ARCADIA

AtRial Cardiopathy and Antithrombotic Drugs In prevention After cryptogenic stroke

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STATISTICAL ANALYSIS PLAN

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1 LIST OF ABBREVIATIONS

AE	Adverse event
AF	Atrial fibrillation
AHA/ASA	American Heart Association/American Stroke Association
ARCADIA	AtRial Cardiopathy and Antithrombotic Drugs In prevention After cryptogenic stroke
CRF	Case report form
CI	Confidence interval
DCU	Data Coordination Unit
DCR	Data clarification request
DSMB	Data and safety monitoring board
ECG	Electrocardiogram
ESUS	Embolic stroke of undetermined source
HR	Hazard ratio
ITT	Intention to treat
MOP	Manual of procedures
NCC	NIH StrokeNet National Coordinating Center
NDMC	NIH StrokeNet National Data Management Center
NIH	National Institutes of Health
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NOAC	Non-vitamin K antagonist oral anticoagulant drug
NT-proBNP	Amino terminal pro-B-type natriuretic peptide
PI	Principal Investigator
PROMIS	Patient-Reported Outcomes Measurement Information System
$PTFV_1$	P-wave terminal force in ECG lead V_1
QVSFS	Questionnaire for Verification of Stroke-Free Status
RCC	Regional Coordinating Center
SAE	Serious adverse event
SD	Standard deviation
SOP	Standard operating procedure

2 KEY ROLES

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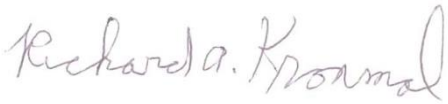

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3 STATISTICAL ANALYSIS PLAN AND STATISTICAL REPORTS

This document provides the details of statistical analyses planned for the ARCADIA trial, including interim analyses for efficacy and futility. In addition, it discusses the statistical issues relevant to these analyses (e.g., sample data to be used, missing data, adjustments for multiplicity, etc.).

The DCU generates two statistical reports: an open report to be distributed to the ARCADIA Executive Committee and the DSMB, and a closed report to be distributed only to the DSMB. The timing of these reports is determined in consultation with the DSMB. Reports will be sent from the DCU to the NINDS liaison two weeks in advance of the scheduled meeting.

Each report provides cumulative summary statistics on enrollment; participant status in the study (e.g., number who completed each study visit assessment); baseline characteristics; protocol violations; data management/quality information (e.g., timeliness and completeness of data entry by the clinical sites; number of DCRs generated and resolved); and safety data, including SAEs by AE code, severity, expectedness (anticipated/unanticipated), and relatedness to the study medication. Aggregate rates of SAEs including atrial fibrillation/flutter and excluding clinical outcomes (stroke [ischemic, hemorrhagic, or undetermined type], symptomatic hemorrhagic transformation of ischemic stroke, intracranial hemorrhage excluding either hemorrhagic stroke or symptomatic hemorrhagic transformation of ischemic stroke, transient ischemic attack, major hemorrhage excluding intracranial hemorrhage, minor hemorrhage, myocardial infarction, systemic embolism, symptomatic deep venous thrombosis, and symptomatic pulmonary embolism) are provided in the open report; data on all other endpoints are restricted to the closed report. The statistics are provided for the overall study as well as by site or RCC, where appropriate, in the open report. For the closed report only, the statistics are also provided by treatment group (A vs B). If a report coincides in timing with a planned interim analysis, the analysis results are appended to the report.

The DCU also generates a blinded quarterly report to be distributed to the ARCADIA Executive Committee which contains updated aggregate data on enrollment, baseline, safety information, and loss to follow-up and crossovers. In addition, this report contains a table that shows what proportion of consented patients were randomized and on which atrial cardiopathy marker(s) these participants were deemed eligible, including summary statistics of the atrial cardiopathy marker values.

4 BACKGROUND AND RATIONALE

In one-third of ischemic strokes, a specific cause cannot be identified. Many of these cryptogenic strokes appear to arise from a distant embolic source. Recent evidence suggests that some cryptogenic strokes may arise from left atrial thromboembolism that goes unrecognized because it has not manifested with atrial fibrillation/flutter (AF). These data suggest that an underlying atrial cardiopathy may provide the substrate for thrombus formation and embolization even in the absence of AF.

In parallel with these new insights about cryptogenic stroke, new therapeutic options for stroke prevention have become available. Non-vitamin K antagonist oral anticoagulant (NOAC) drugs such as apixaban may be more effective than aspirin for treatment of cryptogenic stroke. A benefit is especially likely in cryptogenic stroke patients with atrial cardiopathy because of parallels with AF and because an analysis of data from the WARSS/APASS studies suggests that cryptogenic stroke patients with one marker of atrial cardiopathy (elevated NT-proBNP) and no obvious AF benefit from anticoagulant therapy.[1] Apixaban is a particularly attractive choice because it has a low bleeding risk,[2,3] lowers mortality more than warfarin in patients with AF,[4] and is the only NOAC drug with a Class 1A recommendation in recent AHA/ASA guidelines.[5]

The ARCADIA trial is designed to address several important knowledge gaps. First, it will advance knowledge about stroke pathophysiology by assessing whether atrial cardiopathy is a valid therapeutic target, which may set the stage for a primary prevention trial. Second, this trial will advance knowledge about optimal secondary stroke prevention therapy. One industry-sponsored trial (NAVIGATE-ESUS) that compared rivaroxaban to aspirin in patients with cryptogenic stroke was stopped early for futility. Another industry-sponsored trial (RESPECT-ESUS) is comparing dabigatran versus aspirin in patients with cryptogenic stroke. By including all cryptogenic stroke patients and by including those with up to 6 minutes of AF per day, these trials have mixed patients with heterogeneous stroke mechanisms. These trials may thus fail to show an overall benefit (as in NAVIGATE-ESUS), or may show an overall benefit driven mostly by patients with known AF and atrial cardiopathy, resulting in an overly broad indication for anticoagulant therapy. In the first instance, the ARCADIA trial may ensure that a valuable treatment in a specifically targeted subgroup will not be prematurely abandoned. In the second instance, the results of the ARCADIA trial would provide a compelling rationale to perform subgroup analyses of the industry-sponsored trials and conduct future trials to determine the risks and benefits of anticoagulant therapy across biologically distinct subgroups of stroke patients. Given the expense and risks of bleeding associated with anticoagulant drugs, it is imperative to define as precisely as possible the groups of stroke patients who would and would not benefit from their use. Therefore, the approach taken by the ARCADIA trial comports well with the general move toward precision medicine.

5 SYNOPSIS OF THE STUDY

ARCADIA is a multicenter, biomarker-driven, randomized, double-blind, active controlled Phase III clinical trial of 1,100 participants, conducted at up to 200 enrolling sites. Participants will be followed for a minimum of 1.5 years. Patients who have evidence of atrial cardiopathy and a recent stroke of undetermined source by current criteria will be randomly assigned to apixaban or aspirin (active control therapy) in a 1:1 ratio. Atrial cardiopathy will be defined as one or more of the following biomarkers: P-wave terminal force in electrocardiogram lead V1 >5,000 µV*ms, left atrial size index ≥3.0 cm/m2 on echocardiogram, and serum amino terminal pro-B-type natriuretic peptide >250 pg/mL. Standard heart-rhythm monitoring will be performed before enrollment to exclude as thoroughly as possible those patients with atrial fibrillation. Therapy must be initiated within 3-180 days of enrolling stroke. The primary efficacy endpoint is time to recurrent stroke (ITT analysis). Secondary efficacy endpoints are time to (1) recurrent stroke or death and (2) recurrent ischemic stroke or systemic embolism. There will be a test of the interaction of the atrial cardiopathy selection variables with treatment arm for the primary outcome. The primary safety outcomes are: (1) symptomatic intracranial hemorrhage (including symptomatic hemorrhagic transformation of an ischemic stroke) and (2) major hemorrhage other than intracranial hemorrhage. Additional safety endpoints include all-cause mortality.

6 OUTCOME VARIABLES

6.1 PRIMARY EFFICACY OUTCOME

The primary efficacy outcome is recurrent stroke of any type (ischemic, hemorrhagic, or of undetermined type). Possible stroke events will be centrally adjudicated, and the primary outcome measure will be defined as the centrally adjudicated stroke event, NOT the site determined event.

If a site identifies an event as a possible primary efficacy outcome, the participant will stop study medication. If the adjudication committee determines the event meets the primary efficacy definition, the participant will stop participation in the study, including stopping study drug; otherwise they may stop, continue, or resume study drug at the discretion of the treating physician.

6.2 SECONDARY EFFICACY OUTCOMES

The secondary efficacy outcomes are: (A) composite of recurrent ischemic stroke or systemic embolism, and (B) composite of recurrent stroke of any type or death from any cause. Possible events will be centrally adjudicated, and the outcome measure will be defined as the centrally adjudicated event, NOT the site determined event. Note that recurrent ischemic stroke is a subset of the primary outcome definition and thus the participant would stop participation in the study, including stopping study drug. In the case of systemic embolism, the participant may continue in the trial until the end of follow-up or occurrence of the primary endpoint.

6.3 PRIMARY SAFETY OUTCOMES

The primary safety outcomes are: (A) symptomatic intracranial hemorrhage (including symptomatic hemorrhagic transformation of an ischemic stroke), and (B) major hemorrhage other than intracranial

hemorrhage. Symptomatic intracranial hemorrhage is defined as any extravascular blood within the cranium, including subdural and epidural blood, associated with and identified as the predominant cause of new neurologic symptoms, including headache, or leading to death. Major hemorrhage other than intracranial hemorrhage is defined as clinically overt bleeding accompanied by a ≥2 g/dL decrease in the hemoglobin level during a 24-hour period, transfusion of ≥2 units of whole blood or red cells, involvement of a critical non-intracranial site (intraspinal, intraocular, pericardial, intraarticular, intramuscular with compartment syndrome, or retroperitoneal), or death.

Note that symptomatic intracranial hemorrhage may not meet the primary outcome definition of stroke, in which case the participant is eligible to continue in the trial; this is true for other safety outcomes as well.

6.4 SECONDARY SAFETY OUTCOMES

The secondary safety endpoint is all-cause mortality. Note that death is included as part of a composite secondary efficacy endpoint.

7 SAMPLE SIZE DETERMINATION FOR PRIMARY EFFICACY OBJECTIVE

7.1 SAMPLE SIZE ESTIMATION

The primary outcome variable is the time to recurrent stroke of any type. Preliminary data suggest a 7% annual risk of recurrent stroke of any type in aspirin-treated patients, with a clinically relevant relative risk reduction of 40% (hazard ratio of 0.6, hazard rate of 4.2%) for patients treated with apixaban. Based on preliminary feasibility data and 120 sites, ARCADIA plans to recruit 1,100 participants during a 30-month uniform accrual period and follow these participants to the end of the study (i.e., 18-month minimum follow-up period). In addition, it is anticipated that there will be a 3% annual rate of crossover from blinded aspirin to open-label anticoagulation due to AF detection, and a 3% annual rate of crossover from blinded apixaban to open-label antiplatelet therapy due to bleeding or other adverse events. Finally, it is estimated that there will be a 5% annual rate of loss to follow-up or death in each group.

Given the assumptions above, enrollment of 1,100 participants (approximately 150 recurrent stroke events) will provide 80% power to demonstrate a statistically significant difference in the hazard for recurrent stroke with apixaban compared to aspirin at an alpha level of 0.05 while allowing for one interim look for efficacy (or harm) and futility after 1/2 of expected events have occurred (75 events). To maintain the power as planned and the overall alpha of the study at 0.05, an O'Brien-Fleming type Lan-DeMets error spending function will be used. The sample size was calculated using the "Design: Survival Endpoint: Two-Sample Test – Parallel Design – Logrank" program in EAST® v6 software (Cytel Corporation) (Lan and DeMets, 1983).

7.2 REVISIONS TO THE GROUP SEQUENTIAL BOUNDARIES

It is possible that the information fraction at the interim analysis may be incorrect due to incorrect estimation of the total number of events in the trial. However, given this trial's extremely conservative interim analysis boundaries, it is unlikely that such misestimation would substantively affect the final

test statistic. Furthermore, there is inherent difficulty in accurately estimating the total number of events even at the time of the interim analysis. Therefore, there will be no revisions to the group sequential boundaries.

8 DEFINITION OF TARGET POPULATION AND STUDY SAMPLES

8.1 TARGET POPULATION

The population that will be randomized in the ARCADIA trial comprises patients 45 years of age and older who have a clinical diagnosis of embolic stroke of undetermined source (ESUS) within the prior 180 days, a modified Rankin Scale score ≤4 at the time of screening, and at least one of the following criteria for atrial cardiopathy:

- [ECG]: PTFV1 >5,000 µV*ms on 12-lead ECG.
- [ECHO]: Left atrial size index ≥3 cm/m^2 on echocardiogram.
- [NT-proBNP]: Serum NT-proBNP >250 pg/mL.

8.2 INTENT-TO-TREAT SAMPLE

Under the ITT principle, the evaluable sample includes all participants who are randomized. Each participant will be analyzed according to the treatment group to which they were randomly assigned at the time of randomization.

8.3 SAFETY-ANALYSIS SAMPLE

The safety sample includes all participants who are randomized and receive at least one dose of study medication. The safety sample will include all safety outcomes from randomization until 30 days after permanent discontinuation of study drug. The primary safety analysis will define treatment arm as the treatment to which a participant was randomized. Additional sensitivity analyses will define a time-dependent treatment covariate, and possibly a lagged time-dependent treatment covariate.

8.4 PER-PROTOCOL SAMPLE

In addition to the defined ITT analysis sample, a per-protocol sample is defined as a subset of the ITT sample. This sample will be used for sensitivity analyses of the primary and secondary efficacy outcomes. The per-protocol sample will include all randomized participants who do not have the following protocol deviations:

- Eligibility violation.
- Treatment never started.

For these sensitivity analyses, treatment will first be defined as randomized, but a time-dependent treatment covariate with concurrent treatment effects may be considered.

9 GENERAL STATISTICAL CONSIDERATIONS

9.1 PARTICIPANT ACCOUNTABILITY

A flowchart will be created to present a summary of participants' status. This flowchart will first list the number of patients who were eligible and consented to be screened for the biomarkers. Of consented patients, the chart will then delineate those who did not meet criteria for any of the three biomarkers, those who were positive for at least one biomarker but no longer eligible at the time of randomization or declined randomization or were not randomized for another reason, and those randomized to a treatment group. Then, within each treatment group, it will list the number of participants who were lost to follow-up, the number of participants who withdrew, and the number of participants who were included in the primary analysis.

9.2 RANDOMIZATION

Eligible participants will be allocated in a 1:1 ratio to apixaban or aspirin using a randomization method that controls the maximum tolerated imbalance within each RCC. Implementation details are provided in the randomization plan and validation documentation.

A web-based central randomization system will be developed by the NDMC and installed on the WebDCU™ ARCADIA study website. The randomization algorithm and its implementation will be fully detailed in the ARCADIA Randomization Plan and the Randomization Validation documents. These documents will be developed prior to enrollment initiation and stored in a secure location at the NDMC. The documents will be archived with the study database at the end of the trial.

The participant randomization system is fully integrated into the ARCADIA study database, and presented as a special CRF in the electronic data capture module. Data edit permission for the randomization CRF is centrally managed based on user groups. Site study team members are limited to viewing and editing data only for participants in their own site. Site enrollment status, participant eligibility, and site study drug availability are verified prior to the performance of participant randomization in order to protect data integrity. The participant randomization system checks the drug availability at both the site level and the individual participant level and triggers site study drug shipment and participant drug resupply requests accordingly.

Participant treatment assignment information is masked by using the Study Drug ID, which is a globally unique no-sequence 6-digit number placed on each study drug kit. Access to treatment assignment information is prohibited for all blinded investigators. Authorized emergency unblinding for medical safety purposes is provided through a medical emergency hotline with one of the trial PIs responding to the treating physician. All cases of unblinding are recorded in the study database.

9.3 BLINDING

The study is conducted in a double-blinded manner. Study drug administration, clinical care, and outcomes assessments are made by the clinical site staff blinded to the treatment assignment. Participants will not be told of their actual treatment assignment until the trial is complete, at which point assignments are available upon request, or during the trial in the event of a medical emergency

that requires unblinding. The Executive Committee and the MSM are also blinded to the treatment assignment.

The NDMC biostatistician and statistical programmer will be partially unblinded; they know the participants' group classification (A or B), but not whether A is apixaban or aspirin. During the course of the trial it may be necessary for them to become unblinded if the DSMB requests. The clinical adjudicators will attempt to remain blinded, but information in the narrative may on rare occasions unblind specific cases. The Executive Committee members are completely blinded.

The DCU's unblinded staff generates: (1) a hard copy list (obtained from the WebDCU™) of study drug kit numbers and their corresponding study drug (apixaban or aspirin) as well as the lot numbers and expiration dates; and (2) two identical sealed envelopes that contain identification of treatment codes (e.g., A = apixaban, B = aspirin). Prior to initiation of the trial, one envelope is given to the NINDS liaison to the DSMB. Another is maintained in a locked file cabinet in a limited access central file room along with the hard copy list described in (1).

The central pharmacy personnel at the StrokeNet NCC at the University of Cincinnati are completely unblinded. They have access to the WebDCU™ to match the study drug (apixaban or aspirin) to its corresponding study drug kit number.

The DSMB (as well as the NINDS liaison to the DSMB) is partially unblinded. However, if it so wishes, it may be completely unblinded at any time during the trial. One of the sealed envelopes described in (2) above is given to the NINDS liaison to the DSMB prior to initiation of the trial. If the DSMB wishes to be unblinded on a particular participant only, the NINDS liaison to the DSMB can email the request to the NDMC biostatistician.

In case of a medical emergency for a study participant who requires unblinding, clinical staff can become unblinded only to that participant's treatment by calling the emergency hotline. Clinical staff would then request permission for unblinding to occur and provide a brief clinical summary to one of the responding trial PIs. The PI would perform unblinding via the WebDCU™ ARCADIA study website and inform the clinical staff. Unblinding should only occur if a compelling clinical reason arises, such as active major bleeding or a pending decision on whether to administer intravenous thrombolysis for acute ischemic stroke. Participants who undergo unblinding will stop study drug but continue to be followed as part of the study and will be analyzed according to the intention-to-treat principle.

Details such as how kits will be labeled and how the study drug is blinded are provided in the Study Drug Plan.

9.4 CENSORING PARTICIPANTS

9.4.1 PRIMARY OUTCOME

Participants will be followed until the end of the trial follow-up period, which will be 18 months after enrollment of the last participant. Due to regulatory reasons, participants will have a final telephone visit 30 days after the trial follow-up period to assess SAEs related to discontinuation of study drug, but this 30-day period will not contribute to participants' time-at-risk for the purposes of analysis. Under the ITT principle, all participants who are randomized are included in the analysis. Participants will be censored at the date of their last follow-up visit in cases of loss to follow-up or withdrawn consent, or at the date of death in cases of death.

9.4.2 DEATH

The primary analysis codes death as a censoring event. Sensitivity analyses will be conducted where death is treated as a competing risk.

9.4.3 CROSSOVER TO OPEN-LABEL DRUG

If a participant is known to have permanently crossed over onto open-label drug (e.g., an anticoagulant in the case of AF diagnosis, antiplatelet drug or no antithrombotic therapy in the case of bleeding events on apixaban, antiplatelet therapy for a new coronary event), the participant will continue to be followed and analyzed in the treatment group they are assigned. A sensitivity analysis using a time-dependent treatment effect, as well as an analysis accounting for pill count compliance data, will be considered.

9.4.4 WITHDRAWN CONSENT AND LOSS TO FOLLOW-UP

Using recommended methods,[6] sensitivity analyses will be conducted to assess the effect of any censoring that occurs due to a participant being lost to follow-up or withdrawing consent. Based on other large stroke prevention trials, we anticipate no more than a 4% lost to follow up rate. At the time of each planned analysis (interim and final), the unblinded statistician will report the amount of missing primary outcome data.

9.4.5 SECONDARY AND SAFETY OUTCOMES

In cases where observation of the primary outcome precludes observation of secondary or safety outcomes, the primary analysis will treat the participant as censored. Sensitivity analyses will explore alternative modeling strategies, in particular competing risk, Markov models, or joint modeling approaches.

9.5 TREATMENT GROUP COMPARABILITY

A description of the baseline characteristics of trial participants will be presented by treatment group. Dichotomous variables will be summarized as number (%). Percentages will be calculated based on the number of participants with available data for that variable. Continuous variables will be summarized by the mean and SD. In the case of variables with missing values, the denominator will be stated in the

summary table or in a footnote to the summary table. The baseline characteristics of interest include age, sex, race, time from qualifying stroke, CHA_2DS_2-VASc score, weight, and the value of each of the biomarker inclusion criteria. Statistical significance of covariates will not be assessed. In addition, all of the above covariates, regardless of imbalances between groups, will be included as covariates in multivariable regression in a secondary analysis of the primary aim.

9.6 SITE EFFECTS

Several procedures have been incorporated into the study design (i.e., procedure manual, training and certification programs, protocol violation monitoring, blinding) to reduce trial site effects; however, possible effects will not be ignored for this trial. The distribution of demographics will be examined by RCC using means (SD) and proportions (95% CIs), and RCC and RCC*treatment interaction terms will be included as a random effect in a secondary analysis of the primary outcome.

9.7 PARTICIPANT ADHERENCE

Participants will be requested to bring in their study pill bottles at study visits for pill counts. For each visit interval, adherence by pill count will be measured as (# pills supplied - # remaining pills)/# pills supplied. Adherence will be classified as low (<60%), medium (60-79%), or high (≥80%). A sensitivity analysis of the primary endpoint will use a time-dependent compliance indicator to assess the impact of non-compliance on hazard of stroke. Note that participants are supplied with a buffer of 10 pills per bottle. The calculation listed above will adjust for the number of days between visits (i.e., a participant with 100% compliance and 90 days between study visits will be listed as 100% compliant).

10 EFFICACY ANALYSIS

10.1 PRIMARY EFFICACY ANALYSIS

10.1.1 PARAMETER DESCRIPTION

The primary efficacy analysis will use the ITT sample to assess time to recurrent stroke of any type. Censoring of participants is described in Section 0.

10.1.2 STATISTICAL HYPOTHESES

The null hypothesis (H_0) is that the hazard ratio comparing apixaban versus aspirin for the primary endpoint will be 1. The alternative (H_A) is that the hazard ratio is not 1. That is,

$$H_0: h_{apix}(t) = h_{asp}(t) \qquad \text{versus} \qquad H_A: h_{apix}(t) \neq h_{asp}(t)$$

Where $h_{apix}(t)$ and $h_{asp}(t)$ are the hazard rates at time t for the apixaban and aspirin groups, respectively. The minimum effect size of clinical interest is a relative HR of 0.6. The estimated yearly event rate in the placebo group is assumed to be 7%.

10.1.3 STATISTICAL MODEL

$$Z = \frac{\sum_{i=1}^{D} W(t_i)\left[d_{i1} - Y_{i1}\left(\frac{d_i}{Y_i}\right)\right]}{\sqrt{\sum_{i=1}^{D} W(t_i)^2 \frac{Y_{i1}}{Y_i}\left(1-\frac{Y_{i1}}{Y_i}\right)\left(\frac{Y_i - d_i}{Y_i - 1}\right)d_i}}$$

Which has a standard normal distribution for large samples when H_0 is true. Using this statistic, an α level test of the alternative hypothesis $H_A : h_1(t) \neq h_2(t)$, for some $t \neq \tau$, is rejected when $|Z| > Z_{\alpha/2}$. Let W(t)=1 for all t, which represents the log-rank test.

Efficacy Outcome	**Analysis Method to Compare Apixaban and Aspirin Groups (SAS® v9 Procedure)**
Log-Rank Test of Survival	PROC PHREG

To test the null hypothesis, we will use the log-rank test.
In the above model, an event (Status) and event time (Time) are defined as follows:

First Event	Status	Time
Recurrent stroke of any type	1	Time of first stroke of any type
Systemic embolism	N/A (continue to follow)	
Death	0	Time of death
Diagnosis of AF	N/A (continue to follow)	
Study drug stopped	N/A (continue to follow)	
Loss to follow-up or consent withdrawn	0	Last known contact
Study team requires unblinding and recurrent stroke of any type has not yet occurred	N/A (continue to follow)	

10.1.4 INTERIM ANALYSIS METHOD

The study is designed using one interim look for both efficacy and futility for the primary outcome, and one final look, for a total of two planned analyses of the primary outcome. The interim analysis plan uses the error spending function method with O'Brien-Fleming type stopping guidelines. The error spending function distributes the type I and II error rates across the interim monitoring points giving the flexibility of changing the intervals of monitoring while still preserving the overall type I and II error rates. The O'Brien-Fleming type boundary is considered conservative as its boundaries make it difficult to terminate a study early by requiring extreme early evidence of efficacy or futility. It spends smaller amounts of alpha at the first look and gradually increases the spending as more information is acquired. The trial may be stopped for overwhelming efficacy of one treatment group over the other or for futility at the planned interim analyses if the test statistic crosses the respective boundaries.

The prespecified plan is to conduct the first interim analysis after 75 cumulative adjudicated primary outcome events (estimated information fraction of ½), or approximately 800 participants have been

randomized. Assuming an accrual rate of 36.7 participants per month, it is anticipated that the first look will occur roughly 3.5 years from the start of enrollment. The interval may be altered if requested by the DSMB. The stopping boundaries are defined using the Lans-DeMets O'Brien-Fleming type spending functions (Lan and DeMets, 1983). The futility boundaries are derived as non-binding, meaning that if a futility boundary is crossed it can be overruled without inflation of the type I error rate. If the crossing of an efficacy boundary is overruled, then this decision can impact the type II error rate but not the type I error rate. EAST® v6 software (Cytel Corporation) was used for the boundary calculations. The following table lists the test statistics at a particular look. The NDMC will be responsible for conducting these analyses and compiling the reports for the DSMB. Since several factors need to be taken into consideration before stopping a study, safety and study progress will also be taken into consideration by the DSMB and Executive Committee when deciding whether to stop the study if an efficacy or futility boundary is crossed.

Table. Nominal Critical Values and Alpha Levels for One Interim Analysis for Overwhelming Efficacy and Futility (O'Brien-Fleming, two-sided $\alpha = 0.05$)

	Exp. # events under H_0	Cum. α	Efficacy Boundary		Cum. β	Futility Boundary	
			Z Scale	HR		Z Scale	HR
Interim Analysis	75	0.003	|Z|>2.963	HR>2.123 -OR- HR<0.471	0.04	|Z|<0.356	HR>0.914 -AND- HR<1.095
Final Analysis	150	0.05	|Z|>1.969	HR>1.424 -or- HR<0.702	0.20		

10.1.5 FINAL ANALYSIS METHOD

The primary outcome analysis of the time to stroke of any type will use a log-rank test statistic to assess whether there is a difference in the hazard comparing apixaban to aspirin. Outcome differences will be analyzed under the ITT principle, so all randomized participants will be included in the primary analysis sample. To assess efficacy, the treatment groups will be compared with respect to the hazard of experiencing a primary outcome event. A log-rank test will be performed to compare the treatment group proportions using a cumulative two-tailed significance level of 0.05. An unadjusted HR will be reported with two-sided 95% CIs. At the final analysis, the null hypothesis will be rejected if the HR is greater than 1.424 or less than 0.702; we will fail to reject the null hypothesis at the 0.05 level of significance otherwise.

10.1.6 ADDITIONAL ANALYSES OF THE PRIMARY EFFICACY OUTCOME

10.1.6.1 Adjusting for Key Covariates

In secondary analyses of the primary outcome, we will adjust for the prognostic variables listed in Section 9.5. A Cox proportional hazards model will be used, after the verification of variable and model assumptions and goodness-of-fit assessments accompanying each analysis. Schoenfeld residuals will be used to assess the assumption of proportional hazards, and if significant deviations are found, we will

analyze treatment effect according to time period. These secondary analyses will be reported in the paper reporting the primary results of the trial.

10.1.6.2 Accounting for Crossover to Open-Label Therapy

In a secondary analysis, we will censor participants at the time of AF detection, when they are switched to open-label anticoagulation. This analysis will be used to explore the utility of anticoagulation in participants with atrial cardiopathy before they have manifested AF and therefore remain "cryptogenic" based on the prevailing clinical paradigm. Alongside this analysis, we will also perform an alternative analysis treating AF as a competing risk rather than a censoring point, given very recent theoretical data suggesting that apixaban may reduce the risk of AF itself, not just stroke. Lastly, we will perform another alternative analysis setting the outcome as stroke of any type occurring before the time of AF detection.

10.1.6.3 Accounting for Unblinding

In a secondary analysis, we will censor participants at the time of unblinding, when they are switched to open-label antithrombotic therapy per the discretion of the treating physician.

10.2 INTERACTION OF ATRIAL CARDIOPATHY SEVERITY AND TREATMENT EFFECT

This secondary analysis will explore the relationship between the severity of atrial cardiopathy and the relative efficacy of apixaban over aspirin. The objective of this analysis is two-fold. First, it will be used to assess the degree to which the severity of atrial cardiopathy modifies the efficacy of anticoagulation. Second, it will be used, regardless of whether or not apixaban is superior to aspirin in the overall trial population, to identify high-risk subgroups of participants with severe atrial cardiopathy who substantially benefit from apixaban.

10.2.1 TESTS OF INTERACTION

A Cox regression model will be used to assess interactions between each of the three atrial cardiopathy biomarkers and treatment status. The dependent variable will be recurrent stroke of any type. Independent variables will be a categorical variable for treatment status (apixaban versus aspirin) and three continuous variables representing each atrial cardiopathy biomarker. The atrial cardiopathy biomarkers will be appropriately transformed if necessary (e.g., log-NT-proBNP). Two-way interaction terms will be included between the treatment status variable and each atrial cardiopathy biomarker variable.

The z statistics for the individual interaction hazard ratios will be used to test the significance of the interaction terms. Each of the tests for the three biomarker-treatment interactions will be considered statistically significant if its individual *P* value is <0.016. This conservative *P* value is chosen to account for the multiple hypothesis testing in this exploratory analysis. Given the hypothesis test for the primary endpoint, the tests of the three interactions will exceed an experiment-wide alpha threshold of 0.05, but the alpha threshold of 0.016 for each interaction test will provide a conservative approach to the evaluation of interactions. Given that these tests of interaction are exploratory analyses, formal power calculations are not provided.

10.2.2 IDENTIFICATION OF HIGH-RISK SUBGROUPS

If one or more of the interactions are statistically significant, a prediction score computed from the Cox model will be used to predict the degree of benefit of apixaban over aspirin in a given participant based on the severity of atrial cardiopathy. Such a risk score will be important regardless of the main findings of this trial. If Aim 1 is negative (no benefit of apixaban versus aspirin in the overall trial cohort), the prediction score will be used to identify subgroups of patients with severe atrial cardiopathy who may still benefit from apixaban despite the negative findings in the overall trial population. This would inform follow-up studies of antithrombotic therapy in patients with cryptogenic stroke. If Aim 1 is positive (apixaban proves superior to aspirin in the overall trial cohort), the prediction score will be used to assess whether absolute stroke risk reductions in the highest-risk atrial cardiopathy subgroups are large enough to justify a primary prevention trial.

10.2.3 ADDITIONAL EXPLORATORY MODELING

Since the effect of the atrial cardiopathy biomarkers or their interactions with the treatment assignment may be nonlinear, analyses will also be carried out using the generalized additive model in the Cox regression to allow for nonlinearities in the interactions of the biomarkers with treatment status. For this purpose, the approach and methodology described by Royston (Royston, 2000; Royston and Sauerbrei, 2004) and the associated functions available in Stata (MVRS and FP2) will be used. If there is evidence of important nonlinearities in the biomarker-treatment interactions, the risk score derived from this more complex model will be evaluated and compared to the linear model described in in 11.2.2. Even if there is no effect modification of the atrial cardiopathy variables on the efficacy of apixaban versus aspirin, we will evaluate these markers as predictors of stroke risk in the full sample of participants in whom we have measured these markers and for whom we have follow-up for stroke events. We will construct a prediction risk score which will also include the other prognostic variables defined in Section 9.5. Such a risk score will potentially be valuable for identifying subgroups of patients with atrial cardiopathy in whom the absolute benefit of apixaban is high enough to justify a primary prevention trial.

10.2.4 STATISTICAL MODEL

The following is the right hand side of the full Cox proportional hazards model:

$$= \beta_0 + \beta_1 I_{Tx} + \beta_2 X_{ECG} + \beta_3 X_{Echo} + \beta_4 X_{BNP} + \beta_5 I_{Tx} * X_{ECG} + \beta_6 I_{Tx} * X_{Echo} + \beta_7 I_{Tx} * X_{BNP} + \left[\sum \beta_j X_j\right]$$

Where I_{TX} is an indicator with the value of 1 if the participant is on apixaban or 0 for aspirin, $X_{ECG,\ ECHO,\ BNP}$ are the continuous values of the three biomarkers, and X_j are the values of other prognostic variables.

From the full model, standard backward elimination regression modeling procedures will be used to identify the final model. Specifically, we will first test the interaction term:

H_0: $\beta_5 = 0$; H_0: $\beta_6 = 0$; H_0: $\beta_7 = 0$

If it is not statistically significant at $\alpha = 0.016$, these terms will be dropped from the model and the covariates β_j will be tested in a stepwise approach. For the risk-based modeling, the fitted estimates of the final model will be reported given a participant's covariates, e.g. $\{X_{ECG}, X_{Echo}, X_{BNP}, X_j\}$, under apixaban and under aspirin, as well as the hazard ratio.

10.3 SECONDARY EFFICACY ANALYSIS

10.3.1 PARAMETER DESCRIPTION

The secondary efficacy outcomes are: (A) composite of recurrent ischemic stroke or systemic embolism, and (B) composite of recurrent stroke of any type or death from any cause. Each of these two outcomes will be analyzed separately using the ITT sample (Section 8.2).

10.3.2 STATISTICAL HYPOTHESES

The set of statistical hypotheses can be stated in terms of hazard functions as:

H_0: $h_{apix}(t)=h_{asp}(t)$ versus H_A: $h_{apix}(t) \neq h_{asp}(t)$

Where $h_{apix}(t)$ and $h_{asp}(t)$ are the hazard rates for the apixaban and aspirin groups, respectively.

STATISTICAL MODEL

Each of the secondary efficacy outcomes will be analyzed using the same model as described for the primary efficacy analysis (Section 10.1), but the status and time of events will be defined as follows:

Outcome: Composite of recurrent ischemic stroke or systemic embolism		
First Event	Status	Time
Recurrent ischemic stroke	1	Time of first ischemic stroke
Recurrent stroke of any type	0	Time of first stroke of any type
Systemic embolism	1	Time of first systemic embolism
Death	0	Time of death
Diagnosis of AF	N/A (continue to follow)	
Study drug stopped	N/A (continue to follow)	
Loss to follow-up or consent withdrawn	0	Last known contact

Outcome: Composite of recurrent stroke of any type or death from any cause		
First Event	Status	Time
Recurrent stroke of any type	1	Time of first stroke of any type
Systemic embolism	N/A (continue to follow)	
Death	1	Time of death
Diagnosis of AF	N/A (continue to follow)	
Study drug stopped	N/A (continue to follow)	
Loss to follow-up or consent withdrawn	0	Last known contact

10.3.3 EXPLORATORY EFFICACY ANALYSES

In a tertiary analysis, we will assess the effect of apixaban on the subset of ischemic stroke comprising only cardioembolic or cryptogenic stroke, as determined by Causative Classification of Stroke determinations performed by site investigators.

10.3.4 EXPLORATORY ENDPOINTS

The following exploratory endpoints will also be recorded: AF, any intracranial hemorrhage, major hemorrhage including any intracranial hemorrhage, symptomatic hemorrhagic transformation of an ischemic stroke, transient ischemic attack, myocardial infarction, minor hemorrhage, systemic embolism, symptomatic deep venous thrombosis, symptomatic pulmonary embolism, ischemic vascular death, and hemorrhagic vascular death. The endpoint of any intracranial hemorrhage will be subclassified as: 1) symptomatic versus asymptomatic, and 2) consisting of hemorrhagic transformation of the index brain infarct versus not.

11 SAFETY ANALYSES

11.1 SAFETY MONITORING

The MSM and DSMB will receive periodic safety reports of all SAEs. The detailed guidelines for monitoring for safety by the MSM, and the DSMB will be provided in a separate ARCADIA Safety Monitoring Plan.

11.2 DESCRIPTIVE SUMMARY OF EVENTS

All clinical safety endpoints and SAEs will be summarized by AE code (as provided on the AE CRF) in terms of frequency of the event, number of participants having the event, severity, and relatedness to the study treatment. Clinically important adverse events include:

- Symptomatic intracranial hemorrhage (including symptomatic hemorrhagic transformation of an ischemic stroke).
- Major hemorrhage other than intracranial hemorrhage.
- All-cause mortality.

The proportion of participants experiencing each of these events will be provided in the closed report to the DSMB by treatment arm with two-sided 95% CIs and unadjusted relative risks. Based on prior reports, the following approximate annual rates of major safety endpoints are expected:

Adverse Event	**Apixaban**	**Aspirin**
Symptomatic intracranial hemorrhage	1.5%	1.5%
Major hemorrhage other than intracranial hemorrhage	4.0%	3.0%
All-cause mortality	3.5%	3.5%

Log-rank tests will be utilized to assess the treatment group differences in the rates of: (1) first symptomatic intracranial hemorrhage; (2) first major hemorrhage other than intracranial hemorrhage; and (3) death from any cause.

11.3 ANALYSIS OF SAFETY OUTCOMES AT TRIAL COMPLETION

All safety outcome measurements are analyzed using the safety sample (Section 8.3). Sensitivity analyses will be conducted using the ITT population. At the end of the study, the cumulative incidences

of the pre-specified safety events will be compared between the two treatment groups at a two-sided alpha level of 0.05.

11.3.1 PRIMARY SAFETY ENDPOINTS

The primary safety outcomes are: (A) symptomatic intracranial hemorrhage (including symptomatic hemorrhagic transformation of an ischemic stroke), and (B) major hemorrhage other than intracranial hemorrhage.

11.3.2 SECONDARY SAFETY ENDPOINT

The secondary safety endpoint is all-cause mortality.

11.4 ADJUDICATION OF SAFETY ENDPOINTS

Although regulatory reporting of possible SAEs will be based on the MSM determination of the event, for the publication of the final trial results analyses will use events as determined by the outcomes adjudication committee or site investigator (in the case of endpoints not adjudicated).

12 SUBGROUP ANALYSES

In prespecified subgroup analyses, the analyses in Sections 10.1 (primary efficacy analyses) and 11 (safety analyses) will be stratified by age (≥75 years versus <75 years), sex/gender, race/ethnicity, and weight (<70 kg versus ≥70 kg). In particular, subgroup analyses of previous trials of anticoagulation in ESUS suggest a benefit in patients ≥75 years of age.[7]

13 QUALITY-OF-LIFE ANALYSES

13.1 QUALITY-OF-LIFE MEASURES

Participants' quality of life will be assessed at the Day 540 visit (18 months). Study investigators will elicit quality-of-life data from participants or their proxies using modules from PROMIS, a validated tool for assessing quality-of-life in patients with disorders including stroke. The Global Health Short Form v1.1 and the Physical Function Short Form v1.2 will be used. Missing data for quality-of-life measures will not be imputed.

13.2 ANALYSES OF QUALITY-OF-LIFE MEASURES

The scores from each PROMIS domain will be compared between treatment groups using generalized linear mixed models which can account for correlation between the physical and global health forms.

14 COST-EFFECTIVENESS ANALYSIS

The cost-effectiveness of treatment with apixaban as compared with aspirin in patients with cryptogenic stroke and atrial cardiopathy will be assessed using previously published methods (Kamel, *Neurology*, 2012). In brief, a Markov decision model will be created based on the wholesale cost of apixaban and data from the ARCADIA trial. The model will quantify the cost and quality-adjusted life expectancy resulting from apixaban therapy compared with aspirin. The base case population will be a patient cohort with the mean age of the ARCADIA cohort and a history of prior stroke. The base case will include the following health states: No disability (history of transient ischemic attack or ischemic stroke with full recovery), ischemic stroke (fatal or resulting in moderate-to-severe, mild, or no disability), intracranial hemorrhage (fatal or resulting in moderate-to-severe or mild disability), recurrent stroke or combined stroke and intracranial hemorrhage, extracranial hemorrhage, myocardial infarction, and death. Model inputs on the rates of these adverse events (e.g., recurrent ischemic stroke, intracranial hemorrhage, major hemorrhage, myocardial infarction) will be taken directly from the average annual rates of these events in the apixaban versus aspirin arms of the ARCADIA trial. Costs and quality-of-life estimates will be based on previously published data as well as quality-of-life data from ARCADIA (see Section 13 above). The model will adopt a societal perspective and will exclude costs unrelated to the treatment strategy in question (apixaban versus aspirin) or other disease states besides those listed above. Quality-adjusted life expectancy and net costs will be quantified over 20 years or until death, whichever occurs first. Life-years and costs will be discounted at 3% per year and reported as quality-adjusted life-years (QALYs) and 2023 U.S. dollars (rounded to the nearest $100). TreeAge Pro software (TreeAge Software, Williamstown, Massachusetts) will be used to build the model and perform all analyses.

15 UPDATE OF TRIAL OPERATING PARAMETERS

As a trial with time-to-event outcome, the power can be affected by aspects other than the observed event rate (i.e. recruitment rate leading to decreased follow-up time for participants). At the recommendation of the DSMB, trial design modifications to rectify this problem may be considered, namely:

- Increased follow-up period.
- Increased sample size (i.e. observed accrual rate and increased accrual duration).
- Increased number of sites.

These estimates will be made using the assumptions above (Section 7), namely:

- 7% annual risk of recurrent stroke of any type in aspirin-treated participants.
- Clinically relevant relative risk reduction of 40% (hazard ratio of 0.6, hazard rate of 4.2%) for participants treated with apixaban.
- 3% annual rate of crossover from blinded aspirin to open-label anticoagulation due to AF detection.
- 3% annual rate of crossover from blinded apixaban to open-label antiplatelet therapy due to bleeding or other adverse events.
- 4% annual rate of loss to follow-up or death in each group.

16 CHANGE LOG

Date	Relation to interim or other analysis	Issue	Trigger	Solution
3 Jan 2018	Changes preceded start of recruitment	Elimination of plan for sample size re-estimation	Not logistically feasible	No sample size re-estimation
3 Jan 2018	Changes preceded start of recruitment	Elimination of plan for revision to group sequential boundaries	Inherent difficulty in accurately estimating	No plan for revision of group sequential boundaries
3 Jan 2018	Changes preceded start of recruitment	Randomization method	Balancing variables felt to be arbitrary	Randomization will only control the maximum tolerated imbalance within each RCC
9 Nov 2018	Changes preceded first interim analysis	Typographical error in Section 7.1	Loss-to-follow-up/mortality rate incorrectly written as 4%	Corrected to 5%
31 Mar 2021	Changes preceded first interim analysis	Safety-analysis sample	Protocol change	Safety-analysis sample changed to include safety events from randomization through 30 days after permanent study drug discontinuation
31 Mar 2021	Changes preceded first interim analysis	Subgroup analysis plan	Recent preliminary data that efficacy of aspirin may vary by weight	Subgroup analysis by weight added
31 Mar 2021	Changes preceded first interim analysis	Miscellaneous	Protocol change	Several minor edits to maintain consistency with protocol
29 Oct 2021	Changes preceded first interim analysis	Miscellaneous	Clarifications ahead of interim analysis	Several minor edits to maintain consistency with Manual of Procedures; clarified that log-log plots will be used to examine

				proportional hazards assumption in secondary Cox models; added additional secondary analysis in section 10.1.6.2
15 Feb 2023	Changes preceded final analysis	Subgroup analyses plan	Recent preliminary data that efficacy of apixaban in ESUS may vary by age	Subgroup analysis by age added; also clarified patient accountability flowchart and fixed typographical errors, including inadvertent holdover of section about adjusting for site effects in primary analysis

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