Statistical Analysis Plan

Study Title: Canagliflozin Attenuates CMR-Quantified Myocardial Fibrosis in Patients with Type 2 Diabetes Mellitus at High Cardiovascular Risk: A Randomized Open-Label Controlled Trial

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1. Introduction

This statistical analysis plan (SAP) documents the planned statistical analyses for the study "Canagliflozin Attenuates CMR-Quantified Myocardial Fibrosis in Patients with Type 2 Diabetes Mellitus at High Cardiovascular Risk" and is based on the final protocol (NCT05367063). This SAP

is intended for the use of project team members and should be read in conjunction with the study protocol. This plan was finalized prior to the unblinding of the study data.

2. Study Purpose

The primary purpose of the study is to test whether Canagliflozin (100 mg/day) can attenuate myocardial fibrosis, measured by Extracellular Volume Fraction (ECV), at 26 weeks after randomization compared with the Sitagliptin (100 mg/day) control group, among patients with Type 2 Diabetes Mellitus (T2DM) at high cardiovascular risk.

The secondary purposes of this study are:

1. To evaluate whether Canagliflozin improves cardiac structure (specifically Left Ventricular Mass Index) at 26 weeks after randomization compared with the control group;
2. To evaluate whether Canagliflozin improves resting myocardial blood flow at 26 weeks after randomization compared with the control group.

3. Study Outcomes

3.1 Primary Efficacy Outcome

- Change in Myocardial Fibrosis, measured by **Extracellular Volume Fraction (ECV)** derived from Cardiac Magnetic Resonance (CMR), from baseline to Week 26.

3.2 Secondary Efficacy Outcomes the following are defined as confirmatory secondary endpoints:

- Changes in other CMR parameters: Left Ventricular End-Diastolic Volume (LVEDV), Left Ventricular End-Systolic Volume (LVESV), post-contrast T1, LVEDVI, LVESV, LVESVI.
- Changes in Echocardiographic parameters: LVEDD, LVESD, LVEF, IVS, LVPW, LA. AORD. PASP.
- Changes in metabolic and fibrosis biomarkers: HbA1c, Fasting Blood Glucose (FBG), Lipid profiles, and hs-CRP, BMI.

3.3 Safety Outcome

- Any adverse events (AEs) occurring during the study period.

4. Statistical **Hypotheses**

The primary outcome for this study is the change in ECV from baseline to Week 26. The null hypothesis (H_0) assumes no difference in the change of ECV between the two treatment groups.

H0: $\lambda_1=\lambda_2$

H1: $\lambda_1\neq\lambda_2$

Where λ_1 is the mean change of ECV from baseline to Week 26 in the Canagliflozin group, and λ_2 is the same outcome in the Sitagliptin group. The test will be two-sided at the 5% level of significance.

5. Design

This study is a single-center, randomized, open-label, active-controlled clinical trial with blinded outcome assessment (PROBE design). Eligible patients with T2DM and high cardiovascular risk were randomly assigned in a 1:1 ratio to receive either Canagliflozin (100 mg/day) or Sitagliptin (100 mg/day) for 26 weeks.

6. Sample Size Estimates

The sample size was estimated *a priori* based on the primary endpoint (ECV). Based on previous literature, assuming a standard deviation of change in ECV of -1.4% (SD 2%), a sample size of 22 per group provides 90% power to detect a clinically meaningful difference at a two-sided alpha level of 0.05. Taking into account a potential dropout rate of 20%, a total of 56 subjects were planned for randomization.

7. Analysis Populations

7.1 Intention-to-Treat (ITT) / Full Analysis Set (FAS) According to the basic principle of intention-to-treat (ITT), all randomized subjects will be included in the analysis, regardless of whether they received the assigned treatment or adhered to the protocol. Subjects will be analyzed according to the treatment group to which they were randomized. This population will be the primary population for analyses of efficacy.

7.2 Per Protocol Set (PPS) The PPS is a subset of the FAS and includes all subjects who completed the study without major protocol deviations. Major deviations include failure to meet inclusion criteria, poor compliance (<80%), or use of prohibited concurrent treatments. PPS is a secondary analysis population for robustness checks.

7.3 Safety Set (SS) All patients who received at least one dose of the study medication will be included in the safety population. Throughout the safety results sections, patients will be analyzed according to the actual treatment received.

8. Treatment Comparisons

The treatment comparison of interest in this study is to evaluate whether Canagliflozin improves myocardial fibrosis and cardiac function compared with the Sitagliptin control group.

- **Experimental Group:** Canagliflozin 100 mg/day
- **Control Group:** Sitagliptin 100 mg/day

9. General Considerations for Data Analyses

9.1 Estimands (ICH E9 R1) The primary estimand uses a **Treatment-Policy strategy**. All observed data will be used for analysis regardless of the occurrence of intercurrent events (e.g., discontinuation of study drug, initiation of rescue medication, or poor adherence). This aligns with the ITT principle. The population-level summary is the adjusted mean difference in ECV change between treatment groups.

9.2 Software and General Principles All analyses will be performed using R software (Version 4.3.2 or later). All statistics will be two-sided with a $P < 0.05$ considered significant, subject to multiplicity adjustments. Continuous data will be presented as mean (SD) or median (IQR); categorical data will be presented as n (%).

9.3 Multiple Comparisons and Multiplicity

- **Primary** Endpoint (ECV): Tested at a two-sided alpha of 0.05. No adjustment is applied as there is a single primary endpoint.
- **Secondary Endpoints:** P-values will be reported as nominal (unadjusted) and should be interpreted as hypothesis-generating.

10. Data Handling Conventions

10.1 Premature Withdrawal and Missing Data The primary analysis (MMRM) assumes data are Missing at Random (MAR). Under this assumption, the model provides unbiased estimates using all available data from randomized subjects without explicit imputation. As a sensitivity analysis, Multiple Imputation by Chained Equations (MICE) will be performed to generate m=20 complete datasets, with results pooled using Rubin's Rules.

11. Study Population

11.1 Disposition of Subjects The number of subjects randomized, completed, and prematurely withdrawn from the study will be presented for each treatment group. The primary reasons for withdrawal will also be presented.

11.2 Protocol Deviations Subject data will be examined for evidence of protocol violations.

Subjects with major protocol violations will be excluded from the Per Protocol Population.

11.3 Demographic and Baseline Characteristics Demographic information (age, sex, BMI) and baseline medical history (hypertension, diabetes duration, medication use) will be listed and summarized by treatment group. In accordance with CONSORT guidelines, hypothesis tests (p-values) for baseline imbalance will not be performed.

12. Efficacy **Analyses**

12.1 Primary Efficacy Analysis (MMRM) The primary outcome (Change in ECV) will be analyzed using a **Mixed Model for Repeated Measures (MMRM)** based on the ITT population.

- **Dependent Variable:** The observed outcome value at Week 26.
- **Fixed Effects:** Treatment group (Canagliflozin vs. Sitagliptin) and Baseline ECV value.
- **Covariance Structure:** An unstructured (UN) covariance matrix will be used to model within-subject correlation.
- **Degrees of Freedom:** The Kenward-Roger method will be used.
- **Output:** The Adjusted Mean Difference (AMD) between groups, 95% Confidence Intervals (CIs), and p-values will be derived using Least Squares Means (LS Means).

12.2Sensitivity and Robustness Analyses To assess robustness, the primary MMRM analysis will be repeated:

1. **Covariate Adjustment:** Including additional covariates (Age, Sex, Baseline BMI, Hypertension history, Antihypertensive medication use).
2. **Per-Protocol Analysis:** Using the PPS population.
3. **Missing Data:** Using Multiple Imputation (MICE) datasets.

13. Safety Analyses

All analyses of safety data will be carried out using the Safety Set (SS). Adverse events (AEs) will be coded and grouped by system organ class. The number and percentage of subjects experiencing an AE will be summarized by treatment group. Fisher's Exact