Statistical Analysis Plan

DIAMOND
Dual antiplatelet therapy to Inhibit coronary Atherosclerosis and Myocardial injury in patients with Necrotic high-risk coronary plaque Disease

Version: 1.0

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Signatures

Chief Investigator Prof David Newby	Signature	Date
Trial Statistician Prof Steff Lewis	Signature	Date

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2 List of Abbreviations

CI	Confidence Interval
CONSORT	Consolidated Standards of Reporting Trials
DIAMOND	Trial acronym for 'Dual antiplatelet therapy to Inhibit coronary Atherosclerosis and Myocardial injury in patients with Necrotic high-risk coronary plaque Disease'
DMC	Data Monitoring Committee
ECTU	Edinburgh Clinical Trials Unit
hsTnI	High Sensitivity Cardiac Troponin I
N	Number of patients with an observation
SAP	Statistical Analysis Plan
SD	Standard Deviation
SOP	Standard Operating Procedure

3 Introduction

This document details the criteria to be used for the definition of the analysis populations and the statistical methodology for analysis for the DIAMOND trial, a randomised trial of Ticagrelor to reduce markers of myocardial injury in patients with stable coronary heart disease and high-risk coronary atheroma. This document has been compiled according to the Edinburgh Clinical Trials Unit (ECTU) standard operating procedure (SOP) "Statistical Analysis Plans" and has been written based on information contained in the study protocol version 5.0, dated 31 January 2017.

DIAMOND is a single centre, randomised, parallel-group, double blind, placebo-controlled trial. Randomisation is at the individual level with a 1:1 allocation ratio, and is minimised on age, gender, baseline plasma troponin concentration, presence of coronary 18F-fluoride uptake (as detailed in the Randomisation System Description document). Patients are randomised to either Ticagrelor or matched placebo tablets. The aim is to recruit 220 patients, with a view to having 55 per group in the group with increased coronary 18F uptake.

4 Statistical Methods Section from the Protocol

9.2.1 Description of Analysis Sets

The primary analysis will be to determine whether ticagrelor will reduce plasma high-sensitivity troponin concentrations compared with placebo in patients with stable coronary heart disease and increased coronary 18F-fluoride uptake. For this primary analysis, we will exclude any patient who does not have a blood sample for estimation of the one-month plasma troponin concentration or whose compliance is deemed inadequate (as estimated from pill counts). The required level of medication compliance will be described in the statistical analysis plan.

9.2.2 Methods of Statistical Analysis

For the primary analysis, the change in plasma high-sensitivity troponin concentration from baseline to 30 days will be compared between the two treatment groups (ticagrelor and placebo) using linear regression, adjusting for the minimisation variables, in patients with coronary 18F-fluoride uptake. Prior to analysis, tests for normality will be undertaken and, where data are skewed, logarithmic transformation will be considered prior to analysis. Effect sizes and 95% confidence intervals will be calculated. Similar analyses will be performed for the assessment of troponin at 30 days in patients without coronary 18F-fluoride uptake, and in the study population as a whole; for troponin at 1 year; and for calcium score and plaque volume at 1 year at the site of baseline coronary 18F-fluoride uptake.

Statistical analysis will be performed using SAS. A two-sided P<0・05 will be taken as statistically significant. A full statistical analysis plan will be documented prior to data base lock. This will be overseen by the trial statistician in the Edinburgh Clinical Trials Unit.

5 Description of analysis datasets

The analysis datasets are detailed below. All analyses will be performed on the per protocol population unless otherwise specified.

Per protocol we will exclude any patient who does not have a blood sample for estimation of the plasma troponin concentration or whose compliance is deemed inadequate (as estimated from pill counts) at the 30 day visit. Inadequate compliance is defined as <80% using the formula in Section 7.3. Patients will be analysed according to treatment received.

Safety the Ticagrelor group will include all subjects who received any Ticagrelor study medication, regardless of the group they were originally allocated to. The placebo group will include all patients who received some study medication, but where all of this was placebo, regardless of the group they were originally allocated to. The group who were randomised but did not receive any medication at all will be reported separately.

6 Overall statistical principles

All applicable statistical tests will be 2-sided and will be performed using a 5% significance level. Two-sided 95% confidence intervals (CIs) will be presented. All analyses are seeking to show a difference, rather than equivalence or non-inferiority.

In general terms, categorical data will be presented using counts and percentages, whilst continuous variables will be presented using the mean, standard deviation (SD), median, lower quartile, upper quartile, minimum, maximum, and number of patients with an observation (N). Data will be split by time point where applicable.

Where there is missing data for an outcome variable, in the first instance, those records will be removed from any formal statistical analysis relating to that outcome variable, unless otherwise specified. In tabulations, numbers of missing observations will be provided, but percentages will not include them.

The main statistical analyses for primary and secondary outcomes will be adjusted for the minimisation variables: age, sex, baseline plasma troponin concentration unless doing so causes problems due to collinearity. Baseline troponin will be log transformed. Age and log baseline troponin will be adjusted for as a linear term, unless there is strong evidence of a departure from linearity for groups of related analyses. A group of related analyses is, e.g., the relationship between age and troponin, for all troponin analyses. This decision will be taken without looking at the unblinded treatment data, and taking care not to over-interpret smaller p-values in larger datasets, which may reflect the number of patients in the analysis rather than the magnitude of the effect. If a linear model is not adequate, then a fractional polynomial with two terms will be used. In general, analyses will be presented in subgroups of patients based on the presence of coronary 18F-fluoride uptake, and so this will not be adjusted for unless otherwise specified. The precise details of the minimisation variables are in the DIAMOND randomisation description document. Results of unadjusted statistical analyses will also be presented, although baseline measurements of outcome variables will always be adjusted for.

Distributional assumptions underlying the statistical analyses will be assessed by visual inspection of residual plots. Normality will be examined by normal probability plots. If the distributional assumptions of the parametric approach are not satisfied, further data transformation (to alleviate substantial skewness (i.e. normalizing) or to stabilise the variance), or other suitable methods will be considered, taking into account the level of normality in similar analyses. If analyses do not follow this SAP for any reason, an updated SAP will be written together with the reasoning for the action taken. This will also be documented in the statistical report.

All analyses and data manipulations will be carried out using SAS [1].

7 List of analyses

Note: Throughout this document, "study entry" is defined as the randomisation date.

7.1 Recruitment and retention, and definition of analysis populations

No formal statistical testing will be performed. The date of first and last patient randomised, and the number of patients randomised will be reported.

A CONSORT flow chart will be provided by the statistics team. Reasons for non-inclusion in the study (prior to randomisation) will be categorised. The number of patients discontinued early from the study will be summarised by reason for withdrawal and treatment. The number of patients in each treatment group will be specified for per protocol, and safety populations, with sufficient detail to explain why patients are excluded from each population.

The following will be presented:

- Number and percentage of patients who were ineligible for inclusion in trial (if any – it should not have been possible to randomise such patients).
- Numbers of patients who were randomised but never treated.
- Numbers of patients who received the opposite treatment to that allocated (if there are any)

- A listing of patients where the blind was broken early will be presented, including details of allocation, timing and reason for breaking blind, and outcome.

7.2 Baseline Balance

No formal statistical testing will be performed. These will be produced for the per protocol population, and all patients randomised. The following will be presented and summarised by allocated treatment:

Demographics and Minimisation variables

- Age, as categorised (<65, ≥65) in the minimisation algorithm (this is also the cut-off required for the EUDRACT portal). Age will also be presented as a continuous variable.
- Sex
- BMI
- Baseline plasma troponin concentration as categorised in the minimisation algorithm. This will also be presented as a continuous variable.
- The presence of coronary 18F-fluoride uptake as categorised in the minimisation algorithm.

Other baseline variables:

- Smoking Status
    - Current smoker
        - Average cigarettes smoking per day
    - Smoking history
        - Stopped smoking (<1/≥ 1 year)

- Cardiac History
    - Stable angina
    - History of acute coronary syndrome
    - Days between randomisation and date of most recent ACS
    - Previous PCI
    - Days between randomisation and date of most recent PCI
    - Previous CABG
    - Days between randomisation and date of most recent CABG
    - History of cardiac failure
    - Hypertension
    - High cholesterol/on cholesterol lowering treatment
    - History of atrial fibrillation (paroxysmal or persistent)
    - Type 1 diabetes
    - Type 2 diabetes
    - Treatment for diabetes either Type 1 or Type 2
    - Peripheral vascular disease
    - Previous stroke or TIA
    - Rheumatoid arthritis
    - Family history of coronary heart disease/stroke
    - ASSIGN score[1]

- Concomitant Medication
    - Statin

[1] See ASSIGN score betas.docx for details

    - Beta-blocker
    - ACE inhibitor/angiotensin receptor antagonist

- Physical Examination
    - Heart rate
    - Systolic blood pressure (mmHg)
    - Diastolic blood pressure (mmHg)
- Blood Results at baseline visit
    - Haemoglobin (g/L)
    - Urea (mmol/L)
    - Creatinine (umol/L)
    - WBC ($x10^9$/L)
    - Sodium (mmol/L)
    - eGFR (mL/min) – This will be reported as 31-60 / >60
    - Platelets ($x10^9$/L)
    - Potassium (mmol/L)
    - Total cholesterol (mmol/L)
    - LDL (mmol/L)
    - HDL (mmol/L)
    - Triglyceride (mmol/L)
    - Random glucose (mmol/L)
    - HbA1c (mmol/mol)
    - hsTnl (ng/L)

7.3 Compliance

The number of patients who received <80%, 80-90%, >90% of their study medication, using pill counts, and the following formula.

$$\boldsymbol{Compliance}\ (\%) = \frac{\boldsymbol{Pill\ Taken}}{\boldsymbol{Days}\ \times \mathbf{2}} \times \mathbf{100}$$

Note: Compliance will be calculated based on pill counts at visits. Days will be calculated as days from previous visit date to current visit date. Expected pill taken at visits will be days x2. If participant forgot to bring her/his pill boxes at 30 days visit but bring those at 3 months visits, compliance at 3 months will be used for 30 days compliance.

Reasons for compliance less than 80% will be provided if available.

7.4 Primary outcome: Plasma high sensitivity cardiac troponin I (hsTnI) concentration at 30 days in patients with coronary 18F-fluoride uptake

As Troponin I at 30 days is skewed, it will be log transformed prior to analysis. A value of 0.5ng/l will be used for values below the limit of detection. When reporting summary statistics and results of analyses, the logged and unlogged results will be reported, i.e. if we report a mean of the logged values, we will back-transform this mean to the original unlogged scale for ease of interpretation.

Troponin I at 30 days, in patients with coronary 18F-fluoride uptake at baseline, will be modelled in a linear regression model, adjusting for age, sex, and log baseline troponin I. Unadjusted analyses (still adjusted for log baseline troponin) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 30 days and the change between baseline and 30 days. Numbers with missing data

values at baseline and 30 days will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

7.5 Secondary outcomes

7.5.1 Plasma hsTnI concentrations at 30 days in patients without coronary 18F-fluoride uptake.

As Troponin I at 30 days is skewed, it will be log transformed prior to analysis. A value of 0.5ng/l will be used for values below the limit of detection. When reporting summary statistics and results of analyses, the logged and unlogged results will be reported, i.e. if we report a mean of the logged values, we will back-transform this mean to the original unlogged scale for ease of interpretation.

Troponin I at 30 days, in patients without coronary 18F-fluoride uptake, will be modelled in a linear regression model, adjusted for age, sex, and log baseline troponin I. Unadjusted analyses (still adjusted for log baseline troponin) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 30 days and the change between baseline and 30 days. Numbers with missing data values at baseline and 30 days will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

7.5.2 High sensitivity cardiac troponin I (hsTnI) concentration at 30 days in total study population.

As Troponin I at 30 days is skewed, it will be log transformed prior to analysis. A value of 0.5ng/l will be used for values below the limit of detection. When reporting summary statistics and results of analyses, the logged and unlogged results will be reported, i.e. if we report a mean of the logged values, we will back-transform this mean to the original unlogged scale for ease of interpretation.

Troponin I, in all patients, will be modelled in a linear regression model, adjusting for age, sex, and log baseline troponin I. Unadjusted analyses (still adjusted for log baseline troponin) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 30 days and the change between baseline and 30 days. Numbers with missing data values at baseline and 30 days will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

A term for coronary 18F-fluoride uptake (Y/N) will be added into the model for all patients, and the p-value for the interaction between coronary 18F-fluoride uptake and the treatment effect will be presented.

7.5.3 Plasma hsTnI concentrations over 1 year.

This will be done in three groups: patients with coronary 18F-fluoride uptake, patients without, and in all patients. We will calculate the area under the curve of the plasma hsTnI measurements from 30 days to 1 year, using nominal time points (30 days, 3 months, 6 months, 9 months, 1 year; not visit numbers) rather than actual dates. A value of 0.5ng/l will be used for troponin values below the limit of detection. Where there are missing values between two others, it will be assumed that the missing value lies on the straight line between the measured values either side. Where there are missing values

at the right hand end, the patient will be excluded. This will be modelled in a linear regression model, adjusted for age, sex and log baseline troponin. Unadjusted analyses (still adjusted for log baseline troponin) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for the area under the curve.

A term for coronary 18F-fluoride uptake (Y/N) will be added into the model for all patients, and the p-value for the interaction between coronary 18F-fluoride uptake and the treatment effect will be presented.

7.5.4 Calcium score at 1 year.

This will be done using 4 separate analyses. The method of measurement will be mass.

Plaque calcium score at 1 year at the site of baseline coronary 18F-fluoride uptake in patients with coronary 18F-fluoride uptake will be modelled in a linear regression model, adjusted for age, sex, log baseline troponin I and baseline plaque calcium score. Unadjusted analyses (still adjusted for baseline plaque calcium score) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 1 year and the change between baseline and 1 year. Numbers with missing data values at baseline and 1 year will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

Plaque calcium score at 1 year at the site of referent plaque (a plaque with no baseline coronary 18F-fluoride uptake) in patients with coronary 18F-fluoride uptake will be modelled in a linear regression model, adjusted for age, sex, log baseline troponin I and baseline plaque calcium score. Unadjusted analyses (still adjusted for baseline plaque calcium score) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 1 year and the change between baseline and 1 year. Numbers with missing data values at baseline and 1 year will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

Total calcium score at 1 year in patients with 18F-fluoride uptake will be modelled in a linear regression model, adjusted for age, sex, log baseline troponin I and baseline total calcium score. Unadjusted analyses (still adjusted for baseline total calcium score) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 1 year and the change between baseline and 1 year. Numbers with missing data values at baseline and 1 year will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

Total calcium score at 1 year in patients without 18F-fluoride uptake will be modelled in a linear regression model, adjusted for age, sex, log baseline troponin I and baseline total calcium score. Unadjusted analyses (still adjusted for baseline total calcium score) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 1 year and the change between baseline and 1 year. Numbers with missing data values at baseline and 1 year will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

7.5.5 Plaque volume at 1 year

Plaque volume (mm^3) as originally intended is not available. Instead, we will use plaque calcium volume (mm^3). This will be strongly correlated with plaque calcium mass which is being used as the plaque calcium score.

This will be assessed using 2 separate analyses.

Plaque volume at 1 year at the site of baseline coronary 18F-fluoride uptake, in patients with coronary 18F-fluoride uptake in patients with coronary 18F-fluoride uptake, will be modelled in a linear regression model, adjusted for age, sex, log baseline troponin I and baseline plaque volume. Unadjusted analyses (still adjusted for baseline plaque volume) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 1 year and the change between baseline and 1 year. Numbers with missing data values at baseline and 1 year will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

Plaque volume at 1 year at the site of referent plaque (a plaque with no baseline coronary 18F-fluoride uptake) in patients with coronary 18F-fluoride uptake will be modelled in a linear regression model, adjusted for age, sex, log baseline troponin I and baseline plaque volume. Unadjusted analyses (still adjusted for baseline plaque volume) will also be presented. The difference in means between the treatment groups will be presented, along with a 95% confidence interval and p-value. Descriptive statistics will be presented for baseline, 1 year and the change between baseline and 1 year. Numbers with missing data values at baseline and 1 year will be reported, and descriptive statistics will be presented for the group with no missing data at either time point.

7.5.6 Reproducibility of 18F-fluoride uptake detected on PET imaging at 1 week.

Within the group entered into this sub-study, descriptive statistics will be presented. This will include the proportion of PET positive patients at 1 week, among those who were PET positive at baseline; and the proportion of PET negative patients at 1 week, among those who were PET negative at baseline. Crude overall agreement will be presented, along with a kappa statistic (with the understanding that the margins were fixed in the design of this study).

7.5.7 Natural history of 18F-fluoride uptake over 1 year.

Tables will be produced to summarise the groups of patients measured at baseline and 3 months, baseline and 6 months, and baseline and 1 year. Descriptive statistics will show the proportion of PET positive/negative patients at the later time point, among those who were PET positive at baseline; and the proportion of PET positive/negative patients at the later time point among those who were PET negative at baseline.

7.6 Safety analyses

These analyses will use the safety population. Adverse Events (AEs) will be summarised by treatment received and by seriousness, causality, expectedness, and severity of event. If available, events will be reported by MedDRA preferred terms and grouped by System Organ Class. If a patient has multiple reports of the same event, only the event reported at the highest grade will be included. Each patient will be counted only once in a summary of events by System Organ Class. No formal statistical analysis will be

performed. A listing will be produced detailing each event, and what happened to the patient subsequently.

7.6.1 Bleeding

These analyses will use the safety population. To determine whether the addition of ticagrelor to standard optimal medical therapy is safe and well tolerated in patients with stable coronary heart disease on optimal medical therapy.

All bleeding events will be summarised by treatment received and categorised according to the 2009 PLATO criteria [Wallentin et al, 2009]. No formal statistical analysis will be performed. Time to each bleeding event from study entry and description of the bleeding event will be provided.

PLATO Bleeding Classification:

Major Life-Threatening

- Fatal
- Intracranial
- Intrapericardial with cardiac tamponade
- Resulting in hypovolemic shock or severe hypotension that requires pressors or surgery
- Clinically overt or apparent bleeding associated with decrease in >5 g/dL
- Requiring transfusion of ≥4 U whole blood or PRBCs

Other Major

- Significantly disabling (eg intraocular with permanent vision loss)
- Associated drop in haemoglobin of 3 to 5 g/dL
- Requiring transfusion of 2 to 3 U whole blood or PRBCs

Minor

- Requiring medical intervention to stop or treat bleeding (eg. epistaxis requiring visit to medical facility for packing)

Minimal

- All others (eg. bruising, bleeding gums, oozing from injection sites) not requiring intervention or treatment

7.7 Subgroup analyses

There are no planned subgroup analyses, other than those specified elsewhere in this Statistical Analysis Plan.

7.8 Exploratory analyses

The analyses for the exploratory objectives in the study protocol will not form part of the main statistical report, and will not be performed by the statistical team as part of the main trial analyses

8 Validation

The following will be done by a second statistician:

1. Separate programming and checking of primary outcome results and conclusions.

2. The statistical report will be read and sense-checked.

9 Data sharing

A file, or set of files, containing the final data will be prepared, along with a data dictionary. Files that contain the data that was used in the final analysis will be anonymised according to Edinburgh Clinical Trials Unit approved processes. The meta-data for the DIAMOND study (protocol, data collection forms, data dictionary) will be uploaded to Edinburgh DataShare, and the anonymised datasets will be available on request via this system, following relevant approvals and legal agreements.

10 References

1. SAS® Institute Inc. SAS for Windows. SAS Institute Inc.: Cary, NC, U.S.A