Prolocor, Inc.*

Statistical Analysis Plan

Protocol Number: PRL-0001

Assessment of Individual Risk of Cardiovascular Events by Platelet FcGammaRIIa

NCT05175261

*Prolocor, Inc. is an organization based in the United States of America and is the legal entity acting as the sponsor for this study. The term "sponsor" is used throughout the protocol to represent Prolocor, Inc; the sponsor is identified on the Contact Information page that accompanies the protocol.

Status: Final

Date: July 24, 2023

Prepared by: Prolocor, Inc.

GCP Compliance: This study will be conducted in compliance with Good Clinical Practice, and applicable regulatory requirements.

5 Statistical Methods

5.1 Data Quality Assurance

All clinical entries in the eCRF will be stored in a RedCap Cloud database. The structure of the database is based on the division into sections and entry fields defined in the eCRF. To improve and ensure data quality, data checks will be performed automatically in the eCRF directly on electronic entry at the study site.

Plausible value ranges for numerical data entries and logical data and list entries will be filed in the eCRF. The tests for consistency and completeness based on this will be performed during entry in the eCRF. The validity of the recorded data will therefore be ensured by the validations incorporated in the documentation system, which highlight incorrect or implausible entries to the data entry clerk/doctor.

If corrections are necessary after the data are saved, these will be documented in an audit trail.

All tables, figures, and data listings to be included in the report will be independently checked for consistency, integrity and in accordance with standard procedures for analysis and reporting.

5.2 General Presentation Considerations

The Day 1 visit is the only in-person visit in this study. This will be followed by telephone visits every 6 months, and a final telephone visit after the required number of study endpoints have accrued and at least 18 months have elapsed after the last subject was enrolled.

Continuous data will be summarized in terms of the mean, standard deviation (SD), median, inter-quartile range, minimum, maximum and number of observations, unless otherwise stated. Continuous data exhibiting skewness may also be presented in terms of the maximum, upper quartile, median, lower quartile, minimum and number of observations. The minimum and maximum will be reported to the same number of decimal places as the raw data recorded in the database. The mean, median, lower quartile and upper quartile will be reported to one more decimal place than the raw data recorded in the database.

Categorical data will be summarized in terms of the number of subjects providing data at the relevant time point (n), frequency counts and percentages. The number of subjects with missing information for a specific variable will be displayed if applicable. Any collapsing of categories will be detailed in the data displays. Percentages will be presented to one decimal place. Percentages will be calculated using non-missing data as the denominator.

All report outputs will be produced using R version 4.3.0 or a later version.

5.3 Study Subjects

A clear accounting of the disposition of all subjects who enter the study will be provided, and a summary of the number and percentage of subjects by center will be produced.

A subject is considered to have completed the trial if the subject has adequate follow-up to assess all study outcomes through either the final study telephone visit or the date of death. For subjects who did not complete the trial two different dates are of interest: the last date for which information on nonfatal study outcomes is available and, if later, the date the subject died or was last known to be alive. The

latter can differ from the former when public records are used to determine vital status in a subject who is lost to follow-up.

Completeness of follow-up for the primary endpoint can therefore be assessed in multiple ways, and will be tabulated as follows:

1. Percentage of subjects with complete follow-up for all study outcomes through the final study telephone visit or the date of death
2. Percentage of subjects with complete follow-up for primary study outcomes through the final study visit or the occurrence of a primary study outcome,
3. Percentage of subjects with complete follow-up for vital status
4. Mean percentage of potential follow-up for primary study outcomes as defined in 2 above (defined as 100% for a subject with complete follow-up, or 100 times the number of days of complete follow-up divided by the number of days between the subject's Day 1 visit and the end of study for a subject with incomplete follow-up)
    a. This percentage of follow-up will be subdivided into percentages of follow-up on DAPT and on monotherapy.
5. Mean percentage of potential follow-up for vital status as defined in 3 above (defined as 100% for a subject with complete follow-up or a subject who died, or 100 times the number of days of vital status follow-up divided by the number of days between the subject's Day 1 visit and the end of study for subjects with incomplete follow-up)

5.4 Demographic Characteristics

A table describing baseline conditions and demographics of the study population, including baseline distribution of FcγRIIa, will be provided. In addition, correlations between baseline FcγRIIa and other baseline subject characteristics will be calculated and displayed in scatterplots and boxplots, as appropriate.

5.5 Disease Characteristics Associated with Increased Cardiovascular Risk

We will use clinical characteristics that have been associated with greater risk of subsequent cardiovascular events (MI, stroke, death) in our planned secondary analysis. These clinical characteristics have been derived from large clinical trials (see references).

5.6 Objectives and Endpoints

The primary objective of the study is to determine whether platelet expression of FcγRIIa is associated with risk of MI, stroke, and death.

Secondary objectives are:

- Develop a score that combines clinical characteristics plus platelet expression of FcγRIIa to determine the risk of MI, stroke, and death.
- Determine whether platelet expression of FcγRIIa is associated with risk of major bleeding.

The primary endpoint is the composite of death, MI, and stroke. A secondary endpoint is clinically significant bleeding according to BARC type 2-5.

5.7 Statistical Methods

5.7.1 Analysis of the Primary Objective

The primary analysis of the primary endpoint will be a Cox proportional hazards model. The dependent variable is the number of days from enrolment to the occurrence of a primary endpoint, with censoring at the last day of complete follow-up; independent variables will be FcγRIIa (measured in thousands), age in years, history of diabetes mellitus, prior revascularization. multi-vessel coronary artery disease (MVD) defined as ≥2 vessels or left main with a stenosis ≥50%, chronic kidney disease (CKD) defined as estimated glomerular filtration rate (GFR) < 60 ml/min/1.73 m^2, prior MI, hypertension tobacco use, heart failure or LVEF<30%, previous stroke or transient ischemic attack, and peripheral arterial disease. Interest is in the FcγRIIa parameter: point estimate (increase in risk per 1000-unit increase in FcγRIIa), confidence interval and p-value. FcγRIIa will be declared to be associated with cardiovascular risk if the 2-sided p-value from this model is < 0.05.

A set of exploratory/sensitivity and subgroup analyses based on the Cox model will also be conducted.

- Instead of censoring at the last day of complete follow-up, censor at the last day of vital status follow-up
- FcγRIIa as the only covariate in the model
- Separate analyses of each component of the primary endpoint: MI and stroke, both censored at the last day of complete follow-up, and death, censored at the last day of vital status follow-up
- Run the primary model in subgroups defined by age (in decades), sex, history of diabetes and prior revascularization (including all covariates except the subgroup variable in the model)
- Run the primary model with the use of DAPT (any use prior to a primary endpoint), and its interaction with FcγRIIa, as additional covariates

A final set of exploratory analyses will be done to help determine the optimal threshold value of FcγRIIa for risk stratification. Define a threshold value, x, and define “low” FcγRIIa as a value less than or equal to x, and “high” FcγRIIa as a value greater than x. Next, calculate exposure-adjusted subject incidence rates for the primary endpoint, separately for the “low” and “high” subgroups, as the number of subjects with an event divided by the sum across subjects of the event/censoring times. Then, plot the exposure-adjusted incidence rates as a function of x for each subgroup, as well as the difference between subgroups. In addition, ROC curves will be produced showing the sensitivity and specificity of FcγRIIa to predict the presence or absence of a primary endpoint, as a function of the FcγRIIa threshold.

Flow cytometry considerations

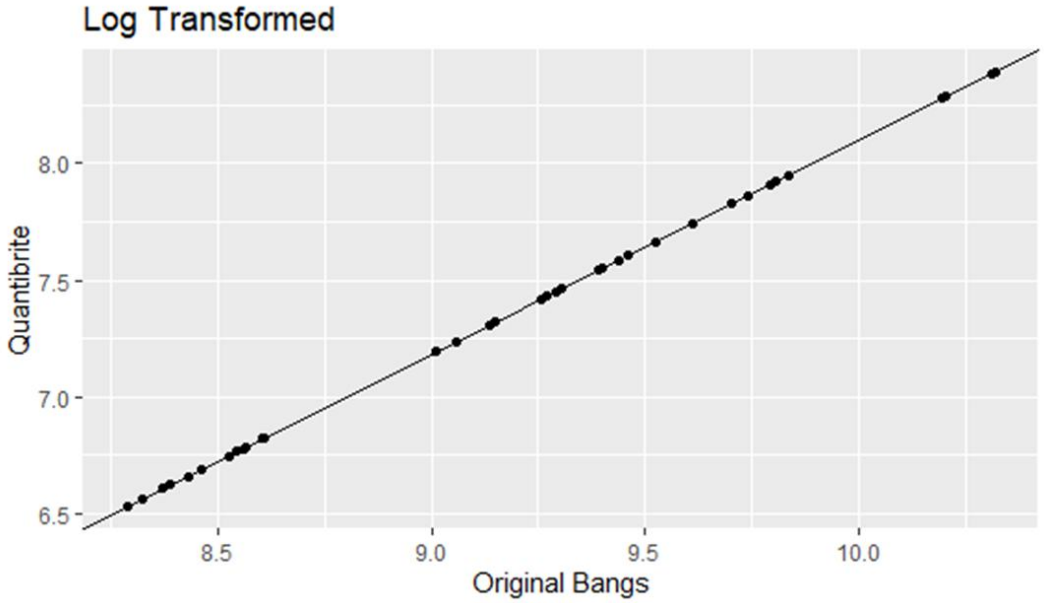
Flow cytometry output, mean fluorescence intensity (MFI), can be influenced by multiple factors. External standards are used to translate MFI into molecules/platelet. In the original study, Bangs Laboratories Quantum MESF beads were used to translate MFI to antibody binding capacity (Schneider et al). Because our antibody is labeled with 1 molecule of phycoerythrin (PE) per antibody, the antibody binding capacity equates to molecules per platelet.

Shortly before initiating the current study, Bangs Laboratory changed their calculation template. The company reported that over time their calculation template had experienced a shift (personal communication). This change led to a substantial change in the quantification. ,The change represents a ‘unit’ change rather than a complete revision of the translation of MFI into molecules/platelet.

Results using Bangs Laboratories Quantum MESF were compared with an independent external standard, BD Quantibrite. The two external standards (Quantum MESF and Quantibrite) yielded similar results. Based on this information, the revision of the formula by Bangs Laboratories was determined to be a more accurate assessment of molecules/platelet.

Results from the original study were analyzed with the use of the Quantibrite formula to relate results obtained using the previous Bangs Laboratories formula with the formula used with Quantibrite beads.

A nearly perfect fit is obtained with log-transformation of the data. The plotted values are the natural logs of the original values. This regression line also doesn't go through zero (see the expanded scale), so the regression line is as follows: log(Quantibrite) = a * log(Original Bangs) + b, where a = 0.9160084 and b = -1.0611595.



The relationship derived was used to translate the original threshold of 11,000 to a new threshold that will be tested in this study. A value of 11,000 obtained with the original calculation template provided by Bangs Beads corresponds to a Quantibrite value of exp(a * log(11,000) + b) = 1742.183. Thus, we will use a threshold rounded to the closest 50, or 1,750, to define subjects with low versus high FcγRIIa.

5.7.2 Analysis of the Secondary Objective

First Secondary Objective: Negative predictive value, positive predictive value, sensitivity, and specificity will be calculated. Prediction will be performed using a Cox regression model with the following predictors: platelet expression of FcγRIIa, sex, age, and clinical risk factors associated with increased cardiovascular risk. Evaluation of the model's error rates will be based on a leave-one-out cross-validation approach. Additional models will evaluate the performance of the predictive model using a continuous measure of FcγRIIa with the performance of predictive models using various binary measures of FcγRIIa.

Second Secondary Objective: The adjusted Cox proportional hazards model described for the primary objective analysis will be used to compare the bleeding risk as a function of FcγRIIa expression. One

model will include FcγRIIa as a continuous variable, and another using the dichotomized version of FcγRIIa selected as the optimal version for risk stratification.

5.8 Safety Data

The only safety information collected in this study is serious adverse events associated with the venipuncture that takes place on Day 1. All adverse events will be listed and, if there is a meaningful number of them, tabulated as well.

5.9 Handling of Missing and Incomplete Data

Subjects with missing data for baseline FcγRIIa will be excluded from all analyses. Missing values for other covariates will be imputed using a median value imputation for continuous variables and most frequent value imputation for categorical variables.

5.9.1 Missing and Partial Dates

The following rules apply when the date of a primary endpoint is fully or partially missing.

Missing Date: The date will be imputed as the last date of complete follow-up for MI and stroke, and the last known alive date for death.

Missing Year, Month, Day: The imputed value will be the largest value such that the date falls within the subject's known follow-up time. If more than one component of the date is missing, the order of imputation is year, month, day.

5.10 Determination of Sample Size

This study is designed to provide a sufficient number of events to build and evaluate a prediction model. The planned sample size of 800 subjects will continue until at least 80 ischemic events have occurred. Under the guideline from Steyerberg of 10 events required per predictor (Steyerberg 2019), this sample size will allow a model with FcγRIIa and up to seven additional predictors to be built. Preliminary results (Schneider 2018) suggest that a 4-fold greater incidence of events will be seen in subjects with high platelet expression of FcγRIIa. For the primary endpoint, there will be at least 95% power to detect a 2-fold greater incidence of cardiovascular endpoints (MI, stroke, and death) with a two-sided $\alpha<0.01$ assuming 8% of subjects experience the endpoint. With the planned sample size of 800 a hazard ratio (HR) of 1.9 with a p=0.04 (lower bound of the 95% CI of 1.2) can be detected. For the secondary endpoint, there will be 95% power to detect a hazard ratio of 1.93 when comparing bleeding risk in subjects with high compared with low FcγRIIa expression when assuming a two-sided $\alpha=0.05$ and 5% of subjects experience bleeding in the low FcγRIIa expression group.

5.11 References

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Prolocor, Inc.
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On behalf of Prolocor, I have reviewed and approved this document.

Chief Scientific Officer:

Name: (typed or printed): David J. Schneider

Signature: David J. Schneider Digitally signed by David J. Schneider Date: 2023.07.25 16:25:58 -04'00' Date: _____
(Day Month Year)

Chief Executive Officer:

Name: (typed or printed): Peter M. DiBattiste, MD

Signature: Peter M. DiBattiste, MD Digitally signed by Peter M. DiBattiste, MD Date: 2023.07.25 15:44:12 -04'00' Date: _____
(Day Month Year)