Statistical Analysis Plan

Version Number: 2.2 Date: 24-Oct-2023

Clinical Investigation Plan (CIP) Title: Safe and Timely Antithrombotic Removal - Ticagrelor (STAR-T): A Prospective, Multicenter, Double-blind, Randomized, Study to Evaluate Reduction in Postoperative Bleeding by Removal of Ticagrelor with the Intraoperative use of the DrugSorb-ATR Device in Patients Undergoing on-pump Cardiothoracic Surgery within Two Days of Ticagrelor Discontinuation

CIP Reference Number: 2021-01

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Type of Study: Prospective, Multicenter, Double-blind, Randomized

Investigational Product: DrugSorb-ATR Device

Investigational Device Exemption Number: G210081

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Statistical Analysis Plan Signatures

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Table of Contents

List of Abbreviations

ADE	Adverse device effect
AE	Adverse event
AUC	Area under the curve
CABG	Coronary artery bypass grafting
CEC	Clinical Events Committee
CI	Confidence Interval
CIP	Clinical Investigation Plan
CIR	Clinical Investigation Report
CPB	Cardiopulmonary bypass
CTD	Chest tube drainage
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram
i-CABG	Isolated coronary artery bypass grafting
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
ITT	Intent-to-Treat
mITT	Modified Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MI	Myocardial infarction
PK	Pharmacokinetic(s)
PT	Preferred Term
PRBC	Packed red blood cells
SADE	Serious adverse device effect
SAE	Serious adverse event
SaO2	Oxygen saturation
SAP	Statistical Analysis Plan
SD	Standard deviation
SoC	Standard of Care
SOC	System Organ Class
STAR-T	Safe and timely antithrombotic removal - ticagrelor
TEAE	Treatment-emergent adverse event
UADE	Unanticipated adverse device effect
UDPB	Universal Definition of Perioperative Bleeding

1 INTRODUCTION

This statistical analysis plan (SAP) describes the statistical methods and data presentation to be used for analyzing and reporting effectiveness and safety data for study Safe and Timely Antithrombotic Removal – Ticagrelor (STAR-T). This document has been prepared based on the Clinical Investigation Plan (CIP) number 2021-01 (Version 5.1, dated: 27-May-2023).

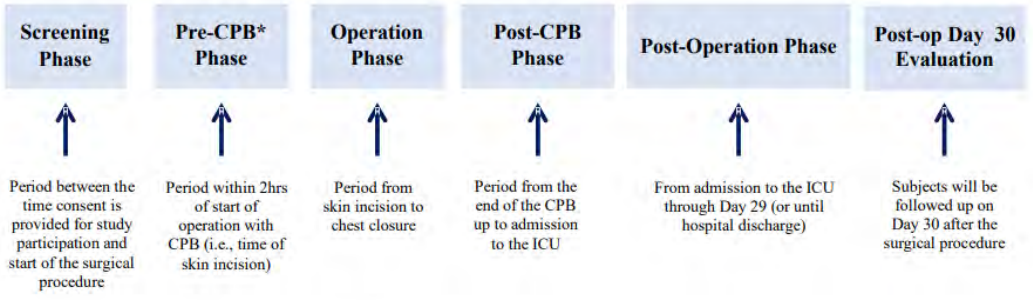
This SAP will be finalized as of the full execution (signatures) of this document. Any additional analyses (*post hoc*), or changes to the planned analyses as described in this SAP will be identified and documented in the Clinical Investigation Report (CIR).

1.1. Study Objectives

- To demonstrate reductions in postoperative bleeding with the intraoperative use of DrugSorb™-ATR in patients undergoing cardiothoracic surgery with CPB within two days of ticagrelor discontinuation
- To demonstrate reductions in ticagrelor blood levels Δ[ticagrelor] with the intraoperative use of DrugSorb™-ATR
- To establish safety of the intraoperative use of DrugSorb™-ATR in the intended population

1.2. Study Design

This is a prospective, multi-center, randomized, double-blind clinical study. Subjects will be randomized in a 1:1 ratio to either standard of care (SoC) or standard of care plus intraoperative treatment with the DrugSorb™-ATR device. The subjects will go through the 6 investigational phases as shown below:



* Pre-CPB phase may overlap with the Screening Phase.

1.3. Study Endpoints

1.3.1. Primary Effectiveness Endpoint

1.3.1.1. Primary Effectiveness Endpoint

Ranked composite of perioperative bleeding events:

(1) Fatal perioperative bleeding (occurring within 48hrs post-index procedure),

(2) UDPB ≥2 (moderate, severe, or massive) perioperative bleeding,

(3) 24-hour chest tube drainage volume (mL)

1.3.1.2. Supplementary Composite Effectiveness Endpoint

Ranked composite of perioperative bleeding events:

(1) Fatal perioperative bleeding (occurring within 48hrs post-index procedure),

(2) UDPB ≥3 (severe or massive) perioperative bleeding,

(3) 24-hour chest tube drainage volume (mL)

is proposed to evaluate the treatment effect on the more severe bleeding events.

1.3.2. Key Secondary Effectiveness Endpoint

Percent difference in pre-CPB vs. post-CPB blood ticagrelor levels as measured by liquid chromatography/tandem mass spectrometry (LC/MS-MS)

1.3.3. Additional Secondary Effectiveness Endpoints

- 24-hour chest tube drainage volume
- Total platelet transfusions from end of operation through the duration of the index hospitalization (volume and units)
- Total PRBC transfusions from end of operation through the duration of the index hospitalization (volume and units)
- Total UDPB events (by class)
- All surgical re-exploration for excessive bleeding
- Total fatal perioperative bleeding events

1.3.4. Exploratory Endpoints

- Percent difference in pre-CPB vs. post-CPB blood ticagrelor active metabolite (TAM) levels as measured by LC/MS-MS
- Area under the concentration time curve (AUC), for intraoperative ticagrelor pharmacokinetic (PK) concentration and for TAM
- Operative time (i.e., duration of index operation, measured from start of skin incision to completion of chest closure)
- Duration of ICU stay
- Duration of hospital stay
- 30-day hospital readmissions

1.3.5. Primary Safety Endpoint

The primary safety endpoint of this trial is to evaluate the product safety profile through the GCP-level assessment of AEs during the study period.

1.3.6. Additional Safety Endpoints:

- 30-day cardiac mortality
- 30-day all-cause mortality
- 30-day postoperative stroke
- 30-day postoperative myocardial infarction (MI)
- Urgent postoperative coronary revascularization during the index hospitalization

- Serious device-related adverse events

1.3.7. Exploratory Subgroup Analyses

The primary effectiveness endpoint and its individual components will be analyzed in the following subgroups:

1. Surgical Characteristics
   a. Procedure duration (skin incision to closure): <4 hours vs. 4-6 hours vs. >6 hours
   b. CPB duration (i.e., device exposure):
      - Tertiles
      - Above and below median
2. Drug Removal
   a. Time from Last Dose:

1. Tertiles
2. 0-24 vs. 24-48 vs. >48 hours
3. above vs below median
4. 0-24 vs. >24 hours
5. 0-48 vs. >48 hours

   b. Ticagrelor Levels

6. Above vs. Below median pre-CPB levels (whole population)
7. Above vs. Below median post-CPB levels (whole population)
8. >67% vs. <67% removal (per intervention arm values)
9. >50 vs. <50% removal (per intervention arm values)
10. >40 vs. <40% removal (per intervention arm values)

The key secondary endpoint will be analyzed according to surgical characteristics and the time from last dose subgroups.

2 SAMPLE SIZE AND STATISTICAL POWER CONSIDERATION

The hypothesis for the primary effectiveness endpoint will be tested using Finkelstein-Schoenfeld test (Finkelstein et al 1999). With the assumed effect sizes and variability for Scenario 1 outlined in Table 1 (Section 11.1 of the CIP), 57 subjects per arm will provide >99% power for the primary effectiveness endpoint based on Finkelstein-Schoenfeld test statistics at the two-sided significant level alpha (α) of 0.05. Taking into consideration higher than anticipated variability observed with the 24hr CTD variable, in scenario 2 we consider the same effect sizes in the 3 components and a larger variability (double of that in Scenario 1). In that instance the needed sample size is 65 per arm to achieve 92% power.

With this same sample size per arm, power for the key secondary endpoint is estimated >99.9%, and for 24-hour chest tube drainage (mL), >99.9%, based on a two-sample t-test assuming equal variance and a two-sided alpha of 0.05. Assuming unequal variances, power for comparisons of platelet transfusions (volume [mL]) and PRBC transfusions (volume [mL]), is estimated at 98.0% and 96.7%, respectively.

Allowing for ~7% dropouts, approximately 140 subjects will be randomized in a 1:1 ratio to the treatment and control arms to ensure that 130 subjects (65 per arm) will be evaluable for the primary effectiveness analysis.

Table 1. Effect Size Assumptions with Power and Sample Size Estimates

Scenario	Sample size Per Group	Effect Size						Power
		Component #1		Component #2		Component #3		
		Fatal Bleeding		UDPB (Class ≥2)		24hr CTD		
		Treatment Event	Control Event	Treatment Event	Control Event	Treatment Mean (SD)	Control Mean (SD)	
1	N= 57	3.0%	3.0%	24.3%	40.5%	480 (253)	800 (253)	**99.7%**
2	N=65	3.0%	3.0%	24.3%	40.5%	480 (500)	800 (500)	**92.1%**

3 GENERAL ANALYSIS DEFINITIONS

3.1 Visit Windows

Nominal visit days/time will be used in the analysis, unless specified otherwise. Refer to the Table 6, Schedule of Assessments in the study protocol for details on study periods.

3.2 Analysis Sets

3.2.1 Modified Intent-to-Treat Population

The modified intent-to-treat (mITT) population will consist of all randomized subjects who undergo the index surgical procedure and receive a study device (whether DrugSorb™-ATR or sham). This is the primary population for all the effectiveness analyses.

3.2.2 Intent-to-Treat Population

The intent-to-treat (ITT) population will consist of all subjects who have been randomized to the study. This population will be used for supplementary effectiveness analyses.

3.2.3 Per Protocol Population

The Per Protocol (PP) Population will include all mITT subjects who have no major protocol deviations. This population will be used for supplementary effectiveness analyses.

3.2.4 Population of Special Interest

The isolated-CABG (i-CABG) populations will consist of mITT or PP subjects who undergo i-CABG as the index surgical procedure. The subjects who undergo all other types of surgery including valve replacement, valve repair, aortic, structural or combination of CABG plus another major procedure will be excluded. Those two populations (i-CABG mITT and i-CABG PP populations) will be used for supplementary effectiveness analyses.

3.2.5 Safety Analysis Population

The safety analysis (SA) population will include all enrolled subjects who are treated with a study device. Similarly, for the population of special interest, i-CABG SA population will consist of SA population who are treated with a study device and undergo an i-CABG procedure.

3.3 Randomization/Blinding

All subjects will be randomized in a one to one (1:1) ratio to either the control arm (SoC) or the treatment arm (SoC + DrugSorb™-ATR device). Pre-randomization stratification will be performed by study site, and by type of intended surgical procedure - either:

- CABG alone (irrespective of number of grafts); or
- All other cardiothoracic procedures (including but not limited to combination procedures [i.e., CABG + aortic/mitral valve procedures, double heart valve procedures, etc.], aortic interventions, and combination aortic + cardiac interventions).

Of note, patients undergoing undergo CABG + minor add-on procedures (e.g., left atrial appendage clipping, IABP or Impella placement, etc.) that do not (in the opinion of the investigator) significantly increase operative complexity or bleeding risk are included in the CABG alone category for Type of planned cardiothoracic surgery**.** Please see Section 6.1 of the CIP for additional details of pre-randomization stratification. Randomization is implemented via an Interactive Voice/Web response (IxRS) system.

Neither the subjects nor the site personnel have the treatment assignment information during the study period, unless specified in **Section 6.3** of the CIP**.** The blinded study team is blocked from every subject's treatment assignment before the data base lock for unblinding. Please see **Section 6.3** of the CIP for additional details of study blinding.

3.4 Definition of Baseline

Baseline is defined as the last non-missing value prior to the index surgical procedure.

3.5 Homogeneity Across Regions

In order to assess homogeneity of results across the two participating countries, assessment of country effect will be carried out for the primary effectiveness endpoint, using Cox proportional hazards regression for the first component (when deemed appropriate), logistic regression for the second components and gamma regression with log link for the third. The individual component of the primary outcome will be the dependent variable and treatment, region and their interaction will be the independent variables.

A treatment-by-country interaction that reaches the 0.05 level of significance will signal that the treatment difference on the component(s) may differ in the two countries and will be followed by an investigation of the direction and magnitude of the treatment effect in each country to assess if any interactions are qualitative in nature.

3.6 Homogeneity Across Subgroups of Interest

The following analysis will be carried out on the mITT, PP, i-CABG mITT, and i-CABG PP populations with no imputation for missing data.

Appropriate descriptive statistics of the primary effectiveness endpoint and its components will be presented by treatment group (treatment vs control) within the subgroup categories below. The purpose of this analysis is not to assess significance of the treatment effect (treatment vs. control on the primary effectiveness endpoint and its components) within subgroups but to assess consistency of treatment effect on the primary effectiveness endpoint and its components across subgroups.

The following is a list of the subgroups to be investigated. For each subgrouping, in order to assess homogeneity of treatment effect on the primary effectiveness endpoint and its components, assessment of treatment-by-subgroup category interaction will be carried out in a similar manner as discussed previously for assessing homogeneity across regions.

We will evaluate the consistency of effectiveness on the primary effectiveness endpoint and its components (homogeneity), in the following subgroups:

1. Sex: Male vs Female
2. Age: <65 vs. ≥65
3. CABG vs. other procedures
4. Pre-existing clinical risk factors
    a. EuroScore II <7%; ≥7%)
    b. STS mortality risk score (<4% [low], 4%-8% [intermediate], >8% [high])
    c. Baseline thrombocytopenia (platelet count <150,000 μ/L vs. ≥150,000 μ/L)

3.7 Multiple Comparisons/Multiplicity

The type I error will be controlled on the multiple outcomes on the primary and secondary effectiveness endpoints through sequential testing procedure in the following order:

Primary endpoint → Key secondary endpoint → Additional Secondary endpoints (24hr CTD → platelet transfusions → PRBC transfusions → UDPB events by class → surgical re-exploration for excessive bleeding → fatal perioperative bleeding event)

The same hierarchy will be applied to the analysis of the population of special interest (i.e., i-CABG).

The significance for the secondary endpoints will only be considered if the primary endpoint is declared statistically significant and the p-value of the previous endpoint is less than 5%, otherwise, all the subsequent p-values will be nominal.

4 SUBJECT INFORMATION

4.1 Disposition Information

The number of subjects in each study population will be summarized by treatment. The number of subjects who did not receive a study device or were discontinued from the study will be summarized by treatment and the primary reasons for discontinuation will be provided.

4.2 Demographics and Baseline Characteristics

Demographics and baseline characteristics such as sex, age, race, weight, body mass index (BMI), vital signs, and clinical laboratory data will be summarized using descriptive statistics by treatment and overall, for the mITT population.

4.3 Protocol Deviations

Sites will report protocol deviations to the Sponsor. All deviations will be collected in a deviation log.

Deviations will be reviewed by blinded Sponsor representatives and characterized as major or minor in accord with the impact to the study conduct and data integrity and as outlined in the protocol deviation plan. Major deviations will be summarized and assessed by treatment and a supportive listing will be provided.

4.4 Medical History

Medical history information will be coded and summarized for the overall study and by treatment for the mITT Population using the most recent version of the Medical Dictionary for Regulatory Activities (MedDRA®).

4.5 Prior and Concomitant Medications

Prior and concomitant medications will be summarized by treatment for the mITT population using the World Health Organization-Anatomical Therapeutic Chemical classification system (WHO Drug Dictionary). Prior medications include those taken within the last 30 days but stopped prior to start of device treatment. Concomitant medications include those that started prior to device treatment (after subject randomization), but continued during treatment, as well as those that started during or after device treatment and up to the end of the 30-day follow-up period.

4.6 Device/Study Exposure and Index Procedure

Extent of exposure to study device during index operation in minutes will be calculated as:

(device stop date/time - device start date/time)

Where device interruptions will be accounted for within the calculation.

Post index operation follow-up duration in days will be calculated as:

(study exit date - device stop date) + 1

Total study duration in days will be calculated similarly as:

(study exit date – Informed consent form (ICF) date) + 1

A summary of device/study exposure and index procedure information will be provided by treatment.

5 STATISTICAL ANALYSIS METHODS

5.1 General Considerations

Unless otherwise specified, all statistical analyses will be performed on the mITT population.

In general, summary statistics (n, mean, SD, median, minimum, and maximum for continuous variables, and number and percentage of subjects in each category for categorical variables) will be provided by treatment. All summary tables will be based on the observed data unless specified otherwise. Source data for the summary tables and statistical analyses will be presented as subject level data listings.

The two-sided significance level of 5% is used for the hypothesis testing, unless specified otherwise.

Fixed sequence testing procedure will be utilized to compare the effectiveness of the treatment vs control in the following order:

Primary endpoint → Key secondary endpoint → Additional Secondary endpoints (24hr CTD → platelet transfusions → PRBC transfusions → UBPB events by class → surgical re-exploration for excessive bleeding → fatal perioperative bleeding event)

The significance for the secondary endpoints will only be considered if the primary endpoint is declared statistically significant and the p-value of the previous endpoint is less than 5%, otherwise, the testing procedure stops, and all the subsequent p-values will be nominal.

5.2 Analysis of the Primary Effectiveness Endpoint

The primary effectiveness endpoint is a hierarchical composite endpoint with three components ranked in descending order of clinical importance as follows:

1. Fatal perioperative bleeding event (occurring within 48hrs post index procedure)
2. Moderate, severe, or massive perioperative bleeding events according to UDPB classification (UDPB ≥2) (Dyke 2014)
3. 24-hour chest tube drainage (CTD) (mL) following surgery.

The hypothesis for the primary effectiveness endpoint will be tested using Finkelstein-Schoenfeld test (Finkelstein et al 1999). In constructing the test, "wins", losses", or "ties" for all pairs of subjects in sequential comparisons according to the hierarchical order of the components of the primary composite endpoint.

Specifically, let the primary effectiveness endpoint Y= (y1, y2, y3), we evaluate win/loss for all subject pairs (i, j) as follows:

Step 1: Pair (i, j) is evaluated at the first component: fatal perioperative bleed occurring within 48 hours of the index procedure, with a binary (yes/no) outcome. It requires one subject not to experience the event and the other subject to experience the event in order to declare a "winner", i.e.,

the subject i wins if y1[i]=no, y1[j]=yes; loses if y1[i]=yes, y1[j]=no.

Otherwise, this is a tie for the pair in the first component and move to Step 2

Step 2: The second component - moderate, severe, or massive perioperative bleeding events according to UDPB classification ≥2 is also a binary (yes/no) outcome and follows the same process as step 1 for determining a win (y2[i]=no, y2[j]=yes) or a loss (y2[i]=yes, y2[j]=no) for the subject i. Otherwise, this is a tie at the second level and move to Step 3.

Step 3: The third component is 24-hour chest tube drainage (mL) following surgery. This is a quantitative (continuous) outcome where a lower drainage is better and therefore the subject with less drainage is declared the winner, i.e.,

the subject i wins if (y3[i]<y3[j]); loses if (y3[i]>y3[j])

Otherwise, they tie on the primary effectiveness endpoint.

5.2.1 Primary Hypotheses

The null hypothesis for the primary composite endpoint is represented by:

H_0: The distribution of the components is the same in the two treatment groups

The alternative hypothesis for the primary composite endpoint is represented by:

H_A: For at least one component, the distribution is different between the treatment groups

The null hypothesis for the primary composite endpoint can also be represented by the following equations:

$$H_0: \pi_{1,DS} = \pi_{1,SOC} \text{ AND}$$

$$\boldsymbol{\pi_{2,DS} = \pi_{2,SOC}} \text{ AND}$$

$$\mu_{3,DS} = \mu_{3,SOC}$$

The alternative hypothesis for the primary composite endpoint is represented by:

$$H_A: \pi_{1,DS} \neq \pi_{1,SOC} \text{ OR}$$

$$\boldsymbol{\pi_{2,DS} \neq \pi_{2,SOC}} \text{ OR}$$

$$\mu_{3,DS} \neq \mu_{3,SOC}$$

where $\pi_{1,DS}$ and $\pi_{1,SOC}$ are the rates for fatal bleeding with DrugSorb™-ATR (DS) and Control (SOC), respectively; $\pi_{2,DS}$ and $\pi_{2,SOC}$ are the rates for UDPB ≥2 with DrugSorb™-ATR (DS) and Control (SOC), respectively; and $\mu_{3,DS}$ and $\mu_{3,SOC}$ are the mean 24hr CTD with DrugSorb™-ATR (DS) and Control (SOC), respectively.

5.2.2 Primary Effectiveness Analysis

The primary effectiveness analysis will be performed on the mITT population. It consists of hypothesis testing based on Finkelstein-Schoenfeld test (Finkelstein et al 1999) and the win ratio estimates following the unmatched win ratio method (Pocock 2012).

5.2.2.1 Significance Test

The primary hypothesis will be tested at the two-sided significance level of 0.05 using Finkelstein-Schoenfeld test statistics (Finkelstein et al 1999). All pairs of subjects are compared, regardless of the treatment groups, and a score $W_{ij} = 1, -1$ or 0 is assigned to the pair (i, j) depending on whether subject i was a winner, a loser, or the comparison was inconclusive.

The test statistic is $T = \sum_i D_i W_i$, where D_i is the indicator variable for the treatment and $W_i = \sum_j W_{ij}$. Under the null distribution of no treatment effect, T is asymptotically normally distributed with mean 0 and variance $\frac{n*m}{N*(N-1)} * \sum_i {W_i}^2$, where n and m are the sample size for the treatment and control group respectively, and $N = n + m$.

Therefore, a significance test based on the standardized T statistic denoted by Z-statistics will be used to test the null hypothesis. The p-value will be provided and compared at the significance level of 0.05.

5.2.2.2 Win Ratio Estimates

In order to evaluate the magnitude of treatment effect in the primary effectiveness endpoint, the unmatched win ratio estimates (Pocock 2012) will be provided as well as the corresponding 95% confidence interval (CI).

The unmatched win ratios are formed by comparing every subject in the treatment group with every subject in the control group. Specifically, if we let n, m be the number of subjects in the treatment and control groups, respectively, then we make $n \times m$ paired comparisons. The win ratio is then calculated as N_W/N_L where N_W and N_L are the total number of pairwise wins and losses, respectively, for the treatment group.

Following Bebu (2016), logarithm of $\widehat{WR} = N_W/N_L$ has an asymptotic normal distribution with mean and variance specified in (2.6) of the paper. Therefore, the estimated WR and the 95% Confidence Interval (CI) will also be derived and presented from the sample distribution of $\log(\widehat{WR})$ using Delta method with the exponential transformation.

5.2.2.3 Missing Data Handling

There is no missing data imputation for the effectiveness analysis of the primary endpoint. Any missing values in the Win/Loss evaluation of pairs for each component will be considered as tie, and the Win/Loss will be determined based on the 3 steps described above.

5.2.3 Sensitivity Analysis

In order to assess the robustness of the primary effectiveness analysis a bootstrap method will be used to estimate the win ratio for the treatment. Instead of using the asymptotic variance for $\log \widehat{WR}$, a bootstrap resampling from the original primary effectiveness endpoint will be performed for 1000 times to obtain a bootstrap estimate of variance. With this bootstrap variance estimate, the 95% CI will be derived similarly as the primary effectiveness analysis.

5.2.4 Supplementary Analyses

5.2.4.1 ITT analysis

The primary effectiveness endpoint analysis will also be performed on the ITT population.

5.2.4.2 Per-protocol Analysis

The primary effectiveness endpoint analysis will also be performed on the per-protocol population.

5.2.4.3 i-CABG Analysis

The primary effectiveness endpoint analysis will also be performed on the i-CABG mITT and i-CABG PP populations.

5.2.5 Supplementary Composite Effectiveness Endpoint Analysis

The supplementary composite effectiveness analysis will be performed on the mITT, PP, i-CABG mITT, and i-CABG PP populations in the same fashion as for primary effectiveness analyses.

5.3 Analysis of Key Secondary Endpoints

All analyses will be performed on the mITT, PP, i-CABG mITT, and i-CABG PP populations.

5.3.1 Key Secondary Effectiveness Endpoints

There is no imputation for the missing pre- or post-CPB blood ticagrelor levels, and analysis will be limited to subjects who have both samples.

Percent difference in pre-CPB vs. post-CPB blood ticagrelor levels as measured by LC/MS-MS is the key secondary effectiveness endpoint.

This endpoint will be evaluated using the two-sample t-test for the percentage change in blood ticagrelor levels between the treatment and the control group. The p-value and 95% CI for this treatment difference will be provided. The model assumption will be checked using the observed data.

If the post-CPB blood ticagrelor level is below the limit of quantification (BLQ), the BLQ value will be assigned.

5.3.2 Sensitivity Analysis

Wilcoxon rank sum test based on the observed data will be performed as a sensitivity analysis. The nonparametric p-value will be provided as well as median treatment difference in percent change between pre-CPB and post-CPB blood ticagrelor levels.

The Last Measurable Observation Carried Forward method will be implemented to test the validity of imputation.

5.4 Analysis of Additional Secondary Effectiveness Endpoints

All analyses will be performed on the mITT, PP, i-CABG mITT, and i-CABG PP populations.

5.4.1 Continuous Secondary Effectiveness Endpoints

The ANCOVA model with treatment group and type of surgery (when deemed appropriate) as covariates will be employed to assess the treatment difference in continuous secondary endpoints, unless specified otherwise.

5.4.1.1 24-hour Chest Tube Drainage Volume

24-hour CTD volume will be evaluated as the cumulative volume in CTD within 24 hours based on the observed data from the end of the index surgery. The subject with missing CTD volume within 24 hours due to death will be excluded from the analysis.

The ANCOVA model with treatment group and type of surgery (when deemed appropriate) as covariates will be employed to assess the treatment difference in 24-hour CTD. The p-

value and estimated mean difference along with the 95% CI will be provided between the treatment and control group.

5.4.1.2 Other Continuous Secondary Effectiveness Endpoints

Total platelet/PRBC transfusions in volumes from end of operation through the duration of the index hospitalization (initial discharge from the hospital) will be analyzed based on the observed data using two-sample t test. The p-value and estimated mean difference along with the 95% CI will be provided between the treatment and control group.

Wilcoxon rank sum test based on the observed data in volumes will be performed if the normality assumption is invalid. The nonparametric p-value will be provided as well as median treatment difference in volumes for total platelet/PRBC transfusions.

The same analyses using transfusion data measured in units will be conducted as the supplemental analyses.

5.4.2 Categorical Secondary Effectiveness Endpoints

5.4.2.1 Total UDPB Events (by class)

The UDPB ≥2 events (Section 3 of the CIP) will be adjudicated by Clinical Events Committee (CEC). The adjudicated events will be analyzed using the Mann-Whitney U test method stratified by class (moderate [UDPB=2], severe [UDPB=3], and massive [UDPB=4]) to assess the risk of having different severity of bleeding events between the treatment and control group.

5.4.2.2 Supplemental Analyses

Two supplemental analyses will be conducted using Fisher exact test on the risk of having UDPB ≥3 and UDPB ≥4 events, respectively.

The estimated rates within each group and odd ratios comparing the treatment groups will be provided along with the 95% confidence intervals.

5.4.2.3 Other Categorical Secondary Effectiveness Endpoints

The following endpoints associated with the bleeding events will be evaluated within 48 hours after the index surgery and adjudicated by CECs according to the CEC charter.

- All surgical re-exploration for excessive bleeding
- Total fatal perioperative bleeding events

The adjudicated endpoints will be analyzed using the Fisher exact test to evaluate the risk of having the events between the treatment groups. The estimated rates within each group and odd ratios comparing the treatment groups will be provided along with the 95% confidence intervals.

5.4.3 Time-to-event Analysis for the Secondary Effectiveness Endpoints

Time-to-event analysis may be applied to the events of interest, along with Kaplan-Meier plot by treatment when deemed appropriate.

5.5 Exploratory Endpoints

All analyses will be performed on the mITT, PP, i-CABG mITT, and i-CABG PP populations.

The descriptive statistics will be summarized by treatment group for the exploratory endpoints.

Percent difference in pre-CPB vs. post-CPB blood TAM levels as measured by LC/MS-MS will be analyzed in the same fashion as blood ticagrelor levels (Section 5.3). Two-sample t-test for the percentage change in blood TAM between the treatment and the control group will be performed and the p-value and 95% CI for this treatment difference will be provided. Wilcoxon rank sum test based on the observed data will be performed as a sensitivity analysis. The nonparametric p-value will be provided as well as median treatment difference in percent change between pre-CPB and post-CPB blood TAM levels.

If the post-CPB blood TAM level is below the limit of quantification (BLQ), the BLQ value will be assigned. The Last Measurable Observation Carried Forward method will be implemented to test the validity of imputation.

ANOVA model will be employed to compare area under the concentration time curve (AUC) between the treatment groups, for intraoperative ticagrelor pharmacokinetic (PK) concentration and TAM based on the observed data.

The boxplots will be presented for the following exploratory endpoints by treatment.

- Operative time (measured from start of skin incision to completion chest closure)
- Duration of ICU stay
- Duration of hospital stay

6 SAFETY ANALYSES

6.1 General Considerations

All safety endpoints will be analyzed by treatment on the SA and i-CABG SA populations unless specified otherwise. All safety data will be listed on the SA Population.

6.2 Adverse Events

An adverse event (AE) is any untoward medical occurrence (signs, symptoms, abnormal laboratory findings) in a subject regardless of relationship to the device or procedure. All AEs that occur starting from subject enrollment through follow-up will be reported. Adverse events (AEs) will be coded using the most recent version of Medical Dictionary for Regulatory Activities (MedDRA). The version of MedDRA® will be provided in a footnote on outputs.

Treatment-emergent AEs (TEAEs) are defined as AEs with onset or worsening in severity after the start of the cardiopulmonary bypass procedure, and within 30 days after study treatment. Analyses of AEs will include only TEAEs. The incidence of treatment-emergent AEs (TEAE) will be summarized by treatment group, and overall. Non-TEAEs (i.e., AEs that

occur following informed consent but prior to the start of CPB) will not be summarized but will instead be provided in a separate listing.

6.2.1 Anticipated Adverse Events

A variety of complications are expected to occur in subjects undergoing CPB and cardiothoracic surgery, regardless of whether or not DrugSorb™-ATR is used. These may include, but are not limited to:

Serious, but less common, risks:

- Bleeding during or after the surgery including bleeding in relation to anticoagulation
- Blood clots that can cause heart attack, stroke, or lung problems
- Heparin-induced thrombocytopenia
- Bypass induced thrombocytopenia
- Reduction in platelets
  Infection, including pneumonia
- Organ injury including:
  - Respiratory damage causing breathing problems or failure to wean from the
  - ventilator
  - Cardiac dysrhythmias/arrhythmias
  - Kidney injury or renal failure
  - Cognitive impairment
- Death

Less serious, but more common risks and adverse events:

- Low-blood pressure
- Nausea and/or vomiting
- Hypothermia and/or Chills
- Allergic response to device materials
- Reduction in platelet count
- Reduction in white blood cell count
- Reduction in proteins (albumin, total protein)
- Coagulation within device
- Heparin-induced thrombocytopenia

6.2.2 Device-Related Adverse Events

Potential adverse device events directly related to DrugSorb™-ATR:

- Removal of drugs and hormones
- Allergic response to device materials
- Reduction in platelet count
- Reduction in white blood cell count
- Reduction in proteins (albumin, total protein) which may increase with CPB duration.
- Reductions in serum calcium (as a consequence of reductions in albumin)
- Coagulation within device
- Infection
- Blood loss

- Hemolysis
- Device leakage
- Circuit leakage
- Death

6.2.3 Adverse Device Effect

An adverse device effect (ADE) is an AE related to the use of an investigational medical device. This definition includes AEs resulting from insufficient or inadequate instructions for use, deployment, installation, or operation, or any malfunction of the investigational medical device. The definition also includes any event resulting from use error or from intentional misuse of the investigational medical device. ADEs will be reported by the site and summarized by treatment group and overall.

6.2.4 Unanticipated Adverse Device Effect

An unanticipated adverse device effect (UADE) is any adverse device effect that by its nature, incidence, severity or outcome has not been identified in the current version of the risk analysis report. UADEs will be reported by the site and summarized by treatment group and overall.

6.3 Analyses of TEAEs

TEAEs will be summarized by the MedDRA® Preferred Term (PT) and by System Organ Class (SOC). Subjects reporting more than one AE within a SOC will be counted only once for that SOC. Subjects reporting the same AE more than once will be counted only once for that PT.

The severity of TEAEs will be presented in the TEAEs by severity table. If a Subject has the same TEAE on multiple occasions, the highest severity will be recorded for the TEAE.

An overview table will be summarized by treatment group and overall, which will include the number of subjects with any TEAEs, serious adverse events (SAEs), procedure related TEAEs, study device related TEAEs, TEAEs by severity, UADEs, device-related SAEs/Serious adverse device effect (SADE), serious UADEs (USADEs), procedure-related SAEs, SAEs leading to device discontinuation, TEAE(s) leading to death.

In addition, TEAEs will be summarized by treatment for the following:

- TEAEs by System Organ Class (SOC) and Preferred Term (PT)
- TEAEs by PT (descending order of frequency)
- TEAEs with ≥5% Incidence Overall by SOC and PT
- TEAEs by SOC, PT, and severity
- Device Related TEAEs/ Adverse Device Effect (ADE) by SOC and PT
- Procedure Related TEAEs by SOC and PT
- UADEs by SOC and PT

- SAEs by SOC and PT
- Device Related SAEs / Serious Adverse Device Effect (SADE) by SOC and PT
- Serious UADEs by SOC and PT
- Procedure Related SAEs by SOC and PT
- SAEs Leading to Device Discontinuation by SOC and PT
- SAEs Leading to Death by SOC and PT

Listings will be provided for all AEs, SAEs, TEAEs leading to device discontinuation, TEAEs leading to death.

6.4 Additional Safety Endpoints

The descriptive statistics will be presented by treatment group and overall, for the following additional safety endpoints.

- 30-day cardiac mortality
- 30-day all-cause mortality
- CEC-adjudicated bleeding event up to 30-day follow-up (30 ± 5 days)
- CEC-adjudicated postoperative stroke up to 30-day follow-up
- CEC-adjudicated postoperative myocardial infarction up to 30-day follow-up
- CEC-adjudicated urgent postoperative coronary revascularization up to 30-day follow-up

Data listings will be provided for the subjects with the observed data on the additional safety endpoints.

6.5 Clinical Laboratory Parameters

Only scheduled laboratory parameters, as specified in Error! Reference source not found.**2**, will be included in the laboratory results summaries, unless specified otherwise. All laboratory data, including those collected at unscheduled visits/time, will be included in the listings.

Quantitative results (actual values and change from baseline) will be summarized for each scheduled time by treatment group and overall. For each parameter, the mean (± SE) at each scheduled time will be plotted for its actual value and its change from baseline. Data collected at the End-of-study/treatment (EOS/ET) time will be included as one time point regardless of subjects' EOS status.

In addition, when deemed necessary, shift tables based on lab normal ranges (e.g., below/within/above the normal range), where the shift is from baseline to last clinic visit will be provided.

Table 2: Clinical laboratory parameters

Clinical chemistry	Hematology

• Alanine aminotransferase • Albumin • Alkaline phosphatase • Aspartate aminotransferase • Blood urea nitrogen • Calcium • Chloride • Creatinine • Direct bilirubin • Glucose • Potassium • Sodium • Total bilirubin • Total protein	Hematocrit Hemoglobin Platelet count Red blood cell count White blood cell (WBC) count
Coagulation tests:	**Other, including cardiac biomarkers**
Coagulation Panel: • Activated partial thromboplastin time • International normalized ratio • Prothrombin time • Fibrinogen Point of Care Test(s): Activated clotting time (ACT) Arterial blood gas (ABG)	• Cardiac troponin (cTn)

6.6 Vital Signs

A descriptive summary of vital sign values, including changes from baseline, will be provided by scheduled time point by treatment group and overall.

The above summary will be provided for the following vital sign variables:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Body temperature (°F)
- Heart rate (beats per minute)
- Respiratory rate (breaths/minute)
- Oxygen saturation (SaO2) (%)

Vital signs data will be listed by subject for all subjects in the safety population.

6.7 12-Lead Electrocardiogram (ECG)

A descriptive summary of ECG values, including changes from baseline (where present), will be provided by time point by treatment group, for the following ECG variables:

- Heart rate (bpm)
- PR interval (msec)

- RR interval (msec)
- QRS duration (msec)
- QT interval (msec)
- QTcB interval (msec)
- QTcF interval (msec)

7 DEFINITIONS AND CONVENTIONS FOR HANDLING OF THE DATA

7.1 Handling of Partial Dates of Concomitant Medication

Partial start dates of prior and concomitant medication will be assumed to be the earliest possible date consistent with the partial date. Partial stop dates of prior and concomitant medications will be assumed to be the latest possible date consistent with the partial date. In the case of completely missing stop date, medication use will be assumed to be ongoing.

7.2 Reporting Precision

Summary statistics will be presented to the degree of precision in **Error! Reference source not found.**3, unless otherwise

Table 3: Degree of Precision

Statistics	Degree of Precision
Mean, Median, Quartiles, Confidence limit boundaries	One more than the raw data, up to 3 decimal places.
Standard deviation, Standard error	One more than the mean, up to 3 decimal places
Minimum, Maximum	The same as the raw data, up to 2 decimal places
p-value	Rounded to 3 decimal places and therefore presented as 0.xxx; p-values smaller than 0.001 as '<0.001'; p-values greater than 0.999 as '>0.999'.
Percentage	One decimal place. A percentage of 100% will be reported as 100%. Percentages of zero will be reported as 0.

Fractional numeric values will be presented with a zero to the left of the decimal point (for example, 0.12 to 0.30).

For weight, height, and body mass index (BMI), one decimal place will be used for summary statistics.

8 MOCK TABLES, LISTINGS, AND GRAPHS (TLGS)

Mock-up tables and listings will be provided in a separate document.

9 PHARMACOKINETIC ANALYSIS

The statistical analysis plan for the pharmacokinetic analysis of plasma ticagrelor and TAM concentration are presented in **Appendix A.**

10 REFERENCES

1. Finkelstein, DM, Schoenfeld, DA. Combining mortality and longitudinal measures in clinical trials. Stat Med, 1999. 18(11): p. 1341-54.
2. Bebu I, Lachin JM. Large sample inference for a win ratio analysis of a composite outcome based on prioritized components. Biostatistics, 2016, 17(1): p 178-187
3. Pocock, SJ, Ariti, CA, Collier, TJ, Wang, D. The win ratio: a new approach to the analysis of composite endpoints in clinical trials based on clinical priorities. Eur Heart J, 2012. 33(2): p. 176-82.
4. Dyke, C, Aronson, S, Dietrich, W, Hofmann, A, Karkouti, K, Levi, M, Murphy, GJ, Sellke, FW, Shore-Lesserson, L, von Heymann, C, Ranucci, M. Universal definition of perioperative bleeding in adult cardiac surgery. J Thorac Cardiovasc Surg, 2014. 147(5): p. 1458-1463.

APPENDIX A: STATISTICAL ANALYSIS PLAN FOR PHARMACOKINETIC ANALYSES

A 1.0 Pharmacokinetic Analyses

The pharmacokinetic (PK) analysis set will include subjects with at least one sample collected and analyzed for plasma drug concentration. The PK analysis set will be the primary set for PK analyses. The PK analyses will be performed for the mITT, PP, i-CABG mITT, and i-CABG PP populations.

A 1.1 Pharmacokinetic Assessment

A 1.1.1 Sample Collections for Pharmacokinetic Analysis

PK Blood samples will be collected for the determination of plasma ticagrelor and desethoxyhydroxy ticagrelor (ticagrelor active metabolite, TAM) concentration. The pre-CPB blood sample will be drawn prior to the start of CPB and can be up to 2hrs prior to skin incision. During the procedure, while the subject is on CPB, serial samples will be drawn at 30±15min following initiation of CPB, at 60min, at 90min, and at 120min of CPB time. If the total CPB time required for the procedure is <120min (2hrs), the serial sampling will only be performed through the required duration of CPB. A post-CPB sample will be obtained as soon as possible following the end of CPB, and no later than 30min after the subject has been disconnected from the CPB circuit. The actual date and time of collection of each PK sample will be recorded.

A 1.1.2 Handling Missing or Below the Lower Limit of Quantification Data

For PK concentration data, if the actual sampling time is missing, but a valid concentration value has been measured, the concentration value will be flagged, and the scheduled time point may be used for the calculation of PK parameters. In cases of missing concentration data, the missing data will not be imputed. For the individual concentration and PK parameter calculation of each group, the following rules will be applied:

- If BLQ values occur between measurable concentrations in a profile, the BLQ should be omitted (set to missing, and the time point is ignored in computations).
- If a BLQ value occurs after the last measurable concentration in a profile, the BLQ should be treated as missing data.
- If two BLQ values occur in succession after the last measurable value, the profile is deemed to have terminated at the first BLQ value and any subsequent concentrations and time points are omitted from pharmacokinetic calculations.

For the concentration summary and mean concentration plot preparation of each group, the following rules will be applied:

2. Mean concentration at any individual time point will only be calculated if at least half of the subjects have valid values (i.e., quantifiable and not missing) at this time point for each arm.
    - In cases where a mean value is not calculated due to the above criterion not being met, the value will be set to missing.
    - BLQ values will be set to zero.

GM and GM CV% will not be calculated if there are zero values for concentrations.

A 1.1.3 Pharmacokinetic Concentration

Plasma concentrations and relevant parameters for ticagrelor and TAM will be summarized by control and group at each nominal time point for the PK Population using descriptive statistics such as mean, median, standard deviation, minimum, and maximum. Geometric mean (GM) and geometric coefficient of variation (GM CV %) will also be provided.

Individual ticagrelor and TAM plasma concentrations will be plotted on a linear and semi-log scale against actual sampling timepoints by. Mean (±SD) concentration of ticagrelor and TAM will be plotted on a linear and semi-logarithmic scale against nominal time points by group. The lower limit of quantification (LLOQ) will be plotted as a reference line on both individual and mean concentration plots.

Actual sampling times that are outside the sampling time windows may be excluded from concentration summary and mean concentration plotting but will still be used in the calculations of PK parameters and individual concentration plotting.

A 1.1.4 Pharmacokinetic Parameters

The following plasma PK parameters of ticagrelor and TAM will be determined with actual sampling time points using noncompartmental methods as appropriate:

Parameters	Description
AUC_{0-t}	Area under the plasma concentration vs time curve (AUC) from pre-dose (time 0) to the last quantifiable plasma concentration (C_{last})

No PK parameters will be calculated for subjects with 2 or fewer detectable concentrations in their PK profile.

AUC and plasma concentration for ticagrelor and TAM at each sampling timepoint (Pre-CPB, 30min, 60min, 90min, 120min, and post-CPB) will be compared between control and DrugSorb using a two-sample t-test, and if parametric assumptions are not met, then via a Mann-Whitney test.

The sample SAS code for this analysis is included here

```
proc ttest data=ADPP sides=2 alpha=0.05 h0=0;
        class TREATMENT;
        var log_PKParm;
        ods output ConfLimits=CL Statistics=Stat Ttests=Ttest;
```

A 2.0 Programming Specifications

The creation of analysis datasets and all analyses will be performed using SAS® (version 9.4 or higher). PK parameters will be calculated via SAS® and confirmed with the results of Phoenix WinNonlin™ (version 8.1 or higher). All results within the tables, figures, and listings (TLFs), including the PK analyses, are validated through independent double programming.