Phase 1 Trial of Bevacizumab Treatment for Severe Retinopathy of Prematurity

PROTOCOL

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RETINOPATHY OF PREMATURITY STUDY 1 (ROP1)

Phase 1 Trial of Bevacizumab Treatment for Severe Retinopathy of Prematurity

PROTOCOL AMENDMENT III (17Apr2018)

Protocol Change # 1

Original Protocol

Unless the study eye meets failure criteria, the fellow eye should not be given more than one injection of bevacizumab within 4 weeks of injection of the study eye (because of crossover effects).

Protocol Change

To add a sentence allowing investigators to call the Protocol Chair to discuss cases where alternative treatment in the fellow eye may be necessary in the best interest of the infant.

The following sentence has been added in Chapter 3.

If there is a strong rationale for treating the fellow eye with more than one injection of bevacizumab, the Protocol Chair or his/her designee should be called to discuss it before treatment is given.

Rationale for Change

Investigators should be allowed to consider non-protocol treatment that is in the best interest of the infant; but are asked to call the Protocol Chair to discuss the case first to come to consensus.

Other Changes

The contact information for the protocol chair has been changed to reflect his move from Duke University to Indiana University.

RETINOPATHY OF PREMATURITY STUDY 1 (ROP1)

Phase 1 Trial of Bevacizumab Treatment for Severe Retinopathy of Prematurity

PROTOCOL AMENDMENT II (11-1-16)

Protocol Change # 1

Original Protocol

If a very high success rate is achieved at every dose tested, then consideration may be given to amending the protocol with DSMC and IRB approval to evaluate doses of 0.016 mg, 0.008 mg, 0.004 mg, 0.002 mg, and 0.001 mg.

Protocol Change

To evaluate a dose of 0.016mg and if success criteria are met, consecutively evaluate the following smaller doses as long as success criteria are met: 0.008mg, 0.004mg, 0.002mg, and 0.001mg.

Rationale for Change

A successful 4-week outcome was achieved for all four dose levels studies under the current protocol (11 of 11 eyes receiving 0.25 mg, 14 of 14 eyes receiving 0.125 mg, 21 of 24 eyes (88%) receiving 0.0625 mg, and 9 of 9 eyes receiving 0.03125 mg). The PEDIG DSMC has reviewed the safety and efficacy data and has approved continuation of the study to evaluate up to five additional lower doses of bevacizumab.

Protocol Change # 2

Original Protocol:

The maximum number of study participants is 112, but it is expected to be approximately 50-60.

Protocol Change

The maximum number of study participants is 201.

Rationale for Change

Sixty-one infants were enrolled to evaluate the first four dose levels of bevacizumab. If as high as 14 infants are enrolled into each of two series of infants for up to an additional five lower dose levels, then the maximum number of infants that will be enrolled is 201 (61 plus 140).

Protocol Change # 3

Original Protocol:

If adverse events occur, they will be recorded and reported throughout the study.

Protocol Change

All adverse events between the time of study eye injection and the 4-week ocular exam or hospital discharge (whichever is later) will be recorded. After the 4-week ocular exam or hospital discharge (whichever is later), only serious adverse events (*see section 5.3),* ocular adverse events, and any events judged by the investigator to be related to injection and/or study treatment will be recorded.

Rationale for Change

Non-serious non-ocular events occur very commonly in premature infants. It is extremely time-consuming to record all of these events, and those occurring after the 4-week ocular exam or

hospital discharge (whichever is later) are not expected to be related to the study injection or treatment, and thus not useful for data analyses, particularly since there is no control arm.

This amendment also provides for the following clarification to the success/failure definition:

The success/failure definition as defined in section 3.4 was edited to provide a more realistic definition of improvement by 3-5 days for infants with pre-treatment zone I, stage 3 without plus disease.

Rationale for Change

Previously, all study eyes with I, stage 3 without plus disease had to demonstrate a significant reduction in the severity and/or extent of neovascularization by 3-5 days, or they were considered failures. However, some eyes show improvement in the posterior pole from pre-plus to normal, and no worsening of neovascularization by 3-5 days, with improvement of neovascularization noted by 1-2 weeks. These eyes will not be considered failures.

RETINOPATHY OF PREMATURITY STUDY 1 (ROP1)

Phase 1 Trial of Bevacizumab Treatment for Severe Retinopathy of Prematurity

PROTOCOL AMENDMENT I (1-27-16)

Proposed Change # 1

Current Protocol

Other than for adverse events, there is no data collection between the 4-week post-injection exam and the visit at 12 months corrected age.

Proposed Change

After the 4-week post-injection exam, sites will report any study infants who develop stage 4 or 5 ROP, or require additional bevacizumab injection, laser treatment, or retinal surgery (vitrectomy or scleral buckle). In addition, at 6 months corrected age (calculated as the estimated date of confinement (EDC), or due date, plus 6 months), medical records will be reviewed to collect data from non-study exams after 4 weeks post-injection.

Rationale for Change

Some cases of ROP that are successfully treated with bevacizumab have recurrence of severe ROP that requires treatment. It is possible that the incidence of ROP recurrence requiring treatment will increase with lower doses of bevacizumab. It is also possible that some of these cases will have sub-optimal outcomes, whether or not additional treatment was required.

These data may be useful to the DSMC when deciding to reduce dosages. It is not feasible to have 6-month data on all infants during DSMC deliberations, because that would require waiting several months after each dosage level is tested before moving to the next dosage level. The primary outcome of successful treatment can be determined after 4 weeks, and late recurrence with suboptimal outcome is expected to be uncommon.

Protocol Change #2

Current Protocol

The visit window for the 2, 3, and 4 week post-injection exams is the target date +/- 2 days.

Proposed Change

Extend the window one day on each side such that the visit window for the 2, 3, and 4 week post-injection exams is the target date +/-3 days.

Rationale for Change

This 7-day window will better match clinical exam schedules, since most centers do ROP rounds once per week. It will help to eliminate extra examinations for infants, which can be stressful.

Protocol Change #3

Current Protocol

The treating investigator and a second person will review the Bevacizumab Study Syringe Preparation Form to confirm which eye will receive the intravitreal injection and will place a mark (with a sticker or marking pen) above the brow of the eye before injection.

Proposed Change

The sentence will be changed to: "For each eye to be treated, the treating investigator and second person will review the Bevacizumab Study Syringe Label to confirm which eye will receive the intravitreal injection." The reference to marking the infant with sticker or pen will be removed.

Rationale for Change

To maintain masking investigators will review the syringe *label* rather than the *prescription form* as the label does not contain the study dosage. Investigators will operate under their own surgical routine with respect to marking or not marking the eye.

CONTACT INFORMATION

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TABLE OF CONTENTS

CHAPTER 1: BACKGROUND AND SUMMARY

This study is being conducted by the Pediatric Eye Disease Investigator Group (PEDIG) and is funded through a cooperative agreement from the National Eye Institute.

1.1 Background

Retinopathy of prematurity (ROP) is a significant cause of childhood vision loss in the United States,[1] characterized by preretinal neovascularization and fibrosis that may ultimately lead to retinal traction and detachment. Low birth weight, gestational age, and supplemental oxygen are important risk factors for ROP, but despite the judicious use of supplemental oxygen and effective screening and management of ROP in most developed countries, it continues to be a significant cause of visual impairment. Severe visual loss is particularly common in middle income countries such as China and India.[1, 2] Treatment for severe ROP has focused on ablation of the peripheral avascular retina with laser photocoagulation.[3] More recently, treatments have targeted blockade of pro-angiogenic growth factors, such as vascular endothelial growth factor (VEGF), or their receptors.[4-6]

Examination findings in ROP are classified according to the international classification of ROP (ICROP).[7] Zone refers to location of disease, from zone I (most posterior) to zone III (most anterior). Stage refers to activity at the vascular/avascular border. Stage 1 is a line, stage 2 is a ridge, stage 3 is neovascular tissue, stage 4 is a partial retinal detachment, and stage 5 is a total retinal detachment. Plus disease is dilation and tortuosity of the posterior retinal vessels meeting or exceeding the amount seen in a standard photograph that has been used in many clinical trials. The Early Treatment for ROP (ETROP) study established "type 1 ROP" as the degree of disease severity for which treatment with laser is indicated. Type 1 ROP is defined as any stage ROP in zone I with plus disease, stage 2 or 3 ROP in zone II with plus disease, or stage 3 ROP in zone I without plus disease.[8]

Bevacizumab (trade name Avastin®; Genentech, Inc., South San Francisco, CA) is a humanized monoclonal antibody which binds to VEGF, prevents coupling of VEGF to its receptor, and inhibits angiogenesis. Initially approved by the FDA for anti-angiogenic treatment of metastatic colorectal cancer, bevacizumab is used increasingly in the US and abroad as an off-label treatment for severe (type 1) ROP, given by intravitreal injection at a small fraction of the systemic dose for cancer. The BEAT-ROP trial (Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity), was a randomized trial of bevacizumab monotherapy versus conventional laser therapy for zone I and posterior zone II, stage 3+ ROP.[5] Results of the BEAT-ROP trial suggested a benefit of bevacizumab treatment over conventional laser therapy for zone I ROP but not for zone II ROP. In addition, high myopia was much less common after bevacizumab compared with laser. However, the BEAT-ROP study had important limitations. The primary outcome was "recurrence of ROP requiring retreatment," and the decisions about retreatment were made by unmasked examiners. The study lacked a functional outcome measure such as visual acuity. The recurrence of ROP requiring retreatment was relatively high (22%). Therefore, additional studies are needed to determine the relative effectiveness of Avastin and laser.

Little is known about the safety of using bevacizumab for ROP,[9] as the BEAT-ROP study provided little short-term and no long-term safety data. In the BEAT-ROP study, 5 infants undergoing bevacizumab injection and 2 infants undergoing laser treatment died before the age of 54 weeks, but the study was not powered to evaluate whether the death rate was any higher

following bevacizumab injection.[5] At 2 ½ years of age the death rate was similar between groups, with a total of 6 infants treated with bevacizumab and 7 infants in the laser treated group dying prior to age 2 ½ years.[10] Angiogenesis is an important process in the normal development of other organ systems such as the lungs, kidneys, brain, and bones. Intravitreal bevacizumab reaches the systemic circulation, so there is significant potential for negative systemic side effects. The dose of bevacizumab used in the BEAT-ROP study (0.625 mg in 25 µl) was chosen empirically as one-half the adult dose used for macular degeneration.[5] Studies looking at dose of intravitreal bevacizumab for treatment of proliferative diabetic retinopathy in adults found consistent biological effects with doses as high as 1.25 mg to as low as 0.00625 mg,[11] suggesting that bevacizumab may be effective at much lower doses than the dose used in the BEAT-ROP study. Ideally, future treatment with bevacizumab would utilize the lowest effective dose, reducing the systemic drug exposure. Nevertheless, the lowest effective dose of bevacizumab for the treatment of severe ROP is unknown. A phase 1 study is needed to find a lower dose of intravitreal bevacizumab injection for the treatment of severe ROP that can be evaluated in future larger studies.

1.2 Anti-VEGF Treatments for Other Retinal Disorders

Intravitreal injection of anti-VEGF agents has become increasingly common for treatment of retinal disorders in adult patients. Macugen (pegaptanib sodium, manufactured by Pfizer) was the first of the anti-VEGF agents for ocular use, approved by the FDA in 2004 for treatment of choroidal neovascularization secondary to wet age-related macular degeneration. Macugen is a pegylated aptamer that works by binding to $VEGF_{165}$ and preventing its binding to VEGF target receptors. In 2006, Lucentis (ranibizumab, manufactured by Genentech) was approved by the FDA for treatment of wet age-related macular degeneration. Lucentis is a monoclonal antibody fragment which binds all forms of VEGF, much like Avastin, preventing binding to target receptors. In 2010, Lucentis became the first FDA-approved anti-VEGF agent for treatment of macular edema resulting from retinal vein occlusion. In 2012, Lucentis became the first and only FDA approved anti-VEGF agent for treatment of diabetic macular edema. Eylea (afibercept, manufactured by Regeneron) was first approved by the FDA in 2011 for treatment of wet AMD and then later in 2012 as a treatment for macular edema following retinal vein occlusion. Eylea is a recombinant fusion protein containing the extracellular domains of human VEGF receptors 1 and 2 fused to a portion of human IgG1, which competes with native VEGF receptors for binding of free VEGF. Despite wide usage of anti-VEGF agents for treatment of retinal disorders in adults, no approval exists for their use in pediatric populations.

1.3 Safety

In a meta-analysis performed by Genentech, Inc. on all clinical trial results using *intravenously* administered bevacizumab (usually dosed as 5 mg/kg every 14 days) in adults, it was found that adult study participants were at an increased risk for certain adverse events, some of which were potentially fatal. These included wound healing complications, bowel perforation, hemorrhage, stroke, myocardial infarction, hypertension, congestive heart failure, and proteinuria. Warnings and precautions included in the bevacizumab package insert for intravenously administered drugs fall under the categories of gastrointestinal perforations, surgery and wound healing complications, hemorrhage, non-gastrointestinal fistula formation, arterial thromboembolic events, hypertension, reversible posterior leukoencephalopathy syndrome, proteinuria, infusion reactions, ovarian failure, and infertility in females with reproductive potential.[12] Data regarding the safety and pharmacokinetics of bevacizumab in pediatric patients have not been established,[12] although there is potential for similar adverse events in infants as in adults.

Doses administered to infants for treatment of ROP (typically 0.3 mg to 0.625 mg) are lower than those administered to adults for treatment of ocular conditions, and much less than intravenous doses used for cancer treatments. A study by Sato et al[13] found serum concentrations of bevacizumab following a 0.5mg intravitreal injection for ROP were well above concentrations required to completely block in vitro VEGF activities in human umbilical vein endothelial cells.[14] There was an inverse relationship between the serum bevacizumab and serum VEGF concentrations. Sato et al concluded that bevacizumab has the ability to cross the blood retinal barrier and enter the systemic circulation in relatively high concentrations.[13] Others have reported similar effects with an even lower dose (0.375mg) of bevacizumab.[15] Because of the vasculoproliferative role that VEGF plays in the normal development of organs, systemic bevacizumab presents a potential risk to the developing organs of the premature neonate.

Ocular side effects have been reported after intravitreal injections of bevacizumab. In some cases, retinal traction may be worsened by injection of bevacizumab, leading to retinal detachment,[16] particularly if membranes have already begun to form.[17] Regression of ROP following bevacizumab treatment may in some cases be transient, with recurrence of ROP occurring later than what is observed with laser treatment.[18] Other complications observed following bevacizumab treatment for ROP include retinal hemorrhage,[19, 20] transient vascular sheathing,[20] choroidal ischemia,[21] abnormalities of the retinal periphery (large avascular areas, abnormal branching, shunts) and of the posterior pole (hyperfluorescent areas, absence of the foveal avascular zone). It is possible but unknown if these changes will have an effect on vision.[22]

It is unclear whether the mortality rate of premature infants receiving intravitreal bevacizumab is any higher than those treated with laser. In the BEAT-ROP study, 7 of 150 infants died prior to the 54-week post-menstrual age outcome examination (5 after intravitreal bevacizumab, 2 after laser).[5] While the mortality was higher in infants receiving bevacizumab, this result was statistically non-significant, although it was acknowledged that the study was grossly underpowered to detect a difference (a sample of 2800 infants would be required to assess a death rate 1.5 times the 5.4% mortality rate observed in the ET-ROP study by 9 months[8] at an alpha of 0.05 and 80% power).[5] Additional follow-up of children enrolled into the BEAT-ROP study showed similar rates of mortality in both groups, with 6 infants treated with bevacizumab and 7 infants in the laser treated group dying prior to age 2 ½ years.[10] Another study reported death of 2 of 7 infants with ROP following a 0.75 mg bevacizumab injection, but attributed the mortality to complications of their previous systemic conditions.[23] However, it is unclear whether systemic bevacizumab may have exacerbated the existing conditions, particularly in cases of multiple organ failure or lung failure. Dosing studies and long-term follow-up studies with a large number of infants are required to determine the safety of intravitreal bevacizumab injection for the treatment of ROP.[5, 9, 24, 25]

1.4 Rationale for the Study

Despite promising initial results using empirical doses of bevacizumab based on half the adult dose for treatment of acute severe ROP, little is known about lower doses of bevacizumab for ROP. An increasing number of ophthalmologists are treating premature infants with severe ROP using bevacizumab. Given the potential systemic and ocular adverse effects of intravitreal bevacizumab injections, determining a lower effective dose of bevacizumab is an important next step. The proposed study will test progressively lower doses to find a dose to take forward to a future larger study.

1.5 Study Objective

To find a dose of intravitreal bevacizumab that is lower than currently used for severe ROP, is effective in this study, and can be tested in future larger studies.

1.6 Synopsis of Study Design

Major eligibility criteria: (See *section 2.2* for a complete listing)

1. Type 1 ROP (as defined in section 2.4) in one or both eyes
2. No previous treatment for ROP (except previous treatment of the fellow eye with laser is allowed)

Treatment Paradigm:

A dosage of 0.625 mg in 25 µl is commonly used in practice by clinicians. This study will evaluate the effectiveness of 0.25 mg and, if there is evidence of effectiveness, will reduce the dose by half until a dosage is found where the evidence suggests insufficient effectiveness. If all doses meet the study effectiveness criteria, then up to 9 different dosages will be evaluated in this study. Effectiveness for the purpose of this study is defined as at least 80% of eyes meeting the study's definition of success (as defined in section 3.4).

Approach to Study Design:

Not all outcomes for every enrolled subject will be obtainable at the 4-week outcome examination for various reasons, including instability of the infant's medical condition, non-drug-related mortality, need to transfer to another medical institution, hospital discharge with poor outpatient follow-up, or obstructed view of the retina. Therefore, additional infants will be enrolled until an outcome has been established for 10 subjects, up to a maximum of 14 subjects per dose.

1. A minimum of 10 subjects, and a maximum of 14 subjects, will be injected with an initial dose of 0.25 mg bevacizumab in 10 µl. For infants with bilateral type 1 ROP requiring treatment, one randomly selected eye will be injected with bevacizumab. The other eye will be treated with laser photocoagulation or with the last effective dose level of bevacizumab (one level higher than the level currently being studied).
    a. Recruitment of subjects at each dose will continue until any one of the following occurs:
        a. There are 4 failures, declared 3 days to 4 weeks post-injection
        b. There are 8 successes, declared 4 weeks post-injection
        c. 14 infants receive injections
        d. The 10th subject for whom an outcome can be assessed is 3 weeks post-injection
    b. After recruitment for a dosage ends based on the above criteria, the Data Safety and Monitoring Committee (DSMC) will review outcomes from the first 10 infants injected as well as preliminary outcomes from any additional (1-4) infants injected, and the committee will make a recommendation to reduce the dosage and test another 10-14 subjects, repeat the same dosage for another 10-14 subjects, wait for outcomes of 1-4 additional infants injected, or stop the study. The DSMC will apply the following guidelines, but may make a different decision based on the specific study data:
        a. If the success rate is 80% or greater, then the study will continue to the next lower dose. Up to 4 different doses will be evaluated.

b. If the success rate is greater than 70% but less than 80%, then 10-14 additional infants will be treated at the same dose.
c. If the success rate is less than 70%, then another 10-14 infants will be evaluated at the next higher dose to confirm efficacy, unless the first dose (0.25 mg) is unsuccessful, or the previous successful dose was already tested on 2 sets of 10-14 infants.

2. The maximum number of study participants is 201.

Exam Schedule

Data will be collected on the day of injection (day 0).

The exam schedule is as follows:

- 1 day post-injection
- If type 1 is still present at day 1, then an exam will occur at 4 days (3 to 5 days) post-injection
- 1 week (6 to 8 days) post-injection
- 2 weeks (11 to 17 days) post-injection
- 3 weeks (18 to 24 days) post-injection
- 4 weeks (25 to 31 days) post-injection

- After 4 weeks, follow-up and treatment will be at investigator discretion, except for the following:
  - If at any time after 4 weeks post-injection an infant develops stage 4 or 5 ROP, or requires additional bevacizumab injection, laser treatment, or retinal surgery (vitrectomy or scleral buckle), medical records will be reviewed to collect data from non-study exams since the 4 week post-injection exam.

  - For all infants:
    - At 6 months (+/-2 weeks) corrected age calculated as the estimated date of confinement (EDC), or due date, plus 6 months, medical records will be reviewed to collect data from non-study exams since the 4 week post-injection exam.
    - At 12 months (+/-2 weeks) corrected age, an examination will be done and medical records will be reviewed to collect data from non-study exams not already collected.

- All adverse events between the time of study eye injection and the 4-week ocular exam or hospital discharge (whichever is later) will be recorded. After the 4-week ocular exam or hospital discharge (whichever is later), only serious adverse events (*see section 5.3),* ocular adverse events, and any events judged by the investigator to be related to injection and/or treatment will be recorded.

Primary Outcome

The outcome for each subject will be defined as *successfully treated* or *not successfully treated* after 4 weeks (*see section 3.4*). Success is defined as improvement* by the 4-day exam and no recurrence of type 1 ROP or severe neovascularization requiring additional treatment within 4 weeks of injection.

* For infants with pre-treatment plus disease, improvement by the 4-day post-injection exam is defined as plus disease no longer being present. For infants with pre-treatment zone I, stage 3,

with pre-plus disease, improvement by the 4-day post-injection exam is defined as: (1) pre-plus no longer present (neither plus nor pre-plus disease), or (2) a reduction in severity and/or extent of extraretinal neovascularization. For infants with pre-treatment zone I, stage 3, with neither plus nor pre-plus disease, improvement by the 4-day post-injection exam is defined as a reduction in severity and/or extent of extraretinal neovascularization.

A dose will be considered effective if it successfully treats at least 80% of subjects.

CHAPTER 2: STUDY PARTICIPANT ELIGIBILITY AND ENROLLMENT

2.1 Eligibility Assessment and Informed Consent

A subject is considered for the study after undergoing a routine examination (as part of standard care) that identifies type 1 ROP that meets the eligibility criteria. The study will be discussed with the infant's parent(s) or guardian(s) (referred to subsequently as parent(s)). Parent(s) who express an interest in the study will be given a copy of the informed consent form to read. Written informed consent must be obtained from the parent prior to performing any study-specific procedures that are not part of the patient's routine care.

2.2 Eligibility Criteria

2.2.1 Participant-level Inclusion Criteria

Study participants are eligible for the study if the following are true:

1. Parent understands the protocol and is willing to provide consent.
2. If hospital discharge is anticipated within the next 4 weeks, parents are able and willing to return to the PEDIG site for outpatient follow-up visits.
3. Transfer to another hospital not covered by study-certified examiners is not anticipated within the next 4 weeks.

2.2.2 Study Eye Inclusion and Exclusion Criteria

The study participant must have at least one eye meeting all of the inclusion criteria and none of the exclusion criteria listed below. Study participants can have only one study eye. If both eyes are eligible at the time of enrollment, then one eye will be randomly selected for injection at the time of enrollment (on the PEDIG website), and the fellow eye will be treated with laser photocoagulation or with the last effective dose level of bevacizumab (one level higher than the level currently being studied). Data will be collected for both the study and non-study eyes.

<u>Inclusion Criteria:</u>

1. Type 1 ROP; defined as:
    - Zone I, any stage ROP with plus disease, or
    - Zone I, stage 3 ROP without plus disease, or
    - Zone II, stage 2 or 3 ROP with plus disease
2. No previous treatment for ROP in the study eye; no previous bevacizumab treatment in the non-study eye

<u>Exclusion Criteria for the Study Eye:</u>

The following exclusions apply to the study eye:

1. Nasolacrimal duct obstruction
2. Major ocular anomalies (e.g., cataract, coloboma)
3. Any opacity that precludes an adequate view of the retina

If purulent ocular discharge is present in either eye, then the infant is ineligible.

2.3 Historical Information

Historical information recorded at enrollment will include any prior treatment for ROP (i.e. laser treatment in the non-study eye), gestational age at birth, birth weight, current weight, head circumference, gender, race, ethnicity, concurrent medical conditions (e.g. IVH, PVL, hydrocephalus), and current medications.

2.4 Enrollment Examination

Classification of ROP on day of examination when type 1 ROP is diagnosed:

ROP will be classified by the investigator using the revised International Classification of Retinopathy of Prematurity (ICROP) criteria.[7, 26]

Location, extent, and stage of disease, as well as presence of pre-plus, plus disease, or aggressive posterior ROP, will be recorded as follows:

Location: Location will be recorded as follows:

- **Zone I**: circle centered on the optic nerve with a radius of twice the distance from the center of the optic nerve to the center of the macula
- **Zone II**: extends centrifugally from the edge of Zone I to the nasal ora serrata and is concentric to zone I

Stage: Disease stage will be recorded as stages 1-5 as defined:

- **Stage 1**: Demarcation line
- **Stage 2**: Ridge
- **Stage 3**: Extraretinal fibrovascular proliferation
- **Stage 4**: Partial retinal detachment. Stage 4 will be further classified based on location of the partial retinal detachment:
  - **Stage 4A**: Extrafoveal
  - **Stage 4B**: Foveal
- **Stage 5**: Total retinal detachment

Extent of Disease (clock hours): Extent of the highest stage will be recorded in 30° increments, or clock hours.

Plus Disease: A diagnosis of plus disease will be made if at least 2 quadrants have abnormal dilation and tortuosity meeting or exceeding the amount shown in the standard photograph of plus disease.[26, 27]

Pre-plus Disease: A diagnosis of pre-plus disease will be made when there is abnormal dilation and tortuosity, but it is insufficient to diagnose plus disease.

Type 1 ROP is the degree of disease severity for which treatment is indicated. It is defined as:

- Zone I, any stage ROP with plus disease, or
- Zone I, stage 3 ROP without plus disease, or
- Zone II, stage 2 or 3 ROP with plus disease

The above classification of ROP in the non-study eye will also be made and data collected at the time of enrollment.

CHAPTER 3: TREATMENT AND FOLLOW-UP

Bevacizumab (Avastin) is made by Genentech, Inc. and is approved for metastatic colorectal cancer, nonsquamous, non-small cell lung cancer, recurrent glioblastoma, and metastatic renal cell carcinoma. The study is being conducted under an Investigational New Drug Application (IND) as intravitreal injection of bevacizumab for ROP in children is an off-label use.

All participants will receive a single intravitreal injection of bevacizumab (*see section 3.1 for dose*) following enrollment into the study. The injection should be given as soon as possible but no later than 72 hours after the diagnosis of type 1 ROP. If there is type 1 ROP in one eye only, then that eye will be injected. If the fellow eye reaches type 1 ROP at a later date within 4 weeks of injection in the study eye, then that eye will be treated with laser photocoagulation or the last effective dose level of bevacizumab (one level higher than the level currently being studied). If there is type 1 ROP in both eyes, then one eye will be randomly selected for injection at the time of enrollment, and the fellow eye will be treated with laser or the last effective dose level of bevacizumab (one level higher than the level currently being studied). If the study eye meets failure criteria, then either eye can be treated at investigator discretion. Unless the study eye meets failure criteria, the fellow eye should not be given more than one injection of bevacizumab within 4 weeks of injection of the study eye (because of crossover effects). If there is a strong rationale for treating the fellow eye with more than one injection of bevacizumab, the Protocol Chair or his/her designee should be called to discuss it before treatment is given. The fellow eye can be treated or re-treated with laser at investigator discretion.

3.1 Bevacizumab Dose and Injection

Study eyes will receive a single dose of bevacizumab provided by the pharmacy at the investigator's institution. A coordinator at each site will be unmasked to dosage as he/she will be required to process the prescription/s to the pharmacy per their usual institutional ordering mechanism. The pharmacy will prepare the bevacizumab in a sterile manner, adhering to USP 797 standards. Syringes containing bevacizumab at the appropriate study concentration will be prepared. If one eye is injected, then two syringes will be prepared. If two eyes are injected, then four syringes will be prepared. One syringe will serve as a backup and will only be used if the other syringe is compromised for any reason; otherwise, the backup syringe will be discarded if unused. The investigator and all other personnel at the site will be masked to the dosage level.

The dosages of injected bevacizumab to be studied are listed in Table 1. The decision of whether to increase, repeat, or decease the dose of bevacizumab will be determined as follows:

1. A minimum of 10 subjects, and a maximum of 14 subjects, will be injected with an initial dose of 0.25 mg bevacizumab in 10 µl. For infants with bilateral type 1 ROP requiring treatment, one randomly selected eye will be injected with bevacizumab. The other eye will be treated with laser photocoagulation or with last effective dose level of bevacizumab (one level higher than the level currently being studied). If the study eye is treated with 0.25 mg, and bevacizumab is used for the non-study eye, then the dose for the non-study eye will be 0.625 mg.
    a. Recruitment of subjects at each dose will continue until any one of the following occurs:
        a. There are 4 failures, declared 3 days to 4 weeks post-injection
        b. There are 8 successes, declared 4 weeks post-injection
        c. 14 infants receive injections
        d. The 10th subject for whom an outcome can be assessed is 3 weeks post-injection.
    b. After recruitment for a dosage ends based on the above criteria, the DSMC will review outcomes from the first 10 infants injected as well as preliminary outcomes from any

additional (1-4) infants injected, and the committee will make a recommendation to reduce the dosage and test another 10-14 subjects, repeat the same dosage for another 10-14 subjects, wait for 4-week outcomes of 1-4 additional infants injected, or stop the study. The DSMC will apply the following guidelines, but may make a different decision based on the specific study data:

a. If the success rate is 80% or greater, then the study will continue to the next lower dose. Up to 4 different doses will be evaluated.
b. If the success rate is greater than 70% but less than 80%, then an additional 10-14 infants will be evaluated at the same dose.
c. If the success rate is less than 70%, then another 10-14 infants will be evaluated at the next higher dose to confirm efficacy, unless the first dose (0.25 mg) is unsuccessful, or the previous successful dose was already tested on 2 sets of 10-14 infants.

2. The maximum number of study participants is 201.

Table 1: Dosages of Injected Bevacizumab to be Studied:

Dose	Volume Injected	Concentration
0.25mg (start)	10µl	25 mg/ml
0.125mg	10µl	12.5 mg/ml
0.0625mg	10µl	6.25 mg/ml
0.03125mg	10µl	3.125 mg/ml
0.016mg	10µl	1.6 mg/ml
0.008mg	10µl	0.8mg/ml
0.004mg	10µl	0.4mg/ml
0.002mg	10µl	0.2mg/ml
0.001mg	10µl	0.1mg/ml

It may not be necessary to enroll 10 subjects for each dose evaluated, because if 8 have success or 4 have failure before 10 are enrolled and treated, then success or failure of that dosage can be declared at that point (and failure may be declared earlier than the 4-week outcome).

3.1.1 Injection Technique

The bevacizumab injection will be given preferably within 24 hours, but no later than 72 hours, after the diagnosis of type 1 ROP. The ophthalmologist may choose to give the intravitreal injection in the operating room or at the bedside, with or without anesthesia given after consultation with the institutional neonatologist. A binocular indirect ophthalmoscope with an appropriate condensing lens should be available, and the pupils should be dilated.

For each eye to be treated, the treating investigator and a second person will review the Bevacizumab Study Syringe Label to confirm which eye will receive the intravitreal injection.

Two individuals must confirm the label information on the study syringe to be used for injection matches the information on the Bevacizumab Study Syringe Preparation Form prior to injection to ensure accuracy.

The investigator who gives the injection must have previous experience giving intravitreal injections. If the injection is the first given by the investigator for ROP, then it must be given with the assistance of an ophthalmologist who has previously given intravitreal injections for ROP.

The injection will be done as outlined in the ROP Injection Procedure Manual.

3.2 Exam Schedule

The exam schedule is as follows:

- 1 day post-injection
- If improvement (defined in sections 1.6 and 3.4) has not occurred at day 1, then an exam is done at 4 days (3 to 5 days) post-injection
- 1 week (6 to 8 days) post-injection
- 2 weeks (11 to 17 days) post-injection
- 3 weeks (18 to 24 days) post-injection
- 4 weeks (25 to 31 days) post-injection (primary outcome)

- After 4 weeks, follow-up and treatment will be at investigator discretion, except for the following:
    - If at any time after 4 weeks post-injection an infant develops stage 4 or 5 ROP, or requires additional bevacizumab injection, laser treatment, or retinal surgery (vitrectomy or scleral buckle), medical records will be reviewed to collect data from non-study exams since the 4 week post-injection exam.

    - For all infants:
        - At 6 months (+/-2 weeks) corrected age calculated as the estimated date of confinement (EDC), or due date, plus 6 months, medical records will be reviewed to collect data from non-study exams since the 4 week post-injection exam.
        - At 12 months (+/-2 weeks) corrected age, a study-mandated examination will be done and medical records will be reviewed to collect data from non-study exams not already collected.

- All adverse events between the time of study eye injection and the 4-week ocular exam or hospital discharge (whichever is later) will be recorded. After the 4-week ocular exam or hospital discharge (whichever is later), only serious adverse events (*see section 5.3),* ocular adverse events, and any events judged by the investigator to be related to injection and/or treatment will be recorded.

- 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.

- Additional (non-study) examinations may be done at investigator discretion.

3.3 Follow-up Exam Procedures

Classification of ROP will be determined at each follow-up exam as described in *section 2.4*. Data will be collected for both the study and non-study eye.

Additional data collected at 6 and 12 months will include the following:

- Additional treatment/s for ROP since the 4-week exam (for both the study and non-study eye)
- Complications since the 4-week exam

Additional data collected at 12-months will include the following:

- Date of initial hospital discharge
- Number of times re-hospitalized by 12 months
- Most recent head circumference (in centimeters) and date obtained
- Most recent weight (in grams) and date obtained
- Current supplemental oxygen requirement, or date supplemental oxygen discontinued
- Date and cause of death (if applicable)
- Presence or history of systemic co-morbidities including:
    - Periventricular leukomalacia
    - Hydrocephalus (with shunt placement)
- Assessment of vision, amblyopia, strabismus, retinal structure, and refractive error

Some data collected at 6 and 12 months will be collected retrospectively from chart review.

3.4 Definition of Success / Failure

Assessment of success/failure will be standardized by certifying investigators as knowledgeable with respect to the revised International Classification of Retinopathy of Prematurity (ICROP) criteria[7], upon which exam findings and failure criteria will be based.

Success is defined as improvement* by the 4-day exam (3 to 5 days), and no recurrence of type 1 ROP or severe neovascularization requiring additional treatment within 4 weeks of injection. If either or both of these criteria are not met, then a second examination will be done by a study-certified examiner. If the second examiner confirms that any success criteria are not met, then treatment for this eye will be considered a failure, and the investigator may give any additional treatment he/she deems necessary. Failure can be declared as early as the 4-day post-injection examination (3 to 5 days), and treatment can then be started at investigator discretion.

If the second examiner does not confirm failure, then they will examine together and reach consensus.

* For infants with pre-treatment plus disease, improvement by the 4-day post-injection exam is defined as plus disease no longer being present. For infants with pre-treatment zone I, stage 3, with pre-plus disease, improvement by the 4-day post-injection exam is defined as: (1) pre-plus no longer present (neither plus nor pre-plus disease), or (2) a reduction in severity and/or extent of extraretinal neovascularization. For infants with pre-treatment zone I, stage 3, with neither plus nor pre-plus disease, improvement by the 4-day post-injection exam is defined as a reduction in severity and/or extent of extraretinal neovascularization.

The investigator will be masked to dosage.

3.5 Side Effects of Bevacizumab

Data will be collected at each follow-up visit to evaluate potential adverse effects of injection as described in *section 5.1*.

3.6 Additional Examinations

Investigators may perform additional examinations at their discretion. Failure may occur at any exam prior to 4 weeks starting with the 4-day post-injection exam. Success cannot be declared until the 4-week exam.

3.7 Additional Treatment

Treatment for the study eye or the fellow eye is at investigator discretion after the 4-week outcome examination, or sooner if the study eye meets criteria for failure and it is confirmed by a second examiner. Investigators should not treat the study eye with laser or additional injections prior to the 4-week outcome examination, unless failure criteria are met and confirmed. If there is a strong rationale for earlier treatment in the study eye in the absence of meeting failure criteria, the Protocol Chair or his/her designee should be called to discuss it before treatment is given.

The fellow eye may be treated with laser photocoagulation or the last effective dose level of bevacizumab (one level higher than the level currently being studied). Unless the study eye meets failure criteria, the fellow eye should not be given more than one injection of bevacizumab within 4 weeks of injection of the study eye (because of crossover effects). If there is a strong rationale for treating the fellow eye with more than one injection of bevacizumab, the Protocol Chair or his/her designee should be called to discuss it before treatment is given. The fellow eye may be treated or re-treated with laser at investigator discretion.

3.8 Plasma levels of VEGF and Avastin

The parents of each infant enrolled in the study will be given the option to participate in a study to measure levels of VEGF and Avastin in the plasma. Participants in this optional study will have blood collected for analysis as described in a separate procedures manual. Scavenged blood may be used if feasible. VEGF and Avastin levels will be determined before injection, and at 2 weeks and 4 weeks post-injection. (The Avastin level before injection will serve as a control.)

CHAPTER 4: MISCELLANEOUS CONSIDERATIONS IN FOLLOW-UP

4.1 Patient Withdrawals

Parents may withdraw their infant from the study at any time.

4.2 Discontinuation of Study

The study may be discontinued by the Steering Committee or by the Data Safety and Monitoring Committee (with approval of the National Eye Institute) prior to the completion of enrollment and follow-up for all participants.

4.3 Participant Payments

The parent/guardian of each participant will be given a $50 merchandise or money card for completion of each protocol-specified outpatient follow-up visit to cover travel and other expenses related to completing the visit. No payment will be made for visits completed while the infant is in the hospital before discharge. If there are extenuating circumstances, and the participant is unable to complete outpatient study visits without additional funds due to travel costs, additional funds may be provided.

4.4 Study Costs

The study will cover the cost of the bevacizumab, but will not cover physician fees and any facility fees associated with the injection, as this is part of standard care of severe ROP. All visits (including study exams and additional exams at the discretion of the investigator) and/or other required treatments that are part of routine care will be the participant's or his/her insurance company's responsibility. Treatment required for complications of the injection itself will also be the participant's or his/her insurance company's responsibility.

4.5 Contacts by the Jaeb Center for Health Research

The Jaeb Center serves as the PEDIG Coordinating Center. The Jaeb Center will be provided with the parent's contact information. The Jaeb Center will contact the parents of the participants only when necessary. Permission for such contacts will be included in the Informed Consent Form. The principal purpose of the contacts will be to help coordinate scheduling of the 12-month examination.

4.6 General Considerations

The study is being conducted in compliance with the policies described in the study policies document, with the ethical principles that have their origin in the Declaration of Helsinki, with the protocol described herein, and with the standards of Good Clinical Practice.

There is no restriction on the number of subjects to be enrolled by each site towards the overall recruitment goal. A risk-based monitoring approach will be followed, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013).

It is the investigators' opinion that the protocol's level of risk falls under DHHS 46.406, which is research involving greater than minimal risk and no prospect of direct benefit to individual subjects, but likely to yield generalizable knowledge about the subject's disorder or condition.

CHAPTER 5: ADVERSE EVENTS AND RISKS

5.1 Definition

An adverse event is any untoward medical occurrence in a study participant, irrespective of whether or not the event is considered treatment-related.

5.2 Recording of Adverse Events

Throughout the course of the study, all efforts will be made to remain alert to possible adverse events or untoward findings. The first concern will be the safety of the study participant, and appropriate medical intervention will be made.

All adverse events between the time of study eye injection and the 4-week ocular exam or hospital discharge (whichever is later), whether discovered by study personnel during questioning or parents, or detected through physical examination, laboratory test, or other means will be reported on an adverse event form online. Each adverse event form is reviewed by the Coordinating Center to verify the coding and the reporting that is required.
The study investigator will assess the relationship of any adverse event to be related or unrelated by determining if there is a reasonable possibility that the adverse event may have been caused by the treatment.

After the 4-week ocular exam or hospital discharge (whichever is later), only serious adverse events (*see section 5.3)*, ocular adverse events, and any adverse event judged by the investigator to be related to injection and/or treatment will be recorded.

To ensure consistency of adverse event causality assessments, investigators should apply the following general guideline when determining whether an adverse event is related:

Yes
There is a plausible temporal relationship between the onset of the adverse event and administration of the study treatment and the adverse event cannot be readily explained by the subject's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study treatment.

No
Evidence exists that the adverse event has an etiology other than the study treatment (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to study treatment administration.

The maximum intensity that occurred since the onset of an adverse event will be rated on a three-point scale: (1) mild, (2) moderate, or (3) severe, categorized as follows:

Mild - Symptom(s) barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptom(s).

Moderate - Symptom(s) of sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptom(s) may be needed.

Severe - Symptom(s) cause severe discomfort; severity may cause cessation of treatment with study medication or device; treatment for symptom(s) may be given and/or subject hospitalized

It is emphasized that the term severe is a measure of intensity: thus, a severe adverse event is not necessarily serious. For example, itching for several days may be rated as severe, but may not be clinically serious.

Adverse events that continue after the study participant's discontinuation or completion of the study will be followed until their medical outcome is determined or until no further change in the condition is expected.

5.3 Reporting Serious or Unexpected Adverse Events

A serious adverse event is any untoward occurrence that:

- Results in death.
- Is life-threatening; (a non-life-threatening event which, had it been more severe, might have become life-threatening, is not necessarily considered a serious adverse event).
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity or substantial disruption of the ability to conduct normal life functions (sight-threatening).
- Is considered a significant medical event by the investigator based on medical judgment (e.g., may jeopardize the participant or may require medical/surgical intervention to prevent one of the outcomes listed above).

Unexpected adverse events are those that are not identified in the current Clinical Investigator's Brochures.

Serious or unexpected adverse events must be reported to the Coordinating Center immediately via completion of the online serious adverse event form.

The Coordinating Center will notify all participating investigators of any adverse event that is both serious and unexpected. Notification will be made within 10 days after the Coordinating Center becomes aware of the event.

Each principal investigator is responsible for reporting serious study-related adverse events and abiding by any other reporting requirements specific to their Institutional Review Board.

5.4 Data and Safety Monitoring Committee Review of Adverse Events

A Data and Safety Monitoring Committee will approve the protocol, template informed consent form, and substantive amendments, and provide independent monitoring of adverse events. Cumulative adverse event data will be tabulated for review by the DSMC at intervals determined by the coordinating center and the DSMC. Following each DSMC data review, a summary will be made available for submission to Institutional Review Boards. A list of specific adverse events to be reported to the DSMC expeditiously will be compiled and included as part of the DSMC Standard Operating Procedures.

5.5 Risks

5.5.1 Potential Adverse Effects of Bevacizumab

As noted in *section 1.3*, the risks of using bevacizumab in infants are largely unknown, but may be similar to risks in adults. Because bevacizumab works by inhibiting vascular growth, any organ system or bodily function that relies on vascular growth or maintenance of vasculature is susceptible to experiencing complications. Administration of bevacizumab during the period of organ development may have negative long-term implications. The systemic risks of bevacizumab when used intravenously include wound healing complications, bowel perforation, hemorrhage, stroke, myocardial infarction, hypertension, congestive heart failure, proteinuria, gastrointestinal perforations, non-gastrointestinal fistula formation, arterial thromboembolic events, reversible posterior leukoencephalopathy syndrome, infusion reactions, and ovarian failure. After intravitreal injection, bevacizumab is present in the blood in low concentrations, and it is unknown if these amounts are sufficient to cause any of the systemic complications listed above. The ocular risks of intravitreal injection include worsened retinal traction, recurrent ROP, retinal hemorrhage, transient vascular sheathing, abnormalities of the retinal periphery (large avascular areas, abnormal branching, shunts) and of the posterior pole (hyperfluorescent areas, absence of the foveal avascular zone). It is possible but unknown if these changes will have an effect on vision.[12, 16-20, 22]

Children with severe ROP often have high myopia (nearsightedness) and astigmatism, and it is common for one eye to have more myopia and/or astigmatism than the other (anisometropia). This can lead to the brain favoring one eye over the other (amblyopia), which is usually treated with patching of the preferred eye to force the brain to use the non-preferred eye. Preliminary studies indicate that use of bevacizumab for ROP seems to result in less myopia (nearsightedness) than laser treatment, especially for zone I eyes. If one eye is treated with laser and the other eye with bevacizumab, it may increase the chances of anisometropia and amblyopia.

5.5.2 Potential Adverse Effects of Intravitreal Injection

Rarely, the topical drugs used to anesthetize the eye before the injections (proparacaine, tetracaine, or xylocaine) can cause an allergic reaction, seizures, and an irregular heartbeat. Generally, infants will have already been exposed to topical anesthetic during diagnostic examinations.

Temporary stinging, burning and conjunctival redness may occur with the use of proparacaine. A rare, severe, immediate-type, apparently hyperallergic corneal reaction characterized by acute, intense and diffuse epithelial keratitis, a gray, ground glass appearance, sloughing of large areas of necrotic epithelium, corneal filaments and iritis with descemetitis has also been reported.

Subconjunctival hemorrhage will commonly occur as a result of the intravitreal injection. Mild discomfort, ocular hyperemia, increased lacrimation, discharge or itching lasting for a few days is also likely.

Immediately following the injection, there may be elevation of intraocular pressure. It usually returns to normal spontaneously, but may need to be treated with topical drugs or a paracentesis to lower the pressure. The likelihood of permanent loss of vision from elevated intraocular pressure is much less than 1%.

A rare complication of injection is endophthalmitis. It can be due to infection with pathogens such as bacteria of fungi or can be noninfectious. Clinical features include eyelid edema, conjunctival injection, corneal edema, anterior chamber and vitreous inflammation and hypopyon. Endophthalmitis is treated by intravitreal injection of antibiotics, and there is a risk of permanent

loss of vision, including blindness. A meta-analysis of 24 studies in adults reporting endophthalmitis after intravitreal injection of an anti-VEGF agent estimated the risk of endophthalmitis per injection to be 0.049% (95% CI, 0.038% to 0.065%). [28]

Retinal detachment is a rare complication of intravitreal injection. If this occurs, surgery may be needed, which is usually successful at reattaching the retina. However, a retinal detachment can produce permanent loss of vision and even blindness. The risk of retinal detachment has been reported to be less than 0.1% in adults.[29]

The risk of vitreous hemorrhage after intravitreal injection has been reported to be less than 1% in adults.[29] When it occurs, it usually resolves spontaneously, but vitrectomy is sometimes needed, and vision loss may result in some cases. Retinal detachment and vitreous hemorrhage can also occur from severe ROP.

CHAPTER 6: STATISTICAL CONSIDERATIONS AND ANALYSES

6.1 Study Design

The goal of the study is to find a dose of bevacizumab that is lower than what is currently considered the standard, and that can be tested in future studies. The approach to the study design is detailed in *section 3.1*, and is summarized below.

In brief, the study will begin by evaluating the effectiveness of 0.25 mg bevacizumab and if there is evidence of effectiveness, will reduce the dose by half until a dosage is found where the evidence suggests insufficient effectiveness. If all doses meet the study effectiveness criteria, then up to 9 total doses will be evaluated in this study. Effectiveness for the purpose of this study is defined as at least 80% of eyes meeting the study's definition of success (as defined in *section 3.4*).

6.2 Classification of Success/Failure and Decision to Increase, Repeat, or Decrease Dosage

At each dosage level, the number of eyes injected and the number and proportion of eyes meeting success criteria as described in *section 3.4* will be evaluated.

The DSMC will review outcomes from the first 10 infants injected as well as preliminary outcomes from any additional (1-4) infants injected, and the committee will make a recommendation to reduce the dosage and test another 10-14 subjects, repeat the same dosage for another 10-14 subjects, wait for outcomes of 1-4 additional infants injected, or stop the study. The DSMC will apply the following guidelines, but may make a different decision based on the specific study data:

- If the success rate is 80% or greater, then the study will continue to the next lower dose.
- If the success rate is greater than 70% but less than 80%, then an additional 10-14 infants will be evaluated at the same dose.
- If the success rate is less than 70%, then another 10-14 infants will be evaluated at the next higher dose to confirm efficacy, unless the first dose (0.25 mg) is unsuccessful, or the previous successful dose was already tested on 2 sets of 10-14 infants.

When reporting study results, both eyes of all infants will be included.

6.3 Additional Analyses

6.3.1 Description of Cohort

At each dosage level, subject level and eye level characteristics will be tabulated including gender, race, gestational age, birth weight, examination findings in the study and non-study eye, and age at diagnosis of type 1 ROP.

6.3.2 Safety

Adverse events reported at any time during the study will be tabulated for all enrolled infants and coded using the MedRA system. For each dosage level, an estimate and 95% confidence interval 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

6.3.3 Power for Analysis of Adverse Effects

For rare side effects, Table 3 below specifies the chance of not observing at least 1 adverse event in a sample of 10 children for various event rates in the population. .

Table 3: Chance of Not Observing at Least One Event in a Sample of 10 Subjects

Actual Probability of an Event	Chance of Not Observing at Least One Event for a Given Dosage
	N=10 Subjects
1%	90%
2%	82%
3%	74%
4%	67%
5%	60%

Hence, with the proposed sample size of 10 subjects at each dosage level, the study has a 60% probability for not observing at least one event for adverse events with 5% occurrence.

6.3.4 Plasma levels of VEGF and Avastin

The parents of each infant enrolled in the study will be given the option to participate in a study to measure levels of VEGF and Avastin in the plasma. Participants in this optional study will have blood collected for analysis The distribution of VEGF and Avastin levels (median, range, and quartiles) will be described before injection, and at 2 weeks and 4 weeks post-injection. For each dosage level, at 2, and 4-weeks post-injection, the change from pre-injection will be calculated, and a 95% confidence interval calculated for the change.

6.3.5 Twelve-month Corrected Age

Descriptive statistics will be calculated to describe the cohort at each dosage level with respect to the following at 12-months corrected age:

- Time since initial hospital discharge
- Number of times re-hospitalized by 12 months
- Number of infants with an increase in oxygen requirement prior to injection (yes, no, unknown)
- Number of deaths
- Number of infants with periventricular leukomalacia
- Number of infants with hydrocephalus (with shunt placement)
- Number of study eye and fellow eyes requiring additional treatment/s for ROP, and if retreated, type of treatment
- Any adverse events or complications since the 4-week exam
- Assessment of vision, amblyopia, strabismus, retinal structure, and refractive error
- Most recent head circumference (in centimeters), reported by z scores
- Most recent weight (in grams), reported by z scores

In addition, the change from pre-injection with respect to z scores of head circumference and weight will be calculated.

Exploratory analyses will evaluate whether these factors differ according to dosage level.

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