Statistical Analysis Plan

Title: In Vivo Effects of Fibrinogen Concentrate (FC) Versus Cryoprecipitate on the Neonatal Fibrin Network Structure After Cardiopulmonary Bypass (CPB)

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Statistical Analysis Plan 04.01.2019 (edited 11.30.23)

Study Populations:

Intention-to-Treat (ITT) Analysis Set
The ITT Analysis Set will consist of all enrolled subjects according to their randomization assignment. Efficacy summarization and analysis will be conducted using the ITT analysis set and this will the primary analysis.

Per Protocol Analysis Set (PPAS):
The subset of subjects in the full analysis (ITT) set who complied with the protocol sufficiently (see below) without any major protocol deviations. Confirmatory analysis will be performed using the PPAS dataset. Final definition of PPAS will be defined prior to data lock. Elements of PPAS may include exclusion criteria such as:

- Violation of inclusion or exclusion criteria
- Did not receive study medication
- Did not go on cardiac bypass during surgical intervention
- Incorrect study medication treatment
- Patients unable to wean from bypass in operating room

Sample Size Considerations

The primary endpoint of the study, on which the statistical power is based, is the difference in clot degradation time at 24 hours post-operatively in neonates randomized to Cyro compared to those randomized to FC, with the primary focus on the overall set of subjects in the ITT population. Based on our previous work comparing adult and neonates, we observed a large difference (>2 standard deviation (SD) difference) in degradation times between the adults and neonates. In this study, we conservatively estimate a 1 SD difference in mean degradation times when comparing degradation times between infants randomized to Cyro vs. those randomized to FC. Assuming a two-sided test at the 5% significance level, 17 completed participants will provide 80% power to detect a 1 SD difference in mean degradation times between our two groups at 24-hours post-operative. Power was calculated using a two-sample t-test. Additionally, assuming an attrition of 1 per group (i.e., death before 24 hour endpoint), 18 participants are// needed for enrollment into the study to ensure an adequate number of subjects complete the primary endpoint. Power calculations were made using PASS v. 14.0.8 (Kaysville, UT).

General Statistical Considerations
Descriptive statistics will be calculated for all variables of interest. For continuous variables this will include means and standard deviations, medians, quartiles (25^{th}, 75^{th}), minimum and maximum values. Categorical data will be described using counts and percentages. Continuous data will be assessed for normality using histograms and normal probability plots and statistically tested using the Anderson-Darling test and/or the Shapiro Wilk test for normality. Non-normal data will be presented using medians accompanied by 25th and 75^{th} percentiles.

No adjustments will be made for testing multiple secondary and exploratory efficacy outcomes. Given the large number of secondary/exploratory outcomes, the p-values associated with such outcomes will be considered descriptive as the likelihood of making a type I error is inflated due to multiple testing. All analyses will be conducted using SAS v. 9.4 (Cary, NC) or CRAN R v. 3.3 or above (Vienna, Austria). All statistical testing will be two-sided and performed using a 0.05 significance level (i.e., alpha =0.05). Missing values at baseline will be imputed using group median values at the specific time points. We will also repeat all analyses without imputation as a sensitivity analysis.

Analysis of Primary and Secondary Outcomes:

The primary endpoint of the study is the percentage change of normalized degradation at 24h post-surgery. Primary comparison will be the difference of the primary endpoint between neonates randomized to Cyro and those randomized to FC, with the primary focus on 24h post-surgery time point. To answer this primary research question, we will utilize two sample t-test or Wilcoxon rank sum tests to compare percent change from pre-surgery (baseline) between groups at 24h post-surgery. We will further employ linear mixed models to analyze the longitudinal endpoints accounting for repeated measures. Specifically, we will examine percentage change of normalized degradation at follow up time points (i.e., post-surgery, ICU admission, and 24h post-surgery) relative to baseline between the two groups. Random intercepts at patient level will be included to account for within-subject correlation. Post-hoc comparison between time points will be conducted by contrasts. Results will be presented as difference in least square means between groups or between time points, with associated 95% confidence intervals. Analysis of secondary outcomes, including measurements collected post-transfusion, will be analyzed using similar methods.

Finally, two sample t-tests or Wilcoxon rank sum tests will be used to examine the difference in clot kinetics (i.e., structure, polymerization, degradation) and clinical outcomes including chest tube output, 24-transfusion needs, and ICU length of stay. Two-sample t-tests or Wilcoxon rank sum tests will be used to compare measures between patients with and without thrombotic events. Multivariable linear regression for continuous outcomes and logistic regression for binary outcomes will be used to examine the association between the clot kinetics and clinical outcome while adjusting for potential confounders including pre-surgical weight, age, cardiac diagnosis/ RACHS score, and pre-operative comorbid conditions, as needed.

Exploratory Subgroup Analysis

Given the sample size of our current study, we are likely underpowered to examine differences in outcomes when stratified by subgroups including race, sex, risk of bleeding and underlying morphology. Nevertheless, we will perform subgroup analyses to examine differences in primary and secondary outcomes within and between subgroups of interest. This will include stratified analyses and by including the interaction between treatment group (FC vs. Cryo) and subgroup in subsequent models.