality of life and body pain endpoints, as well as other potential future analyses of data arising from this trial, will be the subject of future analysis plans.

Currently the trial team have agreed to think about potential publications, but to be flexible, aiming for the highest impact journal(s) possible. This might mean combining endpoints within one manuscript, or having separate manuscripts as appropriate.

Current possibilities for papers include: a 'methods paper' detailing methods and baseline data, a main results paper which would include primary endpoint (HbA1c) and other related (secondary) endpoints such as 25(OH)D level and those achieving levels >75 nmol/l, and safety endpoints, or might also include some of the other secondary endpoints (such as but not limited to anthropometry, BP) within it. There could be a separate paper with CVD endpoints all in one (PWV in London, plus across both sites, BP, lipids, inflammatory markers-CRP, CVD risk score). Also a separate paper analysing quality of life factors (the analyses of the quality of life data will be described and performed at a later stage).

2 Study endpoints

2.1 Primary efficacy endpoint

- Glycated haemoglobin (HbA1c) (mmol/mol IFCC* units). (Note: for ease of interpretation, results will also additionally be presented in units of % (DCCT*) - see section 5.1 for details)

**IFCC: International Federation of Clinical Chemists; DCCT: Diabetes Control and Complications Trial*

2.2 Secondary efficacy endpoints

- Serum 25(OH)D2 concentration (nmol/l)
- Serum 25(OH)D3 concentration (nmol/l)
- Total serum 25(OH)D concentration (nmol/l) – defined as the sum of the D2 and D3 concentrations
- Proportion of participants with serum 25(OH)D3 concentration in the following categories:
    - <25 nmol/l
    - 25 to <50 nmol/l
    - 50 to <75 nmol/l
    - 75 to <150 nmol/l
    - ≥ 150 nmol/l

- BMI (kg/m^2)
- Waist circumference (cm)

- Arterial stiffness assessed by pulse wave velocity (m/s) (London only)

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)

- Total cholesterol (mmol/l)
- HDL cholesterol (mmol/l)
- Total cholesterol/HDL cholesterol ratio (%)

- Apolipoprotein A1 (ApoA1) (mmol/l)
- Apolipoprotein B (ApoB) (mmol/l)
- ApoA1/ApoB ratio (%)

- Modelled CVD risk score (UKPDS) (%)

- hs-CRP (high sensitivity C reactive protein) (mg/l)
- Fructosamine (micromol/l)
- PTH (parathyroid hormone) (pmol/l)

- Liver function tests will include 3 parameters:
    - Alkaline phosphatase (ALP) (IU/l)
    - Alanine aminotransferase (ALT) (IU/l)
    - Aspartate aminotransferase (AST) (IU/l)

2.3 Safety endpoints

- Adverse events (AEs)
- Serious adverse events (SAEs)
- Adverse reactions (ARs)
- Suspected serious adverse reactions (SSARs)
- Suspected unexpected serious adverse reactions (SUSARs)
- Ionised calcium level (on iSTAT)> 1.3 mmol/l or serum corrected calcium > 2.65 mmol/l
- Urine calcium:creatinine ratio > 1.0

2.4 Acceptability/feasibility measures

A participant will be defined as being compliant with the protocol if he/she has taken all 4 doses of IMP. The percentage compliance within each randomised group will be calculated.

3 Analysis population

The primary analysis of efficacy endpoints will use an **Intention To Treat (ITT) population**, which includes all participants in the group to which they were randomised, regardless of the treatment actually received.

A secondary analysis of efficacy endpoints will use a **Per-Protocol (PP) population**. This population will exclude individuals who did not comply with the protocol (i.e. individuals who did not take all doses of the IMP), and will be defined by the responsible PI prior to the start of the trial analysis.

The analysis of safety endpoints will use a **Safety population**, which includes all participants in the group based on treatment actually received. Any individual who received at least 1 dose of either vitamin D2 or vitamin D3 will be included in the vitamin D2 or vitamin D3 group.

4 Descriptive analyses

Baseline characteristics of the study population will be summarised separately within each randomised group.

For continuous variables, means and standard deviations will be presented, unless the variable has a highly skewed distribution, in which case, medians, 25^{th} and 75^{th} percentiles will be presented. For categorical variables, the number and percentage of participants within each category will be presented. For each variable (continuous or categorical), the % of missing values will be reported.

No p-values will be calculated for these tables.

5 Analyses of study endpoints

5.1 Primary efficacy endpoint

The primary efficacy endpoint, HbA1c, will be compared separately between each treatment group and placebo, using analysis of covariance with adjustment for baseline and centre. Where baseline values are missing, the missing indicator method will be used to enable these participants to be included in the analysis. For both vitamin D2 vs placebo and vitamin D3 vs placebo, the difference in mean HbA1c, 95% confidence interval and p-value will be reported. An analysis will be performed to check whether adjusting for age and sex (the stratifiers) in the analysis of covariance model has any impact on the estimated treatment effects; if it has no impact, then they will not be included in the model.

The main results (including p values) for HbA1c will be reported in the new IFCC units mmol/mol [1]. It is noted that all trial participants have data available in these IFCC units.

Additional summary reporting (not including p values) in the DCCT% units will be provided using the following formula which will convert the individual values of HbA1c IFCC mmol/mol data into DCCT% units [2]. It is noted that we will not use the data provided by the lab in the DCCT% units, but rather apply a conversion formula as below.

Conversion formula that will be used to convert individual values of HbA1c from IFCC mmol/mol units to DCCT% units [2]:

HbA1c in % = (0.09148 * (HbA1c in mmol/mol)) + 2.152

5.2 Secondary efficacy endpoints

Differences in means (for continuous endpoints) or proportions (for binary outcomes) between each treatment group and placebo, together with 95% confidence intervals, will be estimated for each of the secondary efficacy endpoints, using the same method described for the primary endpoint. Any continuous endpoints whose distribution is skewed will be log transformed prior to analysis, in which case a ratio of geometric means (and confidence interval) will be reported.

5.3 Safety endpoints

The number and percentage of participants experiencing any of the safety endpoints will be reported separately within each randomised group. No p-values will be calculated.

6 Considerations for analysis

6.1 Missing data

Missing values of endpoints

If an individual has a missing value for an efficacy endpoint, they will be excluded from the analysis. The pattern of missing data will be described. Levels of missing data are expected to be low. Decisions about handling missing data in the analysis will be made once the extent and pattern of missing data are known, but before any between-group comparisons for the primary and secondary endpoints are performed.

Missing baseline values of endpoints

For continuous efficacy endpoints, those participants with a missing baseline value of the variable will be included in the analysis using the missing indicator method, which is a valid method for pre-randomisation measures in trials [3] ensuring that no further participants are excluded while maintaining the advantage of improved precision.

6.2 Subgroup analyses

No *a priori* subgroups were defined in the protocol. For the primary endpoint, interactions between treatment group and baseline HbA1c, and treatment group and baseline vitamin D will be tested by including the appropriate interaction term in the analysis of covariance model. If the p-value for the interaction test is <0.05, the treatment effects and 95% confidence intervals will be estimated within subgroups defined by levels above and below the median value of either HbA1c or vitamin D.

6.3 Multiplicity

For the primary efficacy endpoint, two p-values will be calculated, one for the comparison of vitamin D2 vs placebo and one for the comparison of vitamin D3 vs placebo. Because this is a pilot study, no adjustment for multiple testing will be performed.

For all other efficacy endpoints, the treatment effects (vitamin D2 vs placebo and vitamin D3 vs placebo) will be reported together with a 95% confidence interval. No p-values will be calculated. Interpretation of results for secondary endpoints will be cautious and results that are significant in isolation will be interpreted less strongly than sets of results that are mutually supportive, or which are supported in previous research findings.

For safety endpoints, no p-values will be calculated.

6.4 Exploratory analysis

An exploratory analysis will be performed in which the primary efficacy endpoint (HbA1c) will be compared between the vitamin D2 group and the vitamin D3 group, using the same method described in section 5.1. An estimate of the difference in mean HbA1c (adjusted for baseline), together with a 95% confidence interval, will be reported.

7 References

1. John G, English E (2011) IFCC standardised HbA1c: should the world be as one? Clin.Chem.Lab Med 50: 1243-1248

2. Hoelzel W, Weykamp C, Jeppsson JO, et al (2004) IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. Clin.Chem. 50: 166-174

3. White IR, Thompson SG (2005) Adjusting for partially missing baseline measurements in randomized trials. Stat.Med 24: 993-1007

**IFCC: International Federation of Clinical Chemists;*
**DCCT: Diabetes Control and Complications Trial*