REDUCING WITH METFORMIN VASCULAR ADVERSE LESIONS IN TYPE 1 DIABETES (REMOVAL)

FINAL ANALYSIS
STATISTICAL ANALYSIS PLAN

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1. INTRODUCTION

1.1. STUDY BACKGROUND

Intensive glucose control reduces long term rates of cardiovascular disease (CVD) in people with type 1 diabetes (T1DM) but the majority of individuals affected by the condition do not currently achieve glucose targets with standard insulin therapy. Upward insulin dose titration may lead to weight gain, hypoglycaemia and dyslipidaemia. Metformin has the potential for addressing these issues as it may (i) reduce insulin dose for a given achieved HbA1c; (ii) promote weight stabilization; (iii) be associated with low rates of hypoglycaemia; and (iv) reduce LDL cholesterol – even on a background of statin therapy. It may also have direct and potentially beneficial cardiovascular effects.

Common carotid artery intima-media thickness (cIMT) is increased in type 1 diabetes. cIMT reliably predicted cardiovascular events in DCCT and has been successfully targeted by metformin in a number of small studies in conditions other than type 1 diabetes.

1.2. STUDY OBJECTIVES

The primary objective of the study is to assess whether three years treatment with metformin 1000mg bd added to titrated insulin therapy (towards target HbA1c 7.0% / 53 mmol/mol) reduces atherosclerosis, as measured by progression of cIMT, in adults with confirmed type 1 diabetes aged 40 years and over and at increased risk for CVD.

Secondary and tertiary objectives are to examine over the same period the effect of metformin on other markers of diabetic micro- and macrovascular complications and intermediate disease-related biomarkers.

1.3. STUDY DESIGN

The study is a randomised, double-blind, placebo controlled trial. Subjects will be randomised to either oral metformin or matching placebo. After recruitment each subject will remain in the study for three years during which they will be followed up by telephone and during clinics (telephone follow-up at weeks 1 and 2 and months 15, 21, 27 and 33 and clinic visits at months 3, 4, 6, 9, 12, 18, 24, 30 and 36). The primary outcome will be measured at baseline and at months 12, 24 and 36.

1.4. SAMPLE SIZE AND POWER

Assuming a mean linear progression of 0.044mm over three years (in the control arm) and a standard deviation (SD) for progression of 0.05mm and to have 90% power at a 5% significance level to detect a difference of at least one third of a SD (0.0167 MM) in three year progression of averaged mean far wall cIMT between treatment arms we will require 200 subjects per treatment group. Allowing for 20-25% drop-outs/discontinuations, 500 subjects, in total, will be recruited.

1.5. STUDY POPULATION

1.5.1. INCLUSION CRITERIA

Subjects with a confirmed diagnosis of type 1 diabetes (i.e. diagnosis below age of 40 years and insulin use within one year of diagnosis) for five years or more, aged 40 or over, with $7.0 \leq$ HbA1c $< 10.0\%$ (53 – 86 mmol/mol) and at risk of CVD [i.e. three or more of the following CVD risk factors: BMI ≥ 27 kg/m^2; current HbA1c $> 8.0\%$ (64 mmol/mol); known CVD / peripheral vascular disease; current smoker; eGFR < 90 ml/min/1.73m^2; confirmed micro- (or macro-) albuminuria (according to local assays and reference ranges); hypertension (BP $\geq$ 140/90mmHg, or established hypertensive treatment); dyslipidaemia; strong family history of CVD; and duration of diabetes > 20 years].

1.5.2. EXCLUSION CRITERIA

eGFR < 45 ml/min/1.73m^2; women of childbearing age not on effective contraception; pregnancy and/or lactation; Acute Coronary Syndrome or Stroke/TIA within the last three months; NYHA stage 3 or 4 heart failure; uncontrolled angina; significant hypoglycaemia unawareness; impaired cognitive function / unable to five informed consent; previous carotid surgery or inability to capture adequate carotid images; gastroparesis; history of lactic acidosis; other contraindications to metformin (hepatic impairment, known sensitivity to metformin, acute illness – dehydration, severe infection, shock, acute cardiac failure, suspected tissue hypoxia; any coexistent life threatening condition including prior diagnosis of cancer within two years; history of alcohol problem or drug abuse; diabetes other than type 1 diabetes; and involvement in a clinical trial involving an investigational medicinal product within the last six months.

1.6. STATISTICAL ANALYSIS PLAN (SAP)

1.6.1. SAP OBJECTIVES

The objective of this SAP is to describe the statistical analyses to be carried out for the final analysis of the REMOVAL study.

1.6.2. GENERAL PRINCIPLES

All study data will be summarised as a whole and by treatment group at each study assessment point. Continuous variables will be summarised by the number of observations, number of missing values, mean, standard deviation (SD), median, quartiles and range. Categorical variables will be summarised by the number of observations, number of missing values and the number and percentage of individuals in each category.

Baseline data will be extracted from the Treatment Visit 1 electronic case report form (eCRF) page where possible; if values are missing, then data from the first screening visit (Run-in Visit 1) will be used.

The primary analysis, and all secondary analyses, will be carried out using all randomised individuals who provide sufficient data to conduct the particular analysis, regardless of their adherence to study protocol. For the primary outcome, a sensitivity analysis will be carried out using a Per-Protocol dataset, consisting of those individuals that completed the study according to protocol.

Graphical presentations will be specified and provided according to the results of the final analysis.

1.6.3. CURRENT PROTOCOL

The current version of the protocol at the time of writing is version 3.0, dated 9th November 2015 and the accompanying NHS GG&C R&D protocol filenote. Future amendments to the protocol will be reviewed for their impact on this SAP, which will be updated only if necessary. If no changes are required to this SAP following future amendments to the protocol, this will be documented as part of the Robertson Centre Change Impact Assessment processes.

1.6.4. DEVIATIONS TO THOSE SPECIFIED IN THE PROTOCOL

The biomarker sample assays will be received after the database lock and therefore are not covered by this SAP, but will be detailed in a future SAP.

1.6.5. ADDITIONAL ANALYSES TO THOSE SPECIFIED IN THE PROTOCOL

There are no planned additional analyses to those specified within the Protocol.

1.6.6. SOFTWARE

Analyses will be conducted using SAS for Windows v9.3 or higher, SPlus for Windows v8.1 and/or R for Windows v2.7.0 or higher.

2. ANALYSIS

2.1. STUDY POPULATIONS AND ANALYSIS SETS

The Screened population will consist of all subjects screened for inclusion into the study.

The Intention To Treat (ITT) population will consist of all subjects randomised, regardless of subsequent participation in the study.

The modified-ITT (mITT) analysis set will include all subjects from the ITT population with data as available (without imputation) for analysis for each individual outcome.

The Per Protocol (PP) population will consist of all members of the ITT population with no major protocol deviations (met inclusion and exclusion criteria and no prohibited medications at randomisation), and who have cIMT available at treatment visits 1, 8, 12 and 16. All identified protocol deviations will be reviewed prior to unblinding the dataset, and classified as major or minor.

The Safety population will consist of all members of the ITT population who received at least one dose of study medication.

For any analyses to be carried out in multiple populations, if the populations are identical (e.g. if all members of the ITT population take at least one dose of study medication, then the ITT and Safety population are the same), then the analysis will only be reported once.

2.2. SUBJECT DISPOSITION

The number of patients in the Screened populations will be reported, as well as the number and percentage in the ITT Population. The reasons for screening exclusion will also be reported.

For those in the ITT population, the number and percentage in each treatment group will be reported.

For those in the ITT population, as a whole and by treatment group, the numbers and percentages in the Safety and PP populations will be reported.

Reasons for exclusion for populations will be summarised overall and by treatment group.

The number of subjects in the ITT population who completed or withdrew will be summarised, overall and by treatment group, along with the reasons for and time to withdrawal.

A by subject listing of all withdrawals from study after randomisation, and the reasons for withdrawal, will be provided.

Eligibility criteria and protocol deviations will also be summarised for the ITT population, overall and by treatment group.

A by subject listing of all protocol deviators will be provided.

2.3. BASELINE CHARACTERISTICS

In the ITT population, as a whole and by treatment group, summaries will be provided of the following baseline characteristics as included in the eCRF:

- Age and sex;
- ethnicity;
- smoking and alcohol habits;
- SBP, DBP and heart rate;
- height, weight, BMI and waist circumference;
- time since diabetes diagnosis;
- medical history – macrovascular, microvascular and other;
- family medical history (CV disease and diabetes);
- insulin regimen, including pump therapy;
- concomitant medications – statins, ACE-inhibitors, ARBs, antiplatelet and beta-blockers;
- treatment satisfaction questionnaire;
- hypoglycaemic events – minor and major;
- blood tests – creatinine, eGFR, capillary glucose (3-day average of each of: before breakfast, before mid-day meal, before evening meal and before bed) and C-peptide;
- average mean far wall cIMT (mm) of the common carotid artery;
- Lipid Assays – LDL cholesterol, HDL cholesterol, Total cholesterol and Trigylcerides.

2.4. STUDY OUTCOMES

2.4.1. PRIMARY OUTCOME

The primary outcome will be the rate of progression of average mean far wall cIMT of the common carotid artery over the course of the study (36 months). In the mITT analysis set, as a whole and by treatment group, summary statistics for cIMT and change from baseline will be provided at baseline, 12, 24 and 36 months. The treatment effect estimate, 95% confidence interval (CI) and p-value will be estimated from a repeated measures regression model, with random intercept and random slopes, with cIMT as the outcome, and predictor variables of treatment, baseline cIMT and time. Time will be measured in years from baseline. A time by treatment interaction term will be included in the model, allowing different slopes for the two treatment groups. Adjustments for baseline covariates age, sex, smoking status, systolic blood pressure, BMI, HbA1c and LDL cholesterol will also be included as fixed effects.

A sensitivity analysis will also be carried out extending the model noted above to further adjust for the ultrasound probe frequency used.

Initial analyses will only include the measurements recorded with values < 1.5mm, in line with the Mannheim consensus, but the analyses will be duplicated to include all measurements regardless of value as a sensitivity analysis (see section 2.4.3).

Average mean far wall cIMT of the common carotid artery will be calculated as the average of the left and right mean measurements at each time point, using the following hierarchy:

1. Use all available data for the mean far wall IMT regardless of data availability at other time points.
2. In instances where any duplicate angles are included (resulting in more than six angles per time point), average these duplicate angles first to result in one single measurement per angle.
3. Average next the angles within each side to result in one single measurement for each of the left and right sides
4. Average the remaining left and right measurements to obtain one result per time point

No imputation of the primary outcome will be performed for the main analysis (the model described above will make use of all analysable data available for a subject). However, if any baseline covariates included as adjustments in the model (systolic blood pressure, BMI, HbA1c and LDL cholesterol) have neither treatment visit 1 data, nor run-in visit 1 data available, multiple imputation will be used, using the other baseline covariates included as adjustments with complete data at baseline (including age, sex and smoking status).

The analysis will be repeated for the PP population.

2.4.2. SECONDARY & TERTIARY OUTCOMES

The secondary outcome measures include:

- DCCT-aligned HbA1c (local assays);
- LDL cholesterol (central assay);
- Albuminuria (as per Protocol Appendix 5.0)
- Estimated glomerular filtration rate (eGFR) (ml/min/1.73m2 by MDRD equation);
- Retinopathy stage (ETDRS) (two or more step progression, concatenated score from baseline);

- Weight (kg);
- Insulin dose per 24 hours per kg (units/kg);
- Endothelial function (Reactive Hyperaemia Index by peripheral arterial tonometry).*

*This measurement is only collected in a subset of centres with available equipment.

Each of the above secondary outcome measures will be analysed separately and the individual results will be reported. The protocol has a pre-defined composite interpretation of all secondary outcomes, where results will be considered as clinically meaningful with the potential to influence clinical practice only in the event that a statistically significant improvement in two or more of the individual secondary outcomes is observed on metformin.

Tertiary outcomes include:

- Frequency of major hypoglycaemia (per patient-year) (Steno Questionnaire);
- Frequency of minor hypoglycaemia (per patient-year) (Steno Questionnaire)
- Treatment satisfaction (Diabetes Treatment Satisfaction Questionnaire);
- Progression of averaged maximal distal IMT of the common carotid artery at 12, 24 and 36 months;
- Occurrence of biochemical Vitamin B12 deficiency, defined as values of vitamin B12 < 150pmol/L reported in the lab data in the eCRF.

All secondary and tertiary outcomes will be analysed using the relevant mITT analysis set.

All continuous secondary and tertiary outcomes will be summarised, overall and by treatment group at each time point; change from baseline will also be summarised. Non-Normally distributed variables will be transformed first. Repeated measures models will be applied to all continuous secondary and tertiary outcomes (except for the tertiary outcome "Progression of averaged maximal distal common carotid artery IMT" which will be analysed as per the primary outcome, using the maximal far wall IMT measurements as the main analysis, calculated as detailed below), assuming a general residual covariance structure, with the change from baseline as the outcome, and predictor variables of treatment, visit number (included as a class variable) and the outcome baseline level where available. The main analyses will test the significance of and provide an estimate of the main effect of treatment in this model. The treatment effect estimate, corresponding 95% confidence interval and p-value will be reported.

As an exploratory analysis to investigate whether the treatment effect varies over time, a visit by treatment interaction term will be added to each model and tested for significance. The p-value for the interaction term will be reported. In addition, the treatment effect, 95% confidence interval and p-value will be reported for each visit.

The main analysis of the averaged maximal distal IMT of the common carotid artery will use solely the maximum far wall cIMT measurements, and unlike the main analysis of the primary outcome, will not restrict to values < 1.5mm. A sensitivity analysis will also be performed using both the maximum near wall and the maximum far wall measurements as detailed in section 2.4.3.

The calculation of the averaged maximal distal IMT at each time point will use the following hierarchy:

1. Use all available data for the maximum far wall IMT regardless of data availability at other time points.

2. In instances where any duplicate angles are included (resulting in more than six angles per time point), average these duplicate angles first to result in one single measurement per angle.
3. Average next the angles within each side to result in one single measurement for each of the left and right sides
4. Average the remaining left and right measurements to obtain one result per time point

The frequency of hypoglycaemia will be analysed using a Negative-Binomial regression model, accounting for over-dispersion using the scale option, with the logarithm of time as an offset variable. The frequency of the minor hypoglycaemia will be further adjusted for the method of collection, with results provided for all collection methods combined. Each method of collection will then be analysed and reported separately. In instances where there has been a change in the method of collection, the method that was most commonly used for each subject will be adjusted for.

New onset microalbuminuria will be analysed in a time to event analysis using a Cox Proportional Hazards Model. The results will be summarised graphically using the Kaplan-Meier method. Similar methods will be used to analyse the first occurrence of biochemical vitamin B12 deficiency.

For the retinopathy outcome, logistic regression analysis will be performed. If the numbers of patients with retinopathy are too small to allow logistic regression analysis to be performed, Fisher's exact test will be used instead.

The regression model for the primary outcome will be extended to determine if glucose- and/or lipid-lowering effects of metformin could potentially explain differential effects on the progression of cIMT. This will be accomplished by including baseline and on-treatment HbA1c and LDL-cholesterol values as time-dependent covariates in the model described above for the primary outcome.

2.4.3. IMPUTATION/SENSITIVITY ANALYSIS

The following sensitivity analyses involving imputation of missing data will be carried out:

- A multiple imputation will be used to analyse the primary outcome, with imputation of cIMT measurements that are missing other than because the patient had died, withdrawn consent or was censored due to end of follow-up. The multiple imputation approach will use the baseline covariates included in the adjusted primary outcome model (age, sex, smoking status, systolic blood pressure, BMI, HbA1c and LDL cholesterol) after imputation of any missing values.
- A similar approach will be used to impute missing retinopathy and endothelial function data.

The following sensitivity analyses will also be included:

- Inclusion of all mean far wall cIMT measurements of the common carotid artery for the calculation of the averaged mean for the primary outcome, regardless of whether the individual measurements are < 1.5mm or not, using the same hierarchy as detailed above in section 2.4.1.
- Inclusion of both the maximum near wall and the maximum far wall IMT measurements in the calculation of the averaged maximal distal IMT of the common carotid artery at 12, 24 and 36 months for the tertiary outcome.

The calculation of the averaged maximal distal IMT at each time point for the sensitivity analysis will use the following hierarchy:

1. Use all available data for the maximum near wall and the maximum far wall IMT regardless of data availability at other time points.
2. Take the single absolute maximum value across both the near wall and the far wall for all available angles and sides, resulting in one maximum value at each time point.

2.4.4. SUBGROUP ANALYSIS

The following subgroup analyses for the main analysis of the primary outcome (ie. the analysis using the measurements available, with values < 1.5mm on the mITT population) will be provided:

- Age (≤ / > median)
- Sex (Males/Females)
- Baseline cIMT tertile
- Existing CV Disease (Yes/No)
- Duration of diabetes (≤ / > median)
- Baseline HbA1c (≤ / > median)
- Baseline BMI (≤ / > median)
- Smoker status (Never / Ever)
- LDL cholesterol (≤ / > median)
- SBP (≤ / > median)
- Pump User (Yes/No)

2.4.5. OTHER SAFETY OUTCOMES

2.4.5.1. STUDY TREATMENT

The number of doses of study medication that were taken throughout the entire study (ie. from the date of randomisation to the last treatment visit attended) will be calculated as the total number of doses supplied – the total number of doses returned and will be summarised as a whole and by treatment group.

The number of subjects down-titrating and permanently discontinuing study medication during the study will be summarised overall and by treatment group, along with the reasons for down-titration and discontinuations. Time to permanent discontinuation will also be summarised.

Differences between the two treatment groups in terms of permanent withdrawals of study medication will be tested using Chi-squared tests. No other formal statistical testing will be applied.

2.4.5.2. ADVERSE EVENTS OF MEDICAL INTEREST

Adverse events of medical interest have been defined as:

- Gastrointestinal
    - diarrhoea
    - abdominal pain
    - nausea and vomiting
    - constipation
    - loss of appetite

- Any revascularisation:
    - coronary (angioplasty/stent/CABG)
    - carotid (endarterectomy)
    - peripheral (angioplasty/stent/surgical)
- Foot (left/right)
    - Ulceration
    - amputation – digit, below knee, above knee
    - ulcer debridement
- Eye (left/right)
    - laser treatment
    - vitrectomy
    - cataract surgery
    - vitreous haemorrhage
    - retinal vein or artery occlusion
    - loss of vision in one eye
- Neurological
    - Headache
- Metabolic
    - reduction in eGFR of > 25%
- Other
    - Hypersensitivity reaction to metformin
    - Overdose

The total number (and subjects experiencing at least one) adverse events of medical interest will be summarised. The type, duration, outcome, relationship to study drug and seriousness will also be summarised for all adverse events of medical interest.

A by subject listing of adverse events of medical interest will also be provided.

No formal statistical testing will be applied.

2.4.5.3. SERIOUS ADVERSE EVENTS

The number of serious adverse events will be reported overall and by treatment group for any event, and by classifications of expectedness, relationship to study medication, severity and outcome.

The number and percentage of patients experiencing at least one adverse event will be reported overall and by treatment group for all events, and for events classified by MedDRA System Organ Class and Preferred Term.

A by subject listing of serious adverse events will also be provided.

Listings of all deaths will also be provided.

2.4.5.4. EVENTS OF PARTICULAR INTEREST

Events of particular interest have been defined as:

- Lactic acidosis
- ALT > 3 times upper limit of normal
- eGFR < 30 ml/min/1.73m^2
- Major hypoglycaemic events

- Renal dysfunction (eGFR < 45 ml/min/17.3m^2 or reduction in eGFR > 25%)
- LFTs > 2.5 times upper limit of normal
- Hb < 10.0 g/dL and fall > 1.5 g/dL from baseline
- Occurrence of clinically relevant Vitamin B12 deficiency, defined as values < 110pmol/L reported in the lab data recorded in the eCRF
- Acute coronary syndrome

These events will be grouped according to whether they warrant discontinuation of study medication or not. The first three events above (lactic acidosis, ALT and eGFR < 30 ml/min/1.73m^2) all warrant discontinuation of study medication.

The number and percentage of events of particular interest will be reported. For the events warranting discontinuation of study medication, the number of events occurring whilst taking study medication will be summarised along with these number of events that resulted in discontinuation.

The number and percentage of subjects experiencing at least one event for any and each specific event will also be summarised.

No formal statistical testing will be applied.

2.4.5.5. VITAL SIGNS

SBP, DBP and heart rate will be summarised as a whole, by treatment and by visit. Changes from baseline will also be summarised. Formal statistical comparisons of the changes will be made between the treatment groups using a baseline adjusted ANCOVA.

2.4.5.6. BLOOD TESTS

To identify any potential safety signal, blood tests not included as secondary or tertiary outcomes will be summarised as overall, by treatment group and by visit. Changes from baseline will also be summarised. Formal statistical comparisons of the changes shall be made between the treatment groups using a baseline adjusted ANCOVA. Any laboratory results with evidence of non-Normality of their distribution will be transformed appropriately prior to analysis.

The number of subjects with values of clinical significance will be summarised at each visit for each blood test as appropriate.

3. DOCUMENT HISTORY

This is version 1.1 of the Statistical Analysis Plan (SAP) for the REMOVAL study. The following updates were made to version 1.0:

1. Include pre-specified subgroup analysis for the main primary outcome analysis in section 2.4.4.

2. Slight clarification to the wording of the primary outcome model.

4. TABLES, FIGURES AND LISTINGS

A dummy report will be provided for external review prior to study lock.