DOCUMENT: Statistical Analysis Plan

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Clinical Trial of the On-X® Valve Using Low Dose Anticoagulation

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ABBREVIATIONS:

ACC	American College of Cardiology
ACCP	American College of Chest Physicians
AE	Adverse event
AHA	American Heart Association
AATS	American Association for Thoracic Surgery
ASD	Atrial septal defect
AVP	Aortic valve replacement
BPM	Beats per minute
BSA	Body surface area
CAB	Coronary artery bypass
CI	Cardiac index
CO	Cardiac output
CRF	Case report form
CSA	Left ventricular outflow cross-sectional area in cm^2, calculated from the diameter assuming circular cross-section
DVI	Diastolic velocity integral from the mitral waveform
EOA	Effective orifice area
EOAI	Indexed EOA
FDA	Food and Drug Administration
GOA	Geometric orifice area
INR	International normalized ratio
KM	Kaplan-Meier
LD	Low dose
MOA	Mitral orifice area
MOA1/2	Mitral half-time orifice area
MVR	Mitral valve replacement
NYHA	New York Heart Association
OPC	Objective performance criteria
p_1	Mean pressure drop from the subaortic waveform
p_2	Mean pressure drop from the transaortic waveform
PI	Performance index
PHT	Pressure half-time
PTYR	Patient years
SAS®	Statistical Analysis System
SD	Standard deviation
STS	Society of Thoracic Surgeons
TE	Thromboembolism
v_1	Peak velocity from the subaortic waveform
v_2	Peak velocity from the transaortic waveform
v_m	Peak instantaneous early velocity from the mitral waveform
VSD	Ventricular septal defect
VTI_1	Velocity integral from the subaortic waveform
VTI_2	Velocity integral from the transaortic waveform

1. INTRODUCTION

This document describes the statistical methods and data presentations to be used in the summary and analysis of efficacy and safety data from Protocol 2005-01 (Clinical Trial of the On-X® Valve Using Low Dose Anticoagulation). Background information is provided for the overall study design and objectives. The reader is referred to the study protocol and case report forms (CRFs) for details of study conduct and data collection.

2. STUDY OBJECTIVE(S), TREATMENTS, AND ENDPOINT(S)

2.1 Study Objectives

The main study objective is to determine whether various patient groups with the On-X Valve can be maintained safely on lower doses of Coumadin® or on antiplatelet drugs only rather than the standard dose of Coumadin® and aspirin presently recommended by the ACC/AHA or ACCP professional societies.

2.2 Treatment Comparisons

Isolated Aortic Valve Replacement (AVR) –
Test Group I (high risk AVR) – For the first 3-months post-operation, Coumadin® at an international normalized ratio (INR) target of 2.0 to 3.0 with 81 mg/day aspirin will be used. After 3-months, the Coumadin® dose will be reduced to an INR target of 1.5 to 2.0 with 81 mg/day of aspirin continued.

Test Group II (low risk AVR) – For the first 3-months post-operation, Coumadin® at an INR target of 2.0 to 3.0 with 81 mg/day aspirin will be used. After 3-months, the Coumadin® dose will be removed and clopidogrel will be added using a loading dose of 300 mg followed by 75 mg/day with aspirin at 325 mg/day. AVR low risk patients who do not pass the hypercoagulability or drug response tests were moved to the high risk group until it was fully subscribed after which they became screen failures.

Control Groups – Postoperatively, Coumadin® will be administered for an INR target of 2.0 to 3.0 with 81 mg/day aspirin throughout the study. A separate control group will be used for each test group above.

Mitral Valve Replacement (MVR) –
Test Group III, (regardless of risk) – For the first 3-months post-operation, Coumadin® at an INR target of 2.5 to 3.5 with 81 mg/day aspirin will be used. After 3-months, Coumadin® dose will be reduced to an INR target of 2.0 to 2.5 with 81 mg/day of aspirin continued.

Control Group – Postoperatively, Coumadin® will be administered for an INR target on 2.5 to 3.5 with 81 mg/day aspirin throughout the study.

2.3 Study Endpoints

2.3.1 Safety

The primary study endpoints are the binary measures of occurrence or not of valve thrombosis, thromboembolism (TE), all major and minor bleeding events, whether or not related to drug therapy, reoperation, explant and death. The sum of all TEs (including valve thrombosis events) and major bleeding is used as the primary decision criterion for the primary endpoints.

Secondary safety study endpoints are other valve related adverse events (AEs) including the occurrence or not of endocarditis, hemolysis, hemolytic anemia, paravalvular leak and structural and non-structural dysfunction. All events shall be as defined by the AATS/STS guidelines [Akins CW, Miller DC, Turino MI, et al] for reporting valve study results.

2.3.2 Efficacy

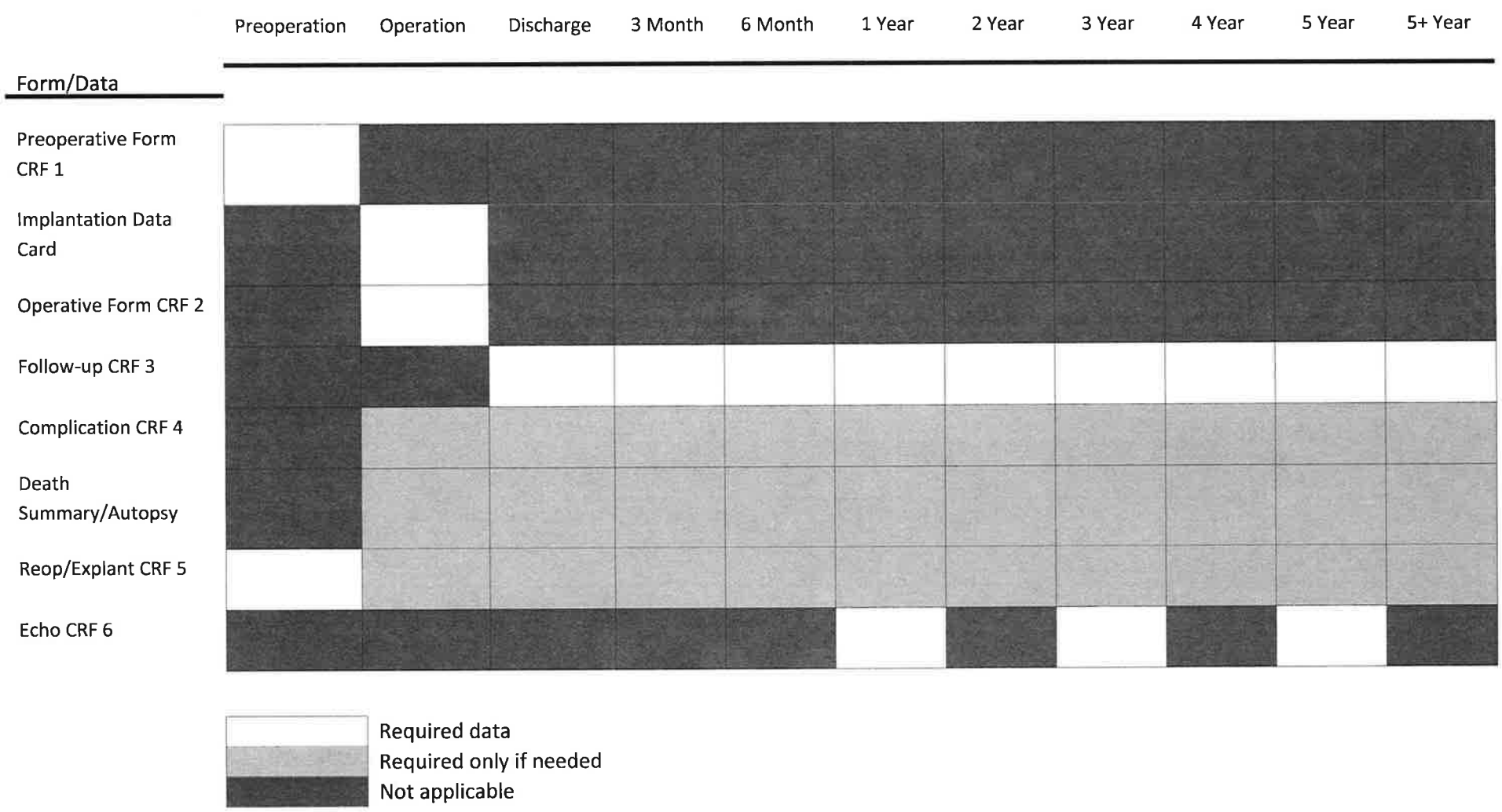
Effectiveness endpoints are also secondary endpoints and are the New York Heart Association (NYHA) functional classification scores and the echocardiographic hemodynamic measures of peak and mean gradient and effective orifice area (EOA). Echocardiographic exams are also used to evaluate indexed EOA, performance index, cardiac output, cardiac index and the amount and location of any valvular regurgitation.

3. STUDY DESIGN

3.1 Overall Study Design and Treatment Groups

This is a longitudinal, randomized (randomization to occur at the 3 month follow-up) study comparing the On-X valve on low dose anticoagulation (test group) to concomitant control groups of On-X valves receiving standard Coumadin®/aspirin therapy, and also to FDA objective performance criteria (OPC) [ISO 5840:2005 Cardiovascular implants – Cardiac valve prostheses and Draft guidance for industry and FDA staff – Heart valves – Investigational Device Exemption (IDE) and Premarket Approval (PMA) applications, January 20, 2010]. It is a multicenter study consisting of 40 centers in the United States enrolling no more than 1200 patients (200 in each of 6 groups)

3.2 Overall Schedule of Time and Events



4. SAMPLE SIZE CONSIDERATIONS

The study is expected to test the safety of low dose (LD) therapy for anticoagulant therapy in On-X valve patients with LD therapy consisting of the use of Coumadin® in combination with aspirin or the use of antiplatelet therapy alone. The sample size for this study was designed as a non-inferiority study requiring only that LD therapy is not inferior to standard Coumadin®/aspirin treatment. It was necessary for the sample size calculation to assume an acceptable event rate for the primary endpoints of thromboembolism, thrombosis and bleeding events derived by summing the FDA OPC's. The incident rate of the sum of these three events needs to be at least as low as the OPC sum, 7.3%.

The test group will need to have less than 6%/ptyr cumulative rate of the three events for the study to reach significance with a patient population of 200 per group followed for 5 years at a statistical power of 80% with type I error of 0.05 and a maximum allowable difference between groups of 1.5%.

5. ANALYSIS POPULATIONS

5.1 Safety Population

The safety population will be the primary population for all analyses and includes all patients who have received at least one dose of study drug.

6. CONSIDERATIONS FOR DATA ANALYSIS

6.1 Programming Environment

All analyses will be conducted using SAS® version 9.2.

6.2 Strata and Covariates

All primary and secondary analyses utilizing Kaplan-Meier life tables will be stratified by risk factors including age and gender. Additionally, MVR treatment groups will be stratified by continent (e.g. North America vs. Italy) for all primary and secondary endpoints.

6.3 Subgroups

There are no planned subgroup analyses.

6.4 Multiple Comparisons and Multiplicity

There are no planned adjustments for multiplicity.

6.5 Significance Level

Unless otherwise noted, all statistical analyses will be conducted with a significance level (α) of 0.05 and utilize two-sided testing.

6.6 Statistical Notation and Methodology

Unless stated otherwise, the term "descriptive statistics" refers to the number of subjects (n), mean, median, standard deviation (SD), minimum, and maximum for continuous data and frequencies and percentages for categorical data. Unless otherwise noted, all data collected during the study will be included in data listings and will be sorted by treatment group, subject number and then by date/time within each subject number.

7. DATA HANDLING METHODS

7.1 Missing Data

7.1.1 Date Values

For the purposes of analysis, imputation of incomplete or missing dates may be performed (i.e. determination of treatment-emergent/post-operative status).

For incomplete or missing dates, the date components will be imputed with the most conservative value possible (i.e. first day of the month if day is missing in a start date or earliest date in a visit window for a missing value).

7.1.2 Non-Date Values

Every effort will be made to obtain required data at each scheduled evaluation from all subjects who have been enrolled. There will be no imputation for missing data.

7.2 Visit Windows

Study periods will be windowed as applicable according to the following:

- Pre-operative
- Operative
- Discharge
- Follow-up:
  - 3 months + 1 month, - 0 months
  - 6 months ± 2 weeks
  - 1, 2, 3, 4, 5 or 5+ yrs (± 1 month)
- Reoperation
- Off schedule

Visits will be presented according to the nominal visit as obtained from the CRF and/or schedule of time and events (section 3.2). If multiple on-schedule assessments are collected for the same characteristic and fall within the same nominal visit window, then the latest value will be used for summary presentation. For off-schedule assessments, those used in the calculation of linearized rates and event

counts will be included in the summary presentations but excluded for those analyzed specifically for on-schedule visits (i.e. NYHA, anticoagulant therapy, etc.).

All assessments, both on-schedule and off-schedule, will be included in the data listings.

7.3 Data Derivations

Preoperative values will be considered as all assessments prior to surgery.

Baseline values will be considered as the last non-missing assessment prior to first dose of study drug.

Treatment-emergent events will be considered as any event occurring after the initiation of study drug.

The following calculations from Appendix F of the study protocol will be used for the echocardiographic assessments:

- EOA (aortic position) = CSA*VTI_1/VTI_2
- Mitral orifice area: MOA = CSA*VTI_1/DVI
- MOA by pressure half-time method: MOA1/2 = 220/PHT
- Peak pressure drop (aortic valve): Peak $\Delta P_a = 4(v_1^2 - v_2^2)$
- Mean pressured difference (aortic valve): Mean $\Delta P = p_1 - p_2$
- Peak pressure drop (mitral valve) Peak $\Delta P_m = 4v_m^2$
- Cardiac output: CO = CSA*VTI_1*BPM
- Indexed EOA: EOAI = EOA/BSA
- Cardiac index: CI = CO/BSA
- Performance Index: PI = EOA/GOA
- Body surface area: BSA (m^2) = $[\text{Weight(kg)}^{0.425} * \text{Height(cm)}^{0.725}] * 0.007184$

8. STUDY POPULATION

8.1 Demographic Characteristics

Demographic data will be summarized with descriptive statistics, by treatment group, for age and gender. Treatment group differences will be assessed with a two-sample Z-test for age and a chi-square test for gender.

8.2 Inclusion/Exclusion Criteria

Inclusion/Exclusion criteria outcomes will be included in a data listing showing whether subjects met all criteria.

8.3 Randomization Deviations

All deviations regarding the randomization of subjects to study drug will be included in a data listing.

8.4 Prior and Concomitant Cardiovascular Procedures

Concomitant cardiovascular procedures will be summarized by type (e.g. CAB, valve repair, ASD, VSD, etc.) and the valve repaired. Treatment group differences will be assessed with a Chi-square test. The data listing will also include the prior cardiovascular procedures.

8.5 Preoperative Characteristics

Predominant cardiac rhythm, disease etiology, valvular lesions, pre-operative anticoagulation, preoperative risk characteristics, and blood tests will be summarized with descriptive statistics by treatment group. Quantitative characteristics will be assessed for treatment group differences with a two-sample Z test and qualitative characteristics will use the Chi-square test of association between treatment groups.

All Preoperative characteristics will be included in data listings. Data included will consist of cardiac rhythm, disease etiology, valvular lesions and location, anticoagulation use, risk characteristics, blood test, and risk group.

8.6 Operation Data

Aortic and Mitral valve size and cross clamp time will be summarized with descriptive statistics by treatment group. Treatment group differences will be assessed with a two-sample Z test for cross clamp time and with the chi-square test for valve size.

All operative data will be included in data listings to include the following: complete valve information, suture technique, cross clamp time, cardioplegia, and Intraoperative drugs.

8.7 Intraoperative Adverse Events

Intraoperative AEs will be summarized with descriptive statistics by treatment group. The chi-square test will be used to assess the association between treatment groups. A data listing will also be included.

9. SAFETY ANALYSIS

9.1 Primary Analysis

The primary study endpoints are the binary measure of occurrence or not of valve thrombosis, thromboembolism, all major and minor bleeding events whether or not related to valve, reoperation, explant and death. Additionally, the sum of all TEs (including valve thrombosis events) and major bleeding will be included with the primary endpoint analyses.

For each of these binary endpoints, a Kaplan-Meier (KM) life table will be fitted and used to compare each valve treatment group and its control group. The KM analysis will adjust for select risk factors

including age and gender. In addition, the linearized rates as well as overall percentages of occurrences will be included in summary tables. Comparisons of overall percentages between each valve treatment group and its control will be made using a chi-square test or Fisher's exact test as appropriate. Linearized rates will be analyzed for differences between treatment and control groups with relative risks and 95% confidence intervals.

Analyses of mitral valve subjects will also be stratified by location (North America versus Italy) in the summary tables.

9.2 Secondary Analysis

Secondary safety study endpoints are the occurrence or not of other valve related adverse events including endocarditis, hemolysis, hemolytic anemia, paravalvular leak and structural and non-structural dysfunction. All events shall be as defined by the AATS/STS guidelines [Akins CW, Miller DC, Turino MI, et al] for reporting valve study results.

For each of these binary endpoints, a Kaplan-Meier life table will be fitted and used to compare each valve treatment group and its control group. The KM analysis will adjust for risk factors age and gender. In addition, the linearized rates as well as overall percentages of occurrences will be included in summary tables. Comparisons of overall frequencies and percentages between each valve treatment group and its control will be made using a chi-square test or Fisher's exact test as appropriate. Linearized rates will be analyzed for differences between treatment and control groups with relative risks and 95% confidence intervals.

Analyses of mitral valve subjects will also be stratified by location (North America and Italy) in the summary tables.

9.3 Complications and Death

All Complications will be included in comprehensive data listings sorted by treatment group and subject. Any AEs resulting in death will be included in a separate data listing.

9.4 Hemolysis Laboratory Evaluations

Descriptive summaries of results will be presented by laboratory test and treatment group.

A data listing will display all laboratory test results and findings.

9.5 Physical Assessments

Descriptive summaries of results heart rate and BSA as a result of physical assessments at the time of echocardiographs will be summarized with descriptive statistics by treatment group and time point.

A data listing will display all results from physical assessments.

10. EFFICACY ANALYSIS

Effectiveness endpoints are all secondary endpoints and are the New York Heart Association (NYHA) functional classification score and the echocardiographic hemodynamic measures of peak and mean gradient and effective orifice area (EOA).

Additional echocardiographic assessments including the indexed EOA, MOA by the pressure half-time method, performance index, cardiac output, cardiac index and amount and location of any valvular regurgitation will be included in the efficacy analyses.

All secondary endpoints and assessments will be summarized with descriptive statistics by treatment group and visit. Mitral valve subjects will also be stratified by location. To assess treatment group differences, quantitative endpoints will use a two-sample Z-test (or t-test for small samples), ordinal endpoints will use a Wilcoxon rank-sum test, and nominal endpoints will use the chi-square test of association.

All data will be included in data listings.

11. INTERIM ANALYSIS

No interim analyses are planned, but they may occur at the request of the Data Safety Monitoring Committee, or the request of the Principal Investigators and approval of the Sponsor as a result of their annual meeting, or the FDA.

12. END-OF-STUDY-ANALYSIS

A final analysis will be conducted after the last patient completes or discontinues the study and the resulting clinical database has been cleaned, quality checked, and locked.

13. REFERENCES

Akins CW, Miller DC, Turino MI, et al. Guidelines for Reporting Morbidity and Mortality after Cardiac Valvular Operations. J Thorac Cardiovasc Surg 2008; 135:732-8.

Cardiovascular implants – Cardiac valve prostheses, ISO 5840:2005(E), International Standards Organization, 2005.

Draft guidance for industry and FDA staff – Heart valves – Investigational Device Exemption (IDE) and Premarket Approval (PMA) applications, U.S. Food and Drug Administration, January 20, 2010.

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