Bevacizumab Treatment For Posterior Zone I ROP

NCT04634578

Statistical Analysis Plan
October 5, 2021

RETINOPATHY OF PREMATURITY 4

(ROP4)

A RANDOMIZED TRIAL OF BEVACIZUMAB TREATMENT FOR POSTERIOR ZONE I RETINOPATHY OF PREMATURITY

Statistical Analysis Plan (version 1.0)

Based on protocol version 1.1

Revision History

VERSION NUMBER		AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION (INCLUDING SECTIONS REVISED)
SAP	Protocol				
1.0	1.1	R. Henderson	Z. Li	05OCT21	Initial version

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1.0 Study Overview

Premature infants (birth weight < 1251 grams) will be randomized 1:1 into the multi-center protocol to evaluate whether Bevacizumab (subsequently referred to as BV) doses of 0.063 mg and 0.25mg are an effective treatment for type 1 ROP, with ROP and retinal vessels all in zone I. Treatment success will be determined at the 4-week visit. One or two eyes per participant will be eligible. Randomization will be stratified by gestational age (≤ 24 weeks vs > 24 weeks).

Schedule of Study Visits and Procedures

	Enroll	1d	4d	1w*	2w*	3w*	4w*	2m*	4m*	6m†	12m†
Informed Consent	X										
Medical/Ocular History	X[a]	X	X	X	X	X	X	X	X	X	X
Classify ROP	X	X	X	X	X	X	X	X	X		
Ocular Exam										X	X
Retinal Imaging	X[b]								X[b]		
Extent of Retinal Vascularization								X	X		
Cycloplegic Refraction											
Plasma VEGF	X[c]				X[c]		X[c]		X[c]		
Ocular Alignment										X	X
Visual Fix & Follow										X	X
Data on Additional Treatments				X	X	X	X	X	X	X	X
Adverse Events		X[d]	X[d]	X[d]	X[d]	X[d]	X[d]	X[e]	X[e]	X[e]	X[e]
Systemic Outcomes										X	X

*Exams through 4 months are timed based on initial treatment (and re-treatment when indicated).
†Exams 6 months and beyond are based on adjusted age.

[a] Medical and ocular history to include concomitant medications and pre-existing medical conditions at time of enrollment
[b] Retinal imaging done at enrollment and prior to any additional treatment. Imaging is also done at 4-months if no additional treatment has been received. OCT and FA are optional.
[c] Plasma VEGF testing is optional.
[d] All adverse events recorded at each visit until 4 weeks post-treatment
[e] Only serious adverse events and ocular adverse events recorded beyond 4 weeks post-treatment

1.1 Primary Outcome Definition – Treatment Success At 6 Months Adjusted Age

The primary outcome is ***Treatment success***, determined at 4 weeks, post injection, and meeting the following three criteria:

- Improvement by the 4-day exam (3 to 5 days)
- No recurrence of type 1 ROP within 4 weeks of injection
- No severe neovascularization requiring additional treatment within 4 weeks of injection.

With a short time to primary outcome, it is expected that lost to follow-up will be minimal and non-informative. However, there may be a few participants who die or are lost to follow up before the 4-week visit. To use the information of these participants as much as possible, a time to event analysis will be performed. Censoring will occur at the time of death or lost to follow up before the 4-week outcome. If an eye fails to meet any of the above criteria at one of the visits before the censoring event, that eye will be counted as a failure at that time. The protocol visit date that reports the failure or censoring event will be

used as the timepoint for the analysis. Direct adjustment methods will be used to estimate the success probability, to be consistent with the hypothesis below.

It is possible for fellow eyes to be enrolled with type 1 ROP in zone 2. It is estimated that ~10% of participants will have or develop zone II ROP in the fellow eye. These eyes will be analyzed separately to estimate success in each treatment group.

1.1.1 Off-protocol treatments and success/Failure

The ROP4 protocol is designed to release eyes to treatment at investigator discretion as quickly as possible if there are signs of non-improvement or worsening. However, if an investigator decides to provide a second treatment after 0.063 or 0.25 BV, the patient will be considered a failure at that date. The reason to consider this a non-response is that any eye treated would probably at least be approaching failure criteria.

1.2 Primary Statistical Hypotheses

The objectives are to estimate the treatment success probability in eyes treated with 0.063 mg and 0.25 mg BV and to evaluate whether the success probability differs between the two dose groups. The null hypothesis is there is no difference in the success probability for BV doses of 0.063 mg and 0.25mg.

1.3 Description/ Calculation of Secondary Outcomes

Many of the secondary outcomes are safety outcomes.

Categorical secondary outcomes are described in two ways:

- Participant level:
  - The outcome is either naturally counted on a participant basis
  - Or, if an ocular outcome, if the event happens in either eye then that participant is counted
- Eye level

Analyses of the outcomes are described in Chapter 2.

1.3.1 Number of re-treatments

The number of re-treatments will be collected throughout the study on a per participant and per eye basis.

1.3.2 Ocular outcomes from ocular exam

At the ocular exams, the following data will be collected.

- The number and proportion of children and eyes with each of the following will be tabulated if known for study eyes:
  - Most severe abnormality in the eye based upon clinical exam:
    - Essentially normal
    - Abnormal angle of temporal vessels (suggestive of traction)
    - Macular ectopia
    - Stage 4A retinal detachment (sparing fovea)
    - Stage 4B retinal detachment (involving fovea)
    - View of macula blocked

        - Total retinal detachment
        - All view of posterior pole and near periphery is blocked due to anterior segment opacity
        - Enucleation due to ROP
        - Enucleation due to other causes
        - Unable to determine (e.g. view impossible due to corneal opacity unrelated to ROP, or to miotic pupil)
    - Normal or abnormal cornea
    - Normal or abnormal anterior segment
    - Normal or abnormal lens
    - Optic nerve atrophy absent, questionable, present, or view obscured
        - A binary indicator will be evaluated as present vs (absent or questionable), not including view obscured

These ocular outcomes will be tabulated at every visit. After the 2-month visit, a category for "ever" having each outcome will be added.

Additional ocular adverse events may be documented on the Adverse Events Form throughout the trial. These will be tabulated separately. Generally, complications of treatment would be adverse events, and outcomes are from the disease process (ROP).

1.3.3 Extent of retinal vascularization

The extent of retinal vascularization will be classified as:

- Posterior Zone I
- Anterior Zone I
- Posterior Zone II
- Mid Zone II
- Anterior Zone II
- Zone III
- Full Vascularization

We will calculate a binary variable for eyes that achieve either Zone III or full vascularization (vs less).

1.3.4 Time from enrollment to hospital discharge

Initial date of hospital discharge will be collected. Participants who are not discharged will be censored at last available follow-up visit.

1.3.5 Number of times re-hospitalized.

The number of re-hospitalizations will be collected. If hospitalizations are not collected for some participants, they will have their own category for missing data. Missing data may not be the same as zero reported hospitalizations.

1.3.6 Physical Measurements of head circumference, body weight (grams)

Head circumference (cm) and body weight (grams) will be collected. The number of missing observations will be displayed below tabulations.

1.3.7 Supplemental oxygen requirement/ discontinuation rate

The status of current supplemental oxygen requirement will be collected. We will categorize:

- The number of participants who are currently on oxygen
- The number of participants who were on oxygen but stopped
- The number of participants never on oxygen

- The number of participants lost to follow up

1.3.8 Mortality rates and causes of death

Date of death will be recorded and used in a time-to-event analysis to estimate morality rate. Participants lost to follow-up will be censored at last available follow-up visit. Reasons for death will be recorded. If the reason for death is missing, a category for missing a reason will be tabulated with the other causes.

1.3.9 Systemic morbidities

Systemic adverse events are collected throughout the trial. These will be listed as they happen.

In addition, the events of interest are different on the 6- and 12-month forms . After the 6-month visit, a category for "ever" will be added for each outcome.

1.3.10 Ocular Alignment, Visual Fix & Follow, & Diagnosis of Amblyopia

Ocular alignment data will be collected. It will include the following (in both eyes where applicable).

- Ocular Alignment on a participant basis:
  - If able to test ocular alignment at <u>distance</u>, the following will be tabulated:
    - Number (%) with intermittent tropia or constant tropia
  - If able to test ocular alignment at <u>near</u>, the following will be tabulated:
    - Number (%) with intermittent tropia or constant tropia
  - Number (%) with tropia defined as intermittent or constant at distance OR near.
  - The number (%) of children with nystagmus absent or present.
- Assessment of Vision on a participant
  - The % of eyes able to fix and follow during fixation preference testing
- Assessment of Amblyopia at participant level:
  - This will be based on fixation preference testing results; the distribution of responses will be tabulated as:
    - Strongly prefers fixation with one eye (will not fix with the other eye for more than 1 sec) - Amblyopia strongly suspected
    - Mild or no fixation preference – Amblyopia not strongly suspected
    - Unable to cooperate for testing

1.3.11 Refractive Error

Refractive error is measured in diopters, to the nearest quarter diopter. The further from zero, the worse the refractive error. Myopia is negative diopters; hyperopia is positive diopters. Normally, babies are mildly hyperopic. Values may range from -30 to +30.

The continuous distribution will be an outcome, as will the categories of myopia and hyperopia. Myopia values will be tabulated as High Hyperopia (>+5.00D) spherical equivalent. non-myopic (5 - >-0.5 D), some myopia (-0.5 D to =>-5.0D), and severe myopia (< -5.0D).

1. 5 Sample Size

The sample size has not been statistically calculated. A sample size of up to 80 participants (40 participants per dose group) is a convenience sample.

1.6 Classifying Visits

Visit	Target Day	Target Window (Around Target Day)	Allowable Window (Around Target Day)
ROP diagnosis Enrollment / Randomization	N/A	N/A	N/A
Randomized Treatment	Day of Randomization	+ 1 day	+ 2 days
1-day post-injection	Treatment Date + 1 day	1 day	0 to 2 days
4-day post-injection if no improvement at 1-day	Treatment Date + 4 days	3 to 5 days	3 to 5 days
1 week post-injection	Treatment Date + 1 week	6 to 8 days	6 to 10 days
2 weeks post-injection	Treatment Date + 2 weeks	11 to 17 days	11 to 17 days
3 weeks post-injection	Treatment Date + 3 weeks	18 to 24 days	18 to 24 days
4 weeks post-injection (primary outcome)	Treatment Date + 4 weeks	25 to 31 days	25 to 53 days
2 months post-injection	Treatment Date + 2 months	54 to 68 days	54 to 83 days
4 months post-injection	Treatment Date + 4 months	115 to 129 days	84 to 168 days
6 months corrected age	EDC + 6 months	169 to 197 days	124 to 242 days
12 months corrected age	EDC + 12 months	335 to 395 days	243 to 547 days

6 and 12 months visits are calculated post corrected age (defined as number of days since the date of birth minus number of days the baby was preterm).

If any study-mandated examination is deferred because of an infant's unstable medical status, then that examination will be done as soon as possible.

The goal will be for all participants to complete all scheduled visits. However, participants who (because of unforeseen circumstances) are unable or unwilling to return for all follow-up visits will be permitted to return for key visits only as an alternative to withdrawal from the study. When a participant is placed into this status, missed visits will not be recorded as protocol deviations (since they would not be recorded as protocol deviations if the participant was dropped from the study).

The allowable window will be the same as the analysis window. Allowable windows were created so that all data would be included.

1.7 Planned Interim Analyses

There will be no interim analyses for futility, efficacy, or re-estimation of sample size.

2.0 Description of Statistical Methods

2.1 General Approach

Analysis methods include survival analyses, generalized linear models, tabulations, and plots. Assumptions will be checked.

The primary analysis will follow an intent-to-treat (ITT) principle.

There are sensitivity analyses for the primary analysis.

2.1.1 Multiplicity

Type I error for the primary outcome analysis will be 5%, as only one analysis is pre-specified as primary. If the P-value for the primary analysis is <0.05, we will pass down the alpha for a treatment comparison of Extent of Retinal vascularization (Zone III or full vs less) at 4 months. The other secondary outcome treatment group comparisons are exploratory.

2.2 Analysis datasets

All datasets are complete case.

2.2.1 Primary Analysis (Eye Level)

The primary efficacy analysis will follow an ITT principle. For the primary analysis, there will be no imputation of data for participants who die or who are lost to follow-up prior to the 4-week outcome visit since they will be treated as censored in the time-to-event analysis. However, participants' eyes can reach failure before the 4-week visit, and they will be included as failures.

The following secondary outcomes on the eye level will also use the ITT eye level dataset:

- Extent of retinal vascularization
- Refractive error
- Visual Fix & Follow
- Ocular exam findings/ complications

Only eyes with Type 1 ROP all in zone I will be included in the primary analysis dataset.

2.2.2 Fellow Eye Primary Analysis

If both eyes are eligible, then both are included as study eyes. If one eye is eligible and the fellow eye has type 1 ROP with ROP or vessels in zone 2, then both eyes will receive the randomly-assigned treatment. If one eye is eligible and the fellow eye later develops type 1 ROP within 4 weeks of injection in the first eye, then the fellow eye will also receive the treatment randomly assigned to the first eye. The fellow eye will follow the same 4-week post-injection study exam schedule, unless the first eye has already met failure criteria, in which case treatment and follow-up for the fellow eye is at investigator discretion. These fellow eyes will be in a separate dataset.

2.2.3 Participant level

Many secondary outcomes are on a participant level:

- Number of re-treatments
- Time from enrollment to hospital discharge
- Number of times hospitalized
- Head circumference
- Weight
- Supplemental oxygen
- Mortality
- Systemic morbidities
- Ocular Alignment/ Amblyopia

There is a contingency plan for the primary analysis if Cox model assumptions fail that would use a binary success/failure outcome for each participant. If a participant has one study eye, that participant is counted as a failure if the study eye fails. If a participant has two study eyes, a failure in either will count the participant as a failure.

2.4 Primary Outcome

2.4.1 Primary analysis

The proportion of study eyes that achieve success will be tabulated by treatment group. The number of events, censorings, and 4-week visits will also be tabulated by treatment group. The denominator will be the number of eyes that were randomized in the study. A tabulation of events with the denominator as the number of eyes that were randomized in the study without deaths and lost to follow-up will also be displayed.

The hazard ratio of failure for dose (0.063 mg:0.25 mg) and a 95% confidence interval (CI) will be calculated with a Cox model with robust variance estimation to control for correlation arising from participants contributing two study eyes to the analysis. The model will include treatment group and adjust for continuous gestational age at randomization. An estimate of the success probability at 4-weeks with 95% CI for each dose group will be calculated with direct adjustment methods. The difference in the success probability between the dose groups (0.063 mg – 0.25 mg) and the 95% CI will be calculated with the normal approximation.

<u>Assumptions</u>
Functional form of continuous gestational age at randomization will be assessed by plotting the observed distribution of cumulative sums of martingale residuals (Y axis) vs the values of gestational age (X axis). Twenty simulated sets of residuals with correctly-specified models will be plotted over our residuals; a seed will be used for reproducibility. If our residuals are very different from the simulated model, this suggests misspecification of the functional form. A Kolmogorov-Type supremum test will be conducted comparing our residuals to 1000 random simulations. A significant result on this test suggests misspecification. Methods that may be used for solving misspecification include transforming or categorizing gestational age at randomization.

Proportional hazards will be checked separately for continuous gestational age at randomization and treatment group. Plots will be created with the observed distribution of cumulative sums of martingale residuals (Y axis) vs the length of follow-up (X axis). Twenty simulated sets of residuals with truly proportional hazards will be plotted over our residuals; a seed will be used for reproducibility. If our residuals are very different from the simulated model, this suggests non-proportional hazards. A Kolmogorov-Type supremum test will be conducted comparing our residuals to 1000 random simulations. A significant result on this test suggests non-proportional hazards. A Kaplan-Meier plot will be generated and examined for proportional hazards violations. If the proportional hazards assumption fails, a model with an interaction between treatment group and a function of time may be an option.

Intermittent missingness is expected to be rare for the primary outcome. These participants are followed very closely because there is risk for blindness.

2.4.2 Tipping Point Analysis for Imaging

To mitigate unmasked assessment bias, wide-angle retinal images will be collected when treatment failure is declared. Images will later be reviewed by masked expert readers to define success or failure. If the site does not have the hardware for wide-angle retinal imaging, a second ocular exam (by a different examiner) will be used in place of the masked image analysis. The number of discrepancies between the

site diagnosis and the masked reviewer diagnosis will be calculated. If there are cases where the investigator declared failure and masked review of the images does not confirm failure, then a sensitivity tipping point analysis will be done to evaluate the number of discrepancies required to tip results from significant to not or vice versa.
Depending on the numbers of disagreements, we will tabulate either all tipping points or summarize by every few cases.

For each treatment group we will create a table like the one below:

Failures tipped to successes	New success probability difference	New 95% CI for success probability difference

2.4.3 Deaths as failures

To check for informative censoring in the primary analysis, a sensitivity analysis will be performed where deaths before failure are counted as failures instead of censoring. If deaths are believed to be independent of treatment, this will overestimate the failure rate in each group.

However, if one group's failure rate meaningfully increases compared to the other, this may indicate informative censoring, or an imbalance in morality risk despite randomization (we have a small sample size). In this case, the primary analysis will have to be interpreted with caution.

2.4.3 Primary outcome contingency plan

If Cox model assumptions fail and cannot be remedied, Barnard's exact test will be performed on the complete case participant level success/failure outcome described in section 2.2.3. Exact 95% CIs for success rates and the difference in success rates will be obtained.

2.5. Secondary Outcomes

We will tabulate or provide summary statistics on all the secondary outcomes overall and by treatment group at each timepoint they are analyzed. N (%) of missingness will be displayed for all outcomes. Most of the secondary outcomes will just be these tabulations or summary statistics at each timepoint below for the participant or eye level from the description of each outcome.

<u>Regression Methods</u>

Binary outcomes will be analyzed using Poisson regression (log link) with generalized estimation equations (exchangeable structure) to control for correlation arising from participants contributing two study eyes to the analysis (if applicable to the outcome). The coefficients, when exponentiated, will estimate relative risk. If the deviance/DF is <0.8, the model is underdispersed and logistic regression will be used to estimate odds ratios.

Continuous/count outcomes will be analyzed using linear regression with generalized estimating equations (exchangeable structure) to control for correlation arising from participants contributing two study eyes to the analysis (if applicable to the outcome). Depending on the empirical distribution, a normal, Poisson, or negative binomial distribution for the model may be used.

Independent covariate adjustments are treatment group and continuous gestational age.

For analyses on an eye level, an exchangeable correlation matrix will be used to account for correlation between eyes for participants who contribute two eyes to the study.

Checking Assumptions

Standard residual diagnostics will be performed for all analyses. The linearity (or log linearity) assumption of covariates will be evaluated using descriptive scatterplots and by categorizing each of the baseline factors in the model to check for approximate linearity (or log linearity) of the coefficients across ordered categories.

For continuous outcomes, potential outliers will be identified, and a sensitivity analysis will be performed to evaluate the effect of these outliers on the primary outcome results If values are highly skewed, then we will consider categorization, transformations, worst-rank regression, MM estimation methods or non-parametric randomization-based method of Koch et al.

For binary outcomes, if the Poisson regression model fails to converge, we reserve the option to use exact logistic regression or Barnard's test to compare treatment groups. We may also choose to use Barnard's test if there are less than 10 outcome events per covariate, which is considered too small for reliable estimation. Barnard's test does not allow for covariate adjustment.

If results differ between models with violated assumptions and models with the changes for robustness, the robust method will be used for trial conclusions and formulation of resulting recommendations.

Patterns of missingness will be analyzed. Suspected informative missingness will be noted and results will be interpreted cautiously.

Time to event

For time to event outcomes, we will attempt to use a time to event model to generate survival time summary statistics accounting for censoring.

The proportion of study eyes that have events will be tabulated by treatment group. The number of events, censorings, and 4-week visits will also be tabulated by treatment group. The denominator will be the number of eyes that randomize in the study. A tabulation of events with the denominator as the number of eyes that randomize in the study without lost to follow-up will also be displayed.

The hazard ratio for BV 0.063 : 0.25 mg and a 95% CI will be calculated with a Cox model with robust variance estimation to control for correlation arising from participants contributing two study eyes to the analysis. Independent covariate adjustments are treatment group and continuous gestational age.

Functional form of continuous gestational age at randomization will be assessed by plotting the observed distribution of cumulative sums of martingale residuals (Y axis) vs the values of gestational age (X axis). Twenty simulated sets of residuals with correctly specified models will be plotted over our residuals; a seed will be used for reproducibility. If our residuals are very different from the simulated model, this suggests misspecification of the functional form. A Kolmogorov-Type supremum test will be conducted comparing our residuals to 1000 random simulations. A significant result on this test suggests misspecification. Methods that may be used for solving misspecification include transforming or categorizing gestational age at randomization.

Proportional hazards will be checked separately for continuous gestational age at randomization and treatment group. Plots will be created with the observed distribution of cumulative sums of martingale residuals (Y axis) vs the length of follow-up (X axis). Twenty simulated sets of residuals with truly proportional hazards will be plotted over our residuals; a seed will be used for reproducibility. If our residuals are very different from the simulated model, this suggests non-proportional hazards. A Kolmogorov-Type supremum test will be conducted comparing our residuals to 1000 random simulations. A significant result on this test suggests non-proportional hazards. A Kaplan-Meier plot will be generated and examined for proportional hazards violations. If the proportional hazards assumption fails, a model with an interaction between treatment group and a function of time may be an option.

As a contingency plan, we will calculate summary statistics after dropping censored values.

Outcome	2-Month	4-Month	6-Month	12-month	Type of outcome	Statistics
Number of retreatments	X	X	X	X	Count	Tabulations
Ocular Outcomes from ocular exam	X	X	X	X	Categorical	Tabulations
Extent of retinal vascularization	X	X			Categorical And Binary	Tabulations of each category At the 4 month outcome only: Estimate and 95% CI for each treatment group and treatment group difference in Zone III /Full Vascularization proportion
Time from enrollment to hospital discharge			X	X	Continuous	Summary Statistics
Number of times re-hospitalized.			X	X	Count	Tabulations
Physical Measurements of head circumference			X	X	Continuous	Summary Statistics

(cm), body weight (grams)						
Supplemental oxygen requirement			X	X	Categorical	Tabulations
Mortality rates and causes of death	X	X	X	X	Time-to-event and descriptive	Exploratory hazard ratio for treatment group comparison testing if the higher dose is associated with higher rates of mortality Tabulations/ Proportion Causes listed
Ocular alignment			X	X	Categorical	Tabulations
Amblyopia			X	X	Categorical	Tabulations Estimate and 95% CI for each treatment group
Refractive Error			X	X	Continuous	Summary statistics Estimate and 95% CI for each treatment group
Proportion with High Hyperopia, High Myopia			X	X	Categorical	Tabulations Estimate and 95% CI for each treatment group
Visual Fix and Follow			X	X	Categorical	Tabulation of each category

2.6 Baseline Characteristics

Subject level and eye level characteristics will be tabulated including gender, race, gestational age, birth weight, examination findings in each eye at time of injection, and age at diagnosis of type 1 ROP.

2.7 Subgroup Analyses

The following subgroup analyses will be considered:

- Plus disease vs not plus

The approach for these analyses will be to estimate the treatment effect and 95% CI within each subgroup using the same analytic methods used for the primary analysis. That is, the subgroup analysis will be performed by including the main effect of the subgroup factor and its interaction effect with the treatment group in the model for the primary analysis. If a potential treatment interaction is indicated, we will also display models for each treatment group separately.

Interpretation of the results will depend on whether the overall analysis demonstrates a significant treatment group difference; in the absence of an overall difference, subgroup analysis results will be interpreted with caution. We will consider the clinical significance of this interaction as well as the effect on the treatment coefficient and AIC. For example, if the interaction effect size is very small (clinically insignificant), but precise (statistically significant), it may be an artifact of modeling/overfitting and clinically misleading to describe. Sometimes this happens when there is some sort of relationship between an unspecified effect and predictors.

2.8 Safety Analyses

Adverse events reported at any time during the study will be tabulated for all enrolled infants and coded using the MedRA system. An estimate and 95% CI of the following proportions will be obtained using the exact binomial method:

- Proportion of infants for whom at least one event was reported
- Proportion of infants with an adverse event thought by investigator to be related to study drug
- Proportion of infants for whom at least one serious adverse event was reported
- Proportion of infant deaths
- Complications after the 4-week exam will be listed.
    - Systemic morbidities

The difference in the above proportions between the dose groups and the exact 95% CI will be calculated.

2.9 Protocol Adherence and Retention

Protocol deviations will be tabulated by randomized treatment group. Visit and call completion will be summarized by randomized treatment group. A flow chart will account for all participant visits and phone calls.

3.0 Plasma Levels of VEGF Optional Study

The parents of each infant enrolled in the study will be given the option to participate in a study to measure levels of VEGF in the plasma. Participants in this optional study will have blood collected for analysis. For each dose group, the distribution of VEGF levels (median, range, and quartiles) will be described before injection, and at 2 weeks, 4 weeks post-injection, and 4 months post-injection. The change from pre-injection will be calculated, and a 95% CI calculated for the change. To fit distributional assumptions, a log transformation or percent change may be used.