Statistical Analysis Plan

TRIAL FULL TITLE	**Impact of EMpagliflozin on cardiac function and biomarkers of heart failure in patients with acute MYocardial infarction (EMMY-Trial)**
SAP VERSION	1.10
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SAP Signatures

I give my approval for the attached SAP entitled Impact of the Empagliflozin on cardiac function and biomarkers of heart failure in patients with acute Myocardial infarction, Version 1.10.

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Abbreviations and Definitions

AE	Adverse Event
CRF	Case Report Form
IMP	Investigational Medical Product
SAP	Statistical Analysis Plan

1 Introduction

1.1 Preface

Type 2 diabetes mellitus (T2DM) is associated with an about two to three-fold increased risk of cardiovascular events as compared with subjects without diabetes. The United Kingdom Prospective Study (UKPDS) was the first to demonstrate a reduction of macrovascular and microvascular complications in subjects with T2DM by an intensified glucose lowering treatment regimen with either insulin/sulfonylurea or metformin after long term follow up. After Rosiglitazone was removed from the European market due to ongoing uncertainty about the cardiovascular safety of this drug, the Federal Drug Administration (FDA) and the European Medicine Agency (EMEA) have issued new guidelines for new antihyperglycaemic drugs to be licensed, requiring a thorough assessment of cardiovascular safety and in most cases the performance of a large cardiovascular outcome trial. Sodium-dependent glucose cotransporter 2 (SGLT-2) is mainly expressed in human kidneys and small intestinal cells. In the proximal tubule of the nephron SGLT-2 is responsible for the reabsorption of approximately 90% of the filtrated glucose. Inhibition of SGLT-2 was shown to increase renal glucose excretion and to lower plasma glucose. Subsequently, a number of SGLT-2 inhibitors were developed and are currently approved for the treatment of T2DM.

Zinman B. et al published the results of the EMPA-REG-OUTCOME trial where the cardiovascular impact of a glucose lowering regimen including Empagliflozin as compared to usual glucose control without an SGLT-2 inhibitor was investigated. The trial demonstrated an unexpected reduction in the primary composite endpoint, comprising cardiovascular death, non-fatal myocardial infarction and non-fatal stroke. The reduction was mainly driven by a 38% relative risk reduction in cardiovascular deaths; moreover they demonstrated an impressive 35% relative risk reduction in the secondary endpoint hospitalization for heart failure. Of note, the beneficial effects observed in the Empagliflozin group seem to occur very rapidly after commencing the treatment, as shown by the early separation of the Kaplan-Meier curves. However, the mechanisms responsible for this finding remain unclear. Diuretic effects with subsequent impact on hemodynamics or potential cardioprotective effects of glucagon, whose levels rise under the treatment with SGLT-2 inhibitors and the resulting rise in ketone bodies or a small increase in haematocrit have been suggested as potential mechanisms.

We hypothesize that Empagliflozin beneficially influences the increased cardiac pre- and afterload as well as reducing the increased sodium reabsorption following myocardial damage with subsequent reduced cardiac output. Whether or not Empagliflozin has direct effects on myocardial remodelling has yet to be elucidated, yet. Moreover, recent animal data suggest that in failing heart ketone bodies seems to be an increasingly important as energy source. Empagliflozin has been shown to increase levels of ketone bodies, which might has a beneficial impact on the metabolism and energy supply of the failing heart. In addition, although SGLT-2 is not expressed in human myocardium, direct, non-SGLT-2 mediated effects on the heart muscle cannot be excluded, given that the molecule is small in size.

1.2 Scope of the analyses

These analyses will assess the efficacy and safety of Empagliflozin in improving cardiac function and biomarkers of heart failure in patients with acute myocardial infarction and will be included in the clinical study report.

2 Study Objectives and Endpoints

2.1 Study Objectives

The aim of this study is to investigate the impact of Empagliflozin on cardiac function and heart failure biomarkers in patients with acute myocardial infarction.

2.1.1 Primary objectives

The primary objective is to investigate the impact of Empagliflozin on NT-proBNP (a heart failure biomarker) in patients with myocardial infarction within 6 months after randomization.

2.1.2 Secondary objectives

Secondary objectives are as follows:
- Short term changes in NT-proBNP levels
- Short term and intermediate term changes in echocardiography parameters
- Change in levels of ketone body concentrations
- Change in HbA1c levels

- Change in body weight
- Number of hospital re-admissions due to heart failure or other causes
- Duration of hospital stay
- All-cause mortality

2.1.3 Safety objectives

- All-cause mortality
- Number of serious adverse events
- Number of severe hypoglycaemic events (i.e. requiring third party assistance)
- Number of genital infections
- Number of ketoacidotic events
- Changes in liver function parameters (AST, ALT, GGT)
- Changes in renal function parameters (creatinine, eGFR)

2.2 Endpoints

2.2.1 Primary endpoint

The primary endpoint is the change in NT-proBNP levels from randomization to week 26.

2.2.2 Secondary endpoints

Secondary endpoints include the following:

- Change of NT-proBNP between treatment groups from randomization to week 6
- Change of ejection fraction (EF) between treatment groups from randomization to week 26
- Change of ejection fraction between treatment groups from randomization to week 6
- Change of left ventricular diastolic function (LVDF) from randomization to week 26
- Change of LVDF from randomization to week 6
- Change of HbA1c between treatment groups from randomization to week 26 (in subjects with known diabetes mellitus Type 2 at randomization)
- Change of body weight between treatment groups from randomization to week 6
- Change of body weight between treatment groups from randomization to week 26
- Change of blood beta-hydroxybutyrate levels between the treatment groups from randomization to week 6

- Change of blood beta-hydroxybutyrate levels between the treatment groups from randomization to week 26
- Difference in hospital re-admissions rate due to heart failure after discharge (of the myocardial infarction event leading to trial inclusion) between the treatment groups
- The difference in the number of hospital re-admissions for any cause between the treatment groups
- The difference in the duration of hospital stay between the treatment groups after randomization

3 Study Methods

3.1 General Study Design and Plan

This is a two arms randomized controlled multi-centre, parallel-group, double-blinded trial. The control group is a placebo and the treated group is Empagliflozin. The aim of the trial is to show superiority of Empagliflozin over placebo. Randomization was carried out by stratifying according to site, T2DM status and sex.

3.2 Inclusion-Exclusion Criteria and General Study Population

3.2.1 Inclusion criteria

1. Myocardial infarction with evidence of significant myocardial necrosis defined as a rise in creatinine kinase >800 U/l and a troponin T-level (or troponin I-level) >10x ULN. In addition, at least 1 of the following criteria must be the met:
   - Symptoms of ischemia.
   - ECG changes indicative of new ischemia (new ST-T changes or new LBBB).
   - Imaging evidence of new regional wall motion abnormality.
2. 18 – 80 years of age.
3. Informed consent has to be given in written form.
4. eGFR > 45 ml/min/1.73m2.
5. Blood pressure before first drug dosing: systolic blood pressure>110mmHg.
6. Blood pressure before first drug dosing: diastolic blood pressure>70mmHg.
7. First intake of study medication ≤72h after myocardial infarction after performance of a

coronary angiography.

3.2.2 Exclusion criteria

1. Any other form of diabetes mellitus than type 2 diabetes mellitus, history of diabetic ketoacidosis.
2. Blood pH < 7,32.
3. Known allergy to SGLT-2 inhibitors.
4. Haemodynamic instability as defined by intravenous administration of catecholamine, calciumsensitizers or phosphodiesterase inhibitors.
5. >1 episode of severe hypoglycaemia within the last 6 months under treatment with insulin or sulfonylurea.
6. Females of child bearing potential without adequate contraceptive methods (i.e. sterilisation, intrauterine device, vasectomised partner; or medical history of hysterectomy).
7. Acute symptomatic urinary tract infection (UTI) or genital infection.
8. Patients currently being treated with any SGLT-2 inhibitor (dapagliflozin, canagliflozin, empagliflozin) or having received treatment with any SGLT-2 inhibitor within the 4 weeks prior to the screening visit.

3.3 Randomization and Blinding

After screening, all eligible patients for the trial were randomized into one of the two arms of the study via Randomiser Software (Institute for Medical Informatics, Statistics and Documentation, Medical University of Graz, http://www.randomizer.at), which is programmed with a randomisation schedule provided by an independent statistician. The randomization was stratified by site, T2DM and by sex. Only the subject number and subject initials were recorded in the case report form (CRF). The investigator maintains a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

Blinding of the medication was done by the pharmacy at the University Hospital in Salzburg, Austria. The pharmacy received the randomization list from the Institute for Medical

Informatics, Statistics and Documentation at the Medical University of Graz, Austria by the independent statistician. The pharmacy distributed then the blinded medication to the study sites. The independent statistician and the pharmacists are otherwise not involved in the EMMY trial.

3.4 Study Assessments

Data are collected according to the following schedule:

	Visit 1 (V1) Screening / Baseline	Visit 2 (V2) (week 6 ± 2)	Visit 3 (V3) (week 12 ± 2)	Visit 4 (V4) (Week 26 ± 2)	Visit 5 (V5) (4 weeks ±1w after V4)
Informed Consent	X				
Inclusion/exclusion criteria	X				
Randomisation	X				
Demography, medical history	X				
Concomitant medication	X	X	X	X	
Vital signs	X	X	X	X	
Height	X				
Weight	X	X	X	X	
Physical examination	X	X		X	
ECG	X				
Cardiac ultrasound	X	X		X	
Biobank sampling	X	X		X	
nt-proBNP (local)	X	X	X	X	
Blood sampling	X	X	X	X	
Liver function parameters (AST, ALT, GGT)	X	X	X	X	
Renal function parameters (creatinine, eGFR)	X	X	X	X	
Adverse Events		X	X	X	X
Dispense medication	X				
Drug accountability				X	
Safety assessment before discharge	X				

4 Sample Size

Previous data showed that NT-proBNP levels decrease by about 50% within 6 months after acute myocardial infarction. To detect a relative 40% larger reduction in NT-proBNP levels

in the Empagliflozin group as compared to the placebo group with a power of 80% and an alpha-level of 0.05%, and assuming a correlation for NT-proBNP levels of 0.85, a sample size of 216 subjects in each group is necessary. To account for a dropout rate of about 10% each group will consist of 238 patients. A detailed document regarding power and sample size calculation is annexed to this SAP.

5 General Analysis Considerations

5.1 Timing of Analyses

The trial will end after the last subject has completed the follow-up telephone assessment (Study visit 5). All patients will be reviewed by a clinician at their last study visit in order to arrange return to appropriate routine clinical care pathways. The data analysis will be performed on data transferred to the file data_emmy_YYMMDD, having been documented as meeting the cleaning and approval requirements and after the finalization and approval of this SAP document.

5.2 Analysis Populations

5.2.1 Intention to Treat Population (ITT)

The ITT population includes all patients who were randomized and received at least one dose of the study medication (i.e. Empagliflozin or Placebo). The ITT population will be used for the primary efficacy analysis.

5.2.2 Per Protocol Population (PP)

The PP population will include all patients in the ITT population who completed week 26 evaluation without any major protocol violations. More specifically, the PP population will include all patients in the ITT population excluding:

a- Patients not fulfilling inclusion/exclusion criteria at baseline, which was identified after randomization and first treatment dosing but who remained in the trial as no safety concern was considered by the principal investigators.
b- Patients who did not complete week 26 evaluation
c- Patients who took study medication for less than 75% of the study duration (study

duration = 26 +/- 2 weeks)

The list of potentially important protocol deviations will be identified, reviewed, and finalized prior to the release of the database.

5.2.3 Safety Population

All patients who were randomized and received at least one confirmed dose of study medication (i.e. Empagliflozin or Placebo) and provided any post-baseline safety information.

5.3 Covariates and Subgroups

5.3.1 Data transformation

Data might be transformed on a logarithmic scale in case of non-normality distribution.

5.3.2 Covariates adjustment

All analyses, including the primary analysis, will be adjusted for the variables used when stratifying the randomization. These variables are Sex and T2DM status. We will not be able to adjust for the variable centre as this might introduce too many categories. Analysis will also be adjusted for baseline levels of the dependent variables.

5.3.3 Subgroup analysis

Subgroup analyses will focus on the evidence for a difference in treatment effects using the interaction term. Subgroup-specific treatment effect estimates will be presented only if the interaction effect is judged to be statistically significant. Interaction effects will be investigated for the following variables:

- T2DM
- Previous cardiovascular events (myocardial infarction or stroke)
- Previous myocardial infarction
- History of heart failure
- Age
- Sex

- Renal function
- LV-EF
- Baseline levels of the dependent variable. Here the interaction will be investigated using the original dependent variable and/or by categorizing this dependent variable into a clinically relevant number of categories. Percentiles will be used if there are no prior clinical cut offs.

Forest plots will be produced to illustrate subgroup effects and interactions. If needed, three way interactions will also be included (e.g. to investigate the interaction between treatment, NT-proBNP and CK).

5.3.4 Multi-centre Studies

Because this study was not designed to detect centre effects with specific power, all centres will be grouped and analyses will be carried out as a whole. However, we will perform an exploratory analysis for centre effects with respect to each of the dependent variables. Qualitative and/or quantitative treatment-by-centre interactions will be investigated in this case. For the purpose of analysis, a small site will be defined as a site with < 4 ITT subjects in either treatment arm. Small sites will be ranked in descending order based on the total number of ITT subjects of the sites. The first small site will be pooled with the next small site, or with more sites if needed, until the pooled site/analysis centre has at least 4 ITT subjects in both the arms. The algorithm will continue down the list, and if the last few small sites are pooled but fail to be adequately large, they will be combined with the previously pooled smallest site/analysis centre.

5.4 Missing Data

Missing data will be imputed for some efficacy analyses described in the next sections (see section: efficacy analyses). Missing values will be imputed at all visits using the Multiple Imputation with Chained Equation (MICE) approach. Ten imputed data sets with all visit values filled in will be generated. The analysis will be performed on each of the 10 imputed datasets, which will produce estimates of treatment effect and the standard error of that estimate. Finally, the set of estimates and standard errors will be analysed by the *mice* R package to produce overall (pooled) estimates, confidence intervals, and P-values for the

treatment effect.

5.5 Interim Analyses and Data Monitoring (as applicable)

No interim analyses are planned for this study

5.6 Multiple Testing

There is no need to control for the overall type 1 error using a multiple testing procedure since there is only one single primary efficacy endpoint.

6 Summary of Study Data

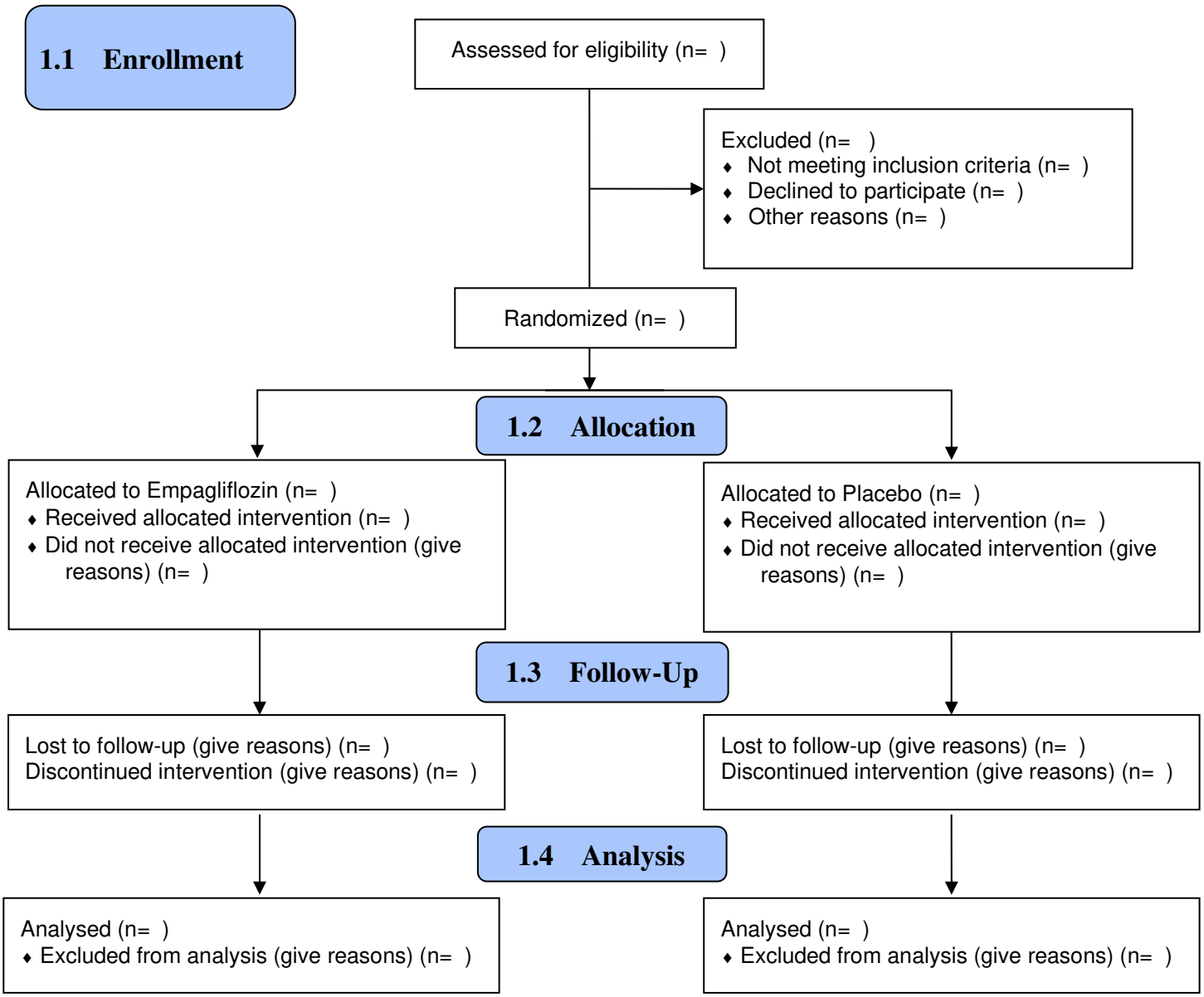
All summary tables will be structured with columns for each treatment and overall in the order (Overall, Placebo, Empagliflozin) and will be annotated with the total population size relevant to that table, including any missing observations. All continuous variables will be summarized using the following descriptive statistics: n (non-missing sample size), mean, standard deviation. The frequency and percentages (based on the non-missing sample size) of observed levels will be reported for all categorical variables. Demographic and clinical characteristics to be summarized will include all baseline variables. P-values will be added to compare baseline characteristics between Placebo and Empagliflozin groups.

Summary tables will be provided overall and also, if adequate, stratified according to: variables used in stratifying the randomization (i.e. Sex, T2DM status and Site), variables used in the subgroup analysis, and by visit.

6.1 Subject Disposition

A graphic showing an overview of the recruitment rate overall and by treatment groups will be provided. Also, a flow diagram will be provided as shown below:

Flow Diagram



Tables showing the number of patients reaching v1 only, v1-v2, v1-v3 and v1-v4 will be

displayed according to the treatment groups. The same information will be provided about missing data on the primary endpoint.

The absolute change in nt-proBNP levels from randomization to week 26 denoted by 'change_proBNP' and will be derived as follows:
change_proBNP = proBNP_w1 - proBNP_w26
where proBNP_w1 stands for the level of nt-proBNP at randomization (w1), and proBNP_w26 stands for the level of nt-proBNP at week 26.
The percentage change in nt-proBNP denoted as 'perc_change_proBNP' will be derived as follows:
perc_change_proBNP = (proBNP_w1 - proBNP_w26)/proBNP_w1
where proBNP_w1, and proBNP_w26 are defined as above.

6.2 Treatment Compliance

Treatment compliance will be assessed by remaining pill count. The pill count is checked at the monitoring visits.

7 Efficacy Analyses

Efficacy analyses performed using the intent-to-treat (ITT) population will be considered as primary, whereas efficacy analyses performed using the per-protocol (PP) population will be considered as supportive.

7.1 Primary Efficacy Analysis

The primary efficacy endpoint is the change in NT-proBNP from baseline to week 26. This primary endpoint will be analysed on the ITT population using a linear mixed effect model (LMEM) where the dependent variable is the NT-proBNP and the fixed effects are treatment, visit, treatment by visit interaction, the stratification factors Sex andT2DM and the level of NT-proBNP at baseline. The random effect component will include patients and unstructured covariance will be assumed for the within-subject errors. In case of non-convergence problems other covariance structures, such as compound symmetry, will be applied until the problem is resolved.

In this analysis, no missing data will be imputed. It will be handled by using the LMEM which assumes the data to be missing at random (MAR process). At week 26, estimates of mean values, the mean differences between the treatment groups and the associated 2-sided 95% confidence interval will be derived from the LMEM model through the use of estimated marginal means (or LS-means).
To claim superiority of Empagliflozin over Placebo, this primary efficacy analysis must show a statistically significant effect of treatment at an alpha level of 5% and two-sided test direction.

7.2 Secondary Efficacy Analyses

To support the interpretation of the primary efficacy analysis, some robustness and/or sensitivity analyses will be performed on the primary efficacy endpoint. However, the conclusion of superiority for comparison of the primary endpoint between the two treatment groups is purely based on results of the primary efficacy analysis.

Secondary efficacy analyses will be performed to assess robustness of the results against missing data, analysis population, and the statistical model used.

7.2.1 Secondary Efficacy analysis I

To assess the sensitivity of the above primary efficacy analysis to missing data, analysis will also be conducted on the ITT population with missing values being imputed at all visits using the Multiple Imputation with Chained Equation (MICE) approach as described in section 5.4. Ten imputed data sets with all visit values filled in will be generated. The primary efficacy analysis described above will then be performed on each of the 10 imputed datasets, which will produce estimates of treatment effect and the standard error of that estimate. Finally, the set of estimates and standard errors will be analyzed by mice package to produce overall (pooled) estimates, confidence intervals, and P-values for the treatment effect.

7.2.2 Secondary Efficacy analysis II

To assess the sensitivity of the above primary efficacy analysis to the analysis population, we will conduct the same primary efficacy analysis as above but by using the Per protocol

population instead of the ITT.

7.2.3 Secondary Efficacy analysis III

In this analysis, we use the absolute change in NT-proBNP from baseline to week 26 as the primary endpoint. This endpoint will be analyzed on the ITT population, with no imputation of missing values, and by using a multiple linear regression model where the dependent variable is the absolute change in NT-proBNP and the independent variables are treatment, Sex, T2DM and baseline level of NT-proBNP.

7.2.4 Secondary Efficacy analysis IV

In this analysis, we use the percent change in NT-proBNP from baseline to week 26 as the primary endpoint. This endpoint will be analyzed on the ITT population, with no imputation of missing values, and by using a multiple linear regression model where the dependent variable is the percent change in NT-proBNP and the independent variables are treatment, Sex, T2DM and baseline level of NT-proBNP.

7.3 Analyses of Secondary Endpoints

The change from randomization to week 26 of all continuous secondary endpoints will be analysed in the same way as the primary efficacy endpoint as follows:
Analysis population: ITT
Missing data: no imputation to be performed. Missing data will be implicitly handled by using the LMEM which assumes the data to be missing at random (MAR process).
Statistical model: LMEM with unstructured covariance matrix. At week 26, estimates of mean values, the mean differences between the treatment groups and the associated 2-sided 95% confidence interval will be derived from the LMEM model through the use of Least square means (LS-means).

8 Safety Analyses

The safety section of the analysis will contain a descriptive listing of the following tables:

All-Cause Mortality: A table of all cases of deaths due to any cause, with number and frequency

of such events in each arm/group of the clinical study.

Serious Adverse Events: A table of all anticipated and unanticipated serious adverse events, grouped by organ system, with number and frequency of such events in each arm/group of the clinical study.

This table will also include the reporting of cases of diabetic ketoacidosis.

8.1 Extent of Exposure

If appropriate use standard text: "The summary statistics will be produced in accordance with section 9."

8.2 Pregnancies

If pregnancies were recorded during the trial, descriptive information on the outcome will be provided.

8.3 Clinical Laboratory Evaluations

Summary statistics on laboratory measurements will be tabulated. For baseline values, differences in normal ranges between sites will be provided.

The primary outcome, NTproBNP, is assessed at the central lab. If measurement in the central lab are not possible (e.g. missing biomarker sample), NTproBNP measurement from the local lab will be accepted for the analysis.

9 Reporting Conventions

P-values ≥0.001 will be reported to 2 significant figures; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, and any other statistics other than quantiles, will be reported to one decimal place greater than the original data. Quantiles, such as median, or minimum and maximum will use the same number of decimal places as the original data.