Phase 1 Trial of Bevacizumab Treatment for Severe Retinopathy of Prematurity (ROP1)

Statistical Analysis Plan
Version 3.0

Statistical Analysis Plan Version: 3.0, 16Mar2020
Protocol Version: 3.1, 17Apr2013

VERSION HISTORY

The following table outlines changes for the analysis plan:

VERSION NUMBER	AUTHOR	APPROVER	EFFECTIVE DATE	REVISION DESCRIPTION*
2.0[a]	T. Dean	M. Melia	08Sep2016	• The primary analysis was updated to incorporate GEE to adjust for the correlation between eyes within a patient. • Clarified which eyes would be used in analyses • Added a secondary analysis that will repeat the primary analysis but look at the total dose received at baseline in lieu of the dose injected into the eye. • Secondary, safety, and exploratory analyses will be performed stratified by the total dose received at baseline and the total dose received by week 4, where applicable, as well as the dose injected in the eye at baseline.
3.0[b]	Trevano Dean I am the author of this document 2020-03-16 16:59-04:00	Michele Melia I am approving this document 2020-03-16 17:41-04:00	16Mar2020	After reviewing the data, it was clear that a dose response curve, the primary analysis, could not be constructed using the proposed methods. Section 8.0 was added to describe the newly proposed primary analysis.

[a] Changes made after patient listings for first 4 doses reviewed with the Data and Safety Monitoring Committee, but prior to data analysis.
[b] Changes made during the data analysis phase.

1.0 Study Design

The goal of the study is to identify an effective dose of bevacizumab lower than the current standard (0.625 mg in 25 μL) that can be used in future studies to test the efficacy of bevacizumab in treating retinopathy of prematurity (ROP).

In brief, the study will begin by evaluating the efficacy of 0.25 mg of bevacizumab and if there is evidence of efficacy, the dose will be reduced by half. This process will continue until a dosage provides insufficient evidence of efficacy or until the ninth dose (0.001 mg) is deemed efficacious. For the purposes of this study, efficacy is defined as an 80% success rate (defined in section 3.1 of the protocol).

2.0 Primary Analysis

A dose response curve will be used to identify the lowest effective dose of bevacizumab. Logistic regression will be used to obtain estimates of the predicted success rate and corresponding 95% confidence intervals for each dosage level. The estimates obtained from the regression will be used to construct a dose response curve (predicted probability of success vs. dose of bevacizumab administered). Doses corresponding to success rates with a lower 95% bound greater than 80% will be identified, and the smallest dose will be selected as the lowest effective dose.

This analysis will make use of data from all study eyes. Eyes will be excluded if censoring occurred due to death but will be considered a failure if non-protocol treatment is received after enrollment but prior to the determination of success at the 4-week exam. The dosage level (in mg) will be included as a continuous covariate and may be transformed (using the $\log_2$ scale for example) to facilitate interpretation.

At least 3 doses will need to be tested in order for a dose response curve to be fit. If only 2 doses are tested, the data and safety monitoring committee will have decided that the second dose was not effective. In this case, the first dose evaluated will be considered the lowest effective dose.

2.1 Model Specification

A plot of the observed vs. fitted values will be compared to assess the appropriateness of the logistic regression model. If the logistic regression model is not appropriate, probit and cumulative log-log regressions will be explored to identify the model that best fits the data.

3.0 Secondary Analyses

3.1 Effect of Bevacizumab on Successful Treatment of Type I ROP

The primary analysis will be repeated using data from study eyes and non-study eyes that were diagnosed with type I ROP at enrollment and received an injection of bevacizumab at the time the study eye was injected. Eyes receiving an injection of 0.625mg of bevacizumab will not be included because this dose is not of interest in this study.

Logistic regression using generalized estimating equations to account for correlation between eyes within an individual will be used to obtain estimates of the predicted success rate and corresponding 95% confidence intervals for each dosage level. Estimates obtained from the

regression will be used to construct a dose response curve (predicted probability of success versus dose of bevacizumab administered) which will be compared with the curve created in the primary analysis.

As specified in the primary analysis, eyes will be excluded if censored due to death but will be considered a failure if non-protocol treatment was received after enrollment but prior to the determination of success at the 4-week exam. The dosage level (in mg) will be included as a continuous covariate and may be transformed to facilitate interpretation.

3.2 Effect of Total Bevacizumab Received on Successful Treatment of Type I ROP

Even though injections are localized, bevacizumab is expected to diffuse into the circulatory system, giving rise to the possibility of the study eye receiving an additional benefit if the non-study eye was injected.

In order to evaluate the effect of the total dose of bevacizumab administered on the successful treatment of type I ROP in study eyes, the primary analysis will be repeated, controlling for the dose of bevacizumab received in the fellow eye. The dose response curve constructed from the model controlling for the dose of bevacizumab injected into the fellow eye will be compared with the curve created for the primary analysis. If clinicians are unable to determine if a significant shift has occurred, a likelihood ratio test will be used to determine if the inclusion of the dose of bevacizumab injected into the fellow eye significantly improves the model fit.

3.3 Potential Confounders

Depending upon sample size and the number of dosage levels studied, a secondary analysis may explore if the following factors confound the relationship between dosage level and success:

- Location of disease (Zone I vs. Zone II)
- Severity of disease (Zone I with plus disease vs. Zone I stage 3 without plus disease vs. Zone II stage 2 or 3 ROP with plus disease)

The success rate for each dosage studied will be tabulated, stratified by each of the factors listed above. In addition, the primary analysis will be rerun adjusting for each of the factors above (models will adjust for one factor at a time as the factors are co-linear). The dose response curve derived from the model including the confounder will be compared with the curve constructed for the primary analysis. If clinicians are unable to determine if the difference between the curves is clinically significant, a likelihood ratio test will be used to determine if the addition of the confounder has a significant improvement on the model fit.

4.0 Exploratory Analyses

Depending upon sample size and the number of dosage levels studied, interaction terms for each potential confounder (section 3.2) and dosage will be added to the multiple logistic regression to evaluate whether the relationship between dosage and success is modified by the factor. In accordance with NIH guidelines, an analysis of treatment effect according to gender, as well as race/ethnicity, will be conducted. We recognize that these analyses are exploratory and may not have sufficient power to detect an interaction.

5.0 Safety

5.1 Adverse Events

Adverse events reported at any time during the study will be coded using the MedRA system and tabulated including all enrolled infants. The number and proportion of infants will be calculated for each of the following, stratified by the total dose of bevacizumab received prior to the time the event was reported:

- Infants for whom at least one event was reported
- Infants with an adverse event thought by the investigator to be related to study drug
- Infants for whom at least one serious adverse event was reported
- Infant deaths

Additionally, adverse events will be categorized as systemic or ocular. Ocular adverse events will be further subdivided into those related to the study eye injected at baseline, non-study eye injected after baseline, or non-study eye receiving no injection.

The Clopper-Pearson interval will be used to obtain 95% confidence intervals for the proportions.

The tabulations above will be repeated 1) limited to events reported through the 4-week post-injection examination to evaluate the safety of the initial study eye injection (treatment was at investigator discretion after the 4-week post-injection examination), and 2) limited to events reported after the 4-week post-injection examination but prior to the adjusted 12-month corrected age examination.

5.2 Physical exam data at each visit

The distribution (median, quartiles, and range) of head circumference (in centimeters) and weight (in grams) will be tabulated at pre-injection and at each follow-up visit. The mean change in head circumference and mean change in weight from pre-injection to each examination will also be calculated, and a 95% confidence interval will be calculated for each.

This analysis will be stratified by the total dose of bevacizumab received prior to the visit. If this data is not available, the analysis will be stratified by the total dose of bevacizumab received at enrollment.

6.0 Plasma Concentrations of VEGF and Bevacizumab

A separate analysis plan will be written to define analyses involving the relationship between VEGF and bevacizumab serum concentrations, as well as change in bevacizumab and VEGF concentration over time.

7.0 Additional Analyses and Tabulations

7.1.1 Description of cohort at Baseline

Subject-level and eye-level characteristics will be summarized for the entire cohort and stratified by dose level and total dose received at time of study eye injection. The following will be tabulated: gender, race, gestational age, birth weight, gestational age at time of study-eye

injection, and examination findings in the study eye and non-study eye at the time of study eye injection.

7.1.2 Description of Cohort at 6-Month Corrected Age

The following data will be tabulated to describe the cohort at the 6-month corrected age data collection:

- Infants requiring additional treatment for ROP in either eye since the 4-week post-injection examination
- Infants developing stage 4 or 5 ROP or vitreous hemorrhage in either eye since the 4-week post-injection examination
- Infants having retinal surgery since the 4-week post-injection examination

Marginal proportions will be reported, but data will also be tabulated and stratified by 1) dose of bevacizumab injected in the eye at baseline, 2) total dose of bevacizumab injected in eye by 4 weeks, and 3) total dose of bevacizumab received (in both eyes) by week 4.

7.1.3 Description at 12-month Corrected Age

Descriptive statistics will be calculated to describe the cohort at 12-month corrected age. The statistics below will be computed for the entire cohort but will also be stratified by 1) total dose of bevacizumab received at baseline and 2) total dose of bevacizumab received by 4 weeks.

The median, quartiles, and range will be calculated for the following data which are not expected to be normally distributed:

- Time since initial hospital discharge
- Number of times re-hospitalized since initial study eye injection
- Magnitude of alignment at near (horizontal and vertical)
- Magnitude of alignment at distance (horizontal and vertical)

The mean and standard deviation will be computed for the following data which are expected to be approximately normally distributed:

- Spherical equivalent refractive error in the study eye
- Spherical equivalent refractive error in the non-study eye

The distribution of the following will be tabulated:

- Number and proportion of infants requiring supplemental oxygen (current or past, current only)
- Number and proportion of infants with periventricular leukomalacia
- Number and proportion of infants with hydrocephalus (with shunt placement)
- Number and proportion of infants requiring additional treatment for ROP in the study eye (and the number of retreatments) if treated
- Number and proportion of infants requiring treatment for ROP in the fellow eye (and the number of treatments) if treated
- Distribution of most severe abnormality in study eye:
    - Essentially normal
    - Abnormal angle of temporal vessels

    - Macular ectopia
    - Stage 4A retinal detachment
    - Stage 4B retinal detachment
    - 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 causes other than ROP
    - Unable to determine
- Distribution of most severe abnormality in fellow eye:
    - Essentially normal
    - Abnormal angle of temporal vessels
    - Macular ectopia
    - Stage 4A retinal detachment
    - Stage 4B retinal detachment
    - 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 causes other than ROP
    - Unable to determine
- Number and proportion of infants with assessment of study eye
    - Cornea normal/abnormal
    - Anterior segment normal/abnormal
    - Lens normal/abnormal
    - Macular Ectopia absent, questionable, present, view obscured
    - Optic Nerve Atrophy absent, questionable, present, view obscured
    - Retinal Fold absent, present, view obscured
    - Retinal detachment absent, present, view obscured
- Number and proportion of infants with assessment of fellow eye
    - Cornea normal/abnormal
    - Anterior segment normal/abnormal
    - Lens normal/abnormal
    - Macular Ectopia absent, questionable, present, view obscured
    - Optic Nerve Atrophy absent, questionable, present, view obscured
    - Retinal Fold absent, present, view obscured
    - Retinal detachment absent, present, view obscured
- Number and proportion of infants with visual fixation in study eye of central, steady, or maintained
- Number and proportion of infants with visual fixation in fellow eye of central, steady, or maintained
- Number and proportion of infants with amblyopia present in study eye
- Number and proportion of infants with amblyopia present in fellow eye
- Number of infants on treatment for amblyopia and types of treatment

- Number and proportion of infants with alignment at distance of orthophoria, phoria, intermittent tropia, or constant tropia
- Number and proportion of infants with alignment at near of orthophoria, phoria, intermittent tropia, or constant tropia
- Number and proportion of infants with nystagmus

8.0 Post-hoc Analyses

8.1 Primary Outcome

After data were collected and reviewed, it was clear that the data did not lend itself to analysis via dose response curve (see section 2.0). Similar success rates were observed for the 7 highest doses, 5 of which resulted in the same observed success rate. Of the 8 doses evaluated, the 6 highest doses appeared to fall within the plateau phase of the dose response curve, the region in which the maximum effect has been achieved. Given that the success rate for only 2 doses did not fall within the plateau phase, there was insufficient data to construct a curve.

Nevertheless, logistic, probit, and cumulative log-log regressions were performed to comply with the analysis plan, but goodness of fit statistics indicated that these models were a poor fit to the data.

Instead of reporting results based on these regressions, the number and proportion of study eyes that met success criteria were tabulated for each dose. For each dose, the number and proportion of study eyes that met success criteria was also tabulated by severity of disease (Zone I with plus disease vs. Zone I stage 3 without plus disease vs. Zone II stage 2 or 3 ROP with plus disease). These tabulations were also performed for fellow eyes.

In addition to tabulations, Bayesian approaches were explored to identify the dose of bevacizumab that will be used in a future randomized trial designed to compare the efficacy of bevacizumab with that of laser photocoagulation for the treatment of type I ROP.