BEVACIZUMAB TREATMENT FOR POSTERIOR TYPE 1 RETINOPATHY OF PREMATURITY

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KEY ROLES

Protocol Chair	
Name, degree	David K. Wallace, M.D., M.P.H.
Title	Chair, Department of Ophthalmology
Institution Name	Indiana University School of Medicine 1160 W. Michigan St, Indianapolis, IN, 46202 Phone: 317-278-2651 Email: dwallac@iu.edu
Signature/ Date	
Protocol Vice-Chair	
Name, degree	G. Baker Hubbard, M.D.
Title	Thomas M. Aaberg Sr. Professor of Ophthalmology
Institution Name	The Emory Eye Center Emory University School of Medicine 1365B Clifton Rd. NE Atlanta, GA 30322 Email: ghubba2@emory.edu
Signature/ Date	
Protocol Vice-Chair	
Name, degree	Daniel F. Martin, M.D.
Title	Chairman
Institution Name	Cole Eye Institute, Cleveland Clinic 9500 Euclid Avenue Cleveland, OH 44195 Email: martind5@ccf.org
Signature/ Date	
Protocol Vice-Chair	
Name, degree	William V. Good, M.D.
Title	Senior Scientist
Institution Name	Smith-Kettlewell Eye Research Institute 2318 Fillmore Street San Francisco, CA 94115 Email: good@ski.org
Signature/ Date	

JCHR Protocol Director	
Name, degree	Robert J. Henderson, M.S.
Title	Biostatistician, PEDIG Coordinating Center
Institution Name	Jaeb Center for Health Research 15310 Amberly Drive, Suite 350 Tampa, FL 33647 Phone: 1-888-797-3344 Email: rhenderson@jaeb.org
Signature/ Date	
JCHR Coordinating Center Director	
Name, degree	Raymond T. Kraker, M.S.P.H.
Title	Director, PEDIG Coordinating Center
Institution Name	Jaeb Center for Health Research 15310 Amberly Drive, Suite 350 Tampa, FL 33647 Phone: 1-888-797-3344 Email: rkraker@jaeb.org
Signature/ Date	
Medical Monitor	
Name, degree	J.A. Sliman, MD, MPH
Title	Chief Medical Officer
Institution Name	The Emmes Company, LLC 401 North Washington Street Rockville, MD 20850 Email: jsliman@emmes.com
Signature/ Date	
Central Laboratory for Blood Testing	
Name, degree	Mary E. Hartnett, M.D.
Title	Lab Director
Institution Name	John A. Moran Eye Center University of Utah Department of Ophthalmology and Visual Sciences 65 Mario Capecchi Drive Salt Lake City, UT 84132
Signature/ Date	

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LIST OF ABBREVIATIONS

ABBREVIATION	DEFINITION
AE	Adverse Event
ATS-HOTV	Amblyopia Treatment Study – HOTV Visual Acuity Testing Protocol
BEAT-ROP	Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity
CFR	Code of Federal Regulations
CI	Confidence interval
D	Diopter
DD	Disc Diopter
DHHS	Department of Health and Human Services
DSMC	Data safety and monitoring committee
eCRF	Electronic Case Report Form
EDC	Estimated Date of Confinement
ETROP	Early Treatment for ROP
EVA	Electronic Visual Acuity Tester
FA	Fluorescein Angiography
FDA	Food and Drug Administration
FDR	False Discovery Rate
GCP	Good clinical practice
GEE	Generalized Estimation Equations
ICH	International Council for Harmonization
ICROP	International Classification of ROP
IND	Investigational New Drug Application
IRB	Institutional Review Board
IQ	Intelligence Quotient
ITT	Intention to Treat
IUGR	Intrauterine growth restriction
JCHR	Jaeb Center for Health Research
NIH	National Institutes of Health
OCT	Optical Coherence Tomography
OSHA	Occupational Safety and Health Administration
PACT	Prism and Alternate Cover Test
PEDIG	Pediatric Eye Disease Investigator Group
PMA	Post-menstrual age
QA	Quality assurance
QC	Quality control
RBM	Risk based monitoring
RCT	Randomized clinical trial
ROP	Retinopathy of Prematurity
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SE	Spherical equivalent refractive error (Sphere + ½ Cylinder)
US	United States
VEGF	Vascular endothelial growth factor

SITE PRINCIPAL INVESTIGATOR STATEMENT OF COMPLIANCE

Protocol Title: Bevacizumab Treatment for Posterior Zone I Retinopathy of Prematurity (ROP4)

Protocol Version/Date: V1.1, 21 June 2021

I have read the protocol specified above. In my formal capacity as a Site Principal Investigator, my duties include ensuring the safety of the study participants enrolled under my supervision and providing the Jaeb Center for Health Research, which serves as the Coordinating Center for the protocol, with complete and timely information, as outlined in the protocol. It is understood that all information pertaining to the study will be held strictly confidential and that this confidentiality requirement applies to all study staff at this site.

This trial will be carried out in accordance with ICH E6 Good Clinical Practice (GCP) and as required by the following United States (US) Code of Federal Regulations (CFR) applicable to clinical studies: 45 CFR Part 46 (protection of human subjects), 21 CFR Part 50 (informed consent and safeguards for children), 21 CFR Part 56 (institutional review boards), and 21 CFR Part 312 (FDA investigational new drug application).

As the Principal Investigator, I will assure that no deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB), except where necessary to eliminate an immediate hazard(s) to the trial participants.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Participants Protection Training and Good Clinical Practice Training. Further, I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Investigator's Signature _____ Date: _____ / _____ / _____
dd mmm yyyy

Investigator's Name: _____

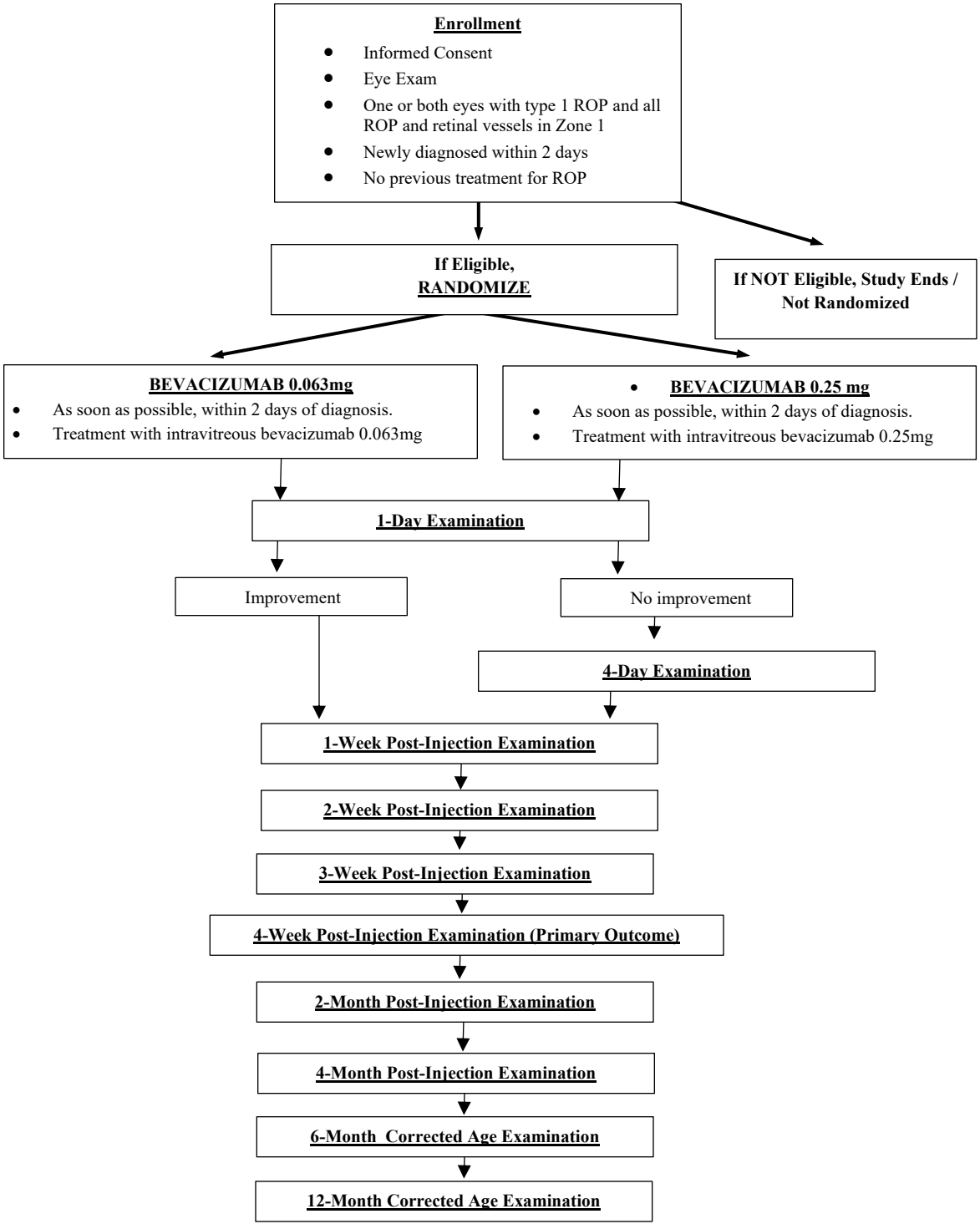
Site Name / Number: _____

PROTOCOL SUMMARY

ITEM	DESCRIPTION
Title	Bevacizumab Treatment for Posterior Type I Retinopathy of Prematurity (ROP4)
Précis	Type 1 retinopathy of prematurity in zone I represents the most severe type of ROP and has the worst prognosis. It is unknown whether low-dose bevacizumab will be successful in these severe cases. Also unknown is the timing and extent of peripheral retinal vascularization after low-dose bevacizumab compared with the standard dose. The current study will evaluate whether doses of 0.063 mg and 0.25mg are effective as treatment for type 1 ROP, with ROP and retinal vessels all in zone I.
Investigational Drug	Bevacizumab.
Objectives	The primary objective of the current protocol is to evaluate whether doses of 0.063 mg and 0.25mg are effective as treatment for type 1 ROP, with ROP and retinal vessels all in zone I. Secondary objectives are to compare 1) safety and efficacy, 2) refractive outcomes, and 3) the extent of retinal vascularization at 4 months post-injection between the two dose groups.
Study Design	Multicenter, randomized clinical trial.
Number of Sites	Up to forty (40) clinical sites in the North America
Outcomes	**Primary Efficacy Outcome** ***Treatment success***, determined at 4 weeks, post injection, and meeting all the following 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. **Secondary Efficacy Outcomes** • Number of re-treatments • Number and types of ocular complications • Extent of retinal vascularization at 2 and 4-months post-injection • Ocular alignment at 6 and 12-months corrected age • Refractive error at 6 and 12-months corrected age • Vision at 6 and 12-months corrected age • Ocular exam findings at 6 and 12-months corrected age **Key Safety Outcomes** • Proportion with at least 1 adverse events • Proportion with at least 1 adverse events related to study drug • Proportion with serious adverse events • Proportions of infant deaths • Complications after 4-week exam

ITEM	DESCRIPTION
Population	**Key Inclusion Criteria:** 1. Birth weight < 1251 grams 2. Newly diagnosed (within 2 days) type 1 ROP, with ROP and retinal vessels all in zone I, in one or both eyes **Key Exclusion Criteria:** 1. Previous treatment for ROP 2. Stage 4 or 5 ROP in either eye 3. Treatment could not be done within 2 days of diagnosis of type 1 ROP 4. Investigator unwilling to randomize or parent unwilling to accept random assignment to either treatment 5. Transfer to another hospital not covered by study-certified examiners anticipated within the next 4 weeks 6. Active ocular infection or purulent nasolacrimal duct obstruction Eye excluded (but the other eye may be eligible) if: 7. Visually significant ocular anomaly (e.g., cataract, coloboma) 8. Opacity that precludes an adequate view of the retina
Sample Size	A sample size of up to 80 participants (40 in each treatment group) is a convenience sample. The study is not powered to evaluate a difference between dose levels.
Phase	Phase II Randomized Clinical Trial
Treatment Groups	Participants will be randomly assigned with equal probability to treatment of one or both eyes with type 1 ROP to either: 1. Intravitreous bevacizumab 0.063 mg 2. Intravitreous bevacizumab 0.25 mg
Participant Duration	Study exams will occur at 1 day, 1, 2, 3, and 4 weeks, and at 2 and 4-months post-treatment (and re-treatment when indicated). If type 1 ROP is still present at day 1, then an exam will occur at 4 days post-injection. Additional study exams will occur at ages (chronological age minus number of weeks born prematurely) of 6 months and 12 months. It is anticipated that non-study visits will occur more frequently, and the timing of these will be at investigator discretion.
Protocol Overview/Synopsis	Infants with type 1 ROP and no prior treatment for ROP will be randomly assigned (1:1) to treatment with either intravitreous bevacizumab 0.063 mg or either intravitreous bevacizumab 0.25 mg. Study exams will be at 1 day, 4 days (if no improvement on day 1), 1, 2, 3, and 4 weeks, and at 2 and 4-months post-treatment (and re-treatment when indicated). Additional study exams will occur at corrected age 6 and 12 months. Non-study examinations will be at clinician discretion and are likely to occur more often. The primary outcome will be treatment success within each dose group, defined as improvement by the day 4 exam and no recurrence of type 1 ROP or severe neovascularization requiring additional treatment within 4 weeks of injection. Important secondary outcomes include safety and efficacy. refractive outcomes, and the extent of retinal vascularization at 2- and 4-months post-injection in each of the two dose groups.

STUDY SUMMARY FLOWCHART



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
Ocular Exam/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										X	X
Visual Acuity										X	X
Data on Additional treatments				X	X	X	X	X	X	X	X
Adverse Events		X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X[d]	X[d]	X[d]	X[d]
Systemic Outcomes										X	X
Optional:											
Fluorescein Angiography	X[b]										
OCT	X[b]										
Plasma VEGF	X				X		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 corrected 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 is optional.
[c] All adverse events recorded at each visit until 4 weeks post-treatment
[d] Only serious adverse events and ocular adverse events recorded beyond 4 weeks post-treatment

Chapter 1: Background Information

1.1 Introduction

1.1.1 Retinopathy of Prematurity

Retinopathy of prematurity (ROP) is a significant cause of childhood vision loss in the United States.[1] It is characterized by retinal vascular abnormal alterations that may occur during the first several months after premature delivery. The vascular changes may be mild and regress with time, or they may continue to progress to preretinal neovascularization and fibrosis that may ultimately lead to retinal traction and detachment. Other sequelae include tortuosity of the retinal vessels, dragged retina, displacement of the macula, temporal folds of the retina, equatorial folds, chorioretinal scarring, retinal pigment abnormalities, retinoschisis, Coats'-like changes, increased incidence of lattice degeneration, vitreous hemorrhage, elevated retinal vessels, vitreous membranes, exudative and tractional retinal detachments, retinal breaks, amblyopia, myopia, strabismus, glaucoma, cataract, band keratopathy, microphthalmia, nystagmus, shallow anterior chamber, and steep corneal curvature.[2-13]

Important risk factors for ROP include low birth weight, young gestational age, twin status, outborn status, and supplemental oxygen. 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.[1,14] In severe cases of ROP, vascular endothelial growth factor (VEGF) accumulates in the retina and vitreous, leading to retinal neovascularization and cicatrization. Peripheral retinal photocoagulation destroys the avascular retina that produces VEGF. More recently, treatments have targeted blockade of pro-angiogenic growth factors, such as vascular endothelial growth factor (VEGF). Anti-VEGF treatment of severe ROP may provide a more direct and effective approach to reducing intraocular VEGF levels and preventing retinal detachment. One such anti-VEGF antibody is bevacizumab, which binds several VEGF isoforms. 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 intravitreous injection at a small fraction of the systemic dose for cancer. Intravitreous injection of bevacizumab is also used in the treatment of other ocular diseases involving neovascularization, including age-related macular degeneration and diabetic retinopathy.

Examination findings in ROP are classified according to the international classification of ROP (ICROP)[15] and the revised ICROP.[16] 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 demarcation line, stage 2 is a ridge, stage 3 is neovascular tissue, stage 4 is partial retinal detachment, and stage 5 is 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.[17]

1.1.2 Clinical Studies of ROP Treatment

The CRYO-ROP Study first demonstrated the effectiveness of peripheral retinal ablation in curtailing progression of ROP to cicatricial stages.[18] However, the impact of peripheral retinal ablation on visual outcome was less dramatic than the structural benefit. The ETROP Study showed that treatment of eyes at high-risk prethreshold resulted in better visual and structural outcomes than treatment at threshold.[17] Based on these results, laser was established as the standard of care in the US for treatment of type 1 ROP. Although laser usually prevents retinal detachment, it does not promote ongoing retinal vascular development, leaving areas of avascular peripheral retina which seem to be nonfunctional. Also, visual impairment and high myopia are common outcomes even among those infants successfully treated with laser.[19,20] Finally, although peripheral retinal ablation significantly reduces the incidence of retinal detachment and blindness, fewer than 20% of 5½ year old children whose eyes reached threshold for treatment in the CRYO-ROP study achieved 20/40 vision.[21] In the ETROP Study, only about 35% of children had visual acuity better than 20/40, even with earlier treatment.[22] Many premature infants treated for ROP also develop other significant ocular defects. These data, coupled with improved survival rates of very low birth weight infants, support the need to develop better treatment strategies for severe ROP.

In the past decade, intravitreous injections of anti-VEGF drugs have been used increasingly to treat severe ROP. The most commonly used agent is bevacizumab, and several case series and results from one randomized trial have shown that bevacizumab is effective in treating severe ROP.[23-31] The BEAT-ROP study (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.[23] The primary outcome was recurrence of ROP in one or both eyes requiring retreatment before 54 weeks' postmenstrual age. ROP recurred in 4 of 70 infants (6%) in the bevacizumab group and 19 of 73 infants (26%) in the laser group (P = 0.002). When stratified by zone, results 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. Among nonrandomized studies, Wu et al found that 37 of 41 eyes (90%) with stage 3 ROP regressed after a single bevacizumab injection. Four eyes required additional laser treatment, and 4 eyes had a vitreous or pre-retinal hemorrhage.[26] Quiroz-Mercado et al observed neovascular regression in 17 of 18 eyes with high-risk prethreshold, threshold, or stage 4 ROP after intravitreous bevacizumab.[28] Travassos et al injected the worse eye of three patients with aggressive, posterior retinopathy of prematurity with 0.75 mg of bevacizumab as monotherapy or complementary to laser therapy. All injected eyes showed rapid regression of tunica vasculosa lentis and iris vessel engorgement, disappearance of iris rigidity, and regression of plus disease and retinal proliferation.[30]

The RAINBOW study randomized 225 infants with ROP to treatment with the anti-VEGF agent ranibizumab 0.2 mg, ranibizumab 0.1 mg, or laser therapy.[32] The primary outcome, treatment success, was defined as survival with no active retinopathy, no unfavorable structural outcomes, and no need for a different treatment modality at or before 24 weeks. Treatment success occurred

in 56 (80%) of 70 infants receiving ranibizumab 0.2 mg compared with 57 (75%) of 76 infants receiving ranibizumab 0.1 mg and 45 (66%) of 68 infants after laser therapy. The odds ratio (OR) of treatment success following ranibizumab 0.2 mg compared with laser was 2.19 (95% Cl 0.99−4.82, p=0.051), for ranibizumab 0.1 mg compared with laser the OR was 1.57 (95% Cl 0.76−3.26); for ranibizumab 0.2 mg compared with 0.1 mg the OR was 1.35 (95% Cl 0.61−2.98). Death, serious and non-serious systemic adverse events, and ocular adverse events were evenly distributed between the three groups.

1.1.3 Phase 1 Dosing De-escalation Study

BEAT-ROP used a dose of 0.625 mg, which was chosen empirically as half of the adult dose. Other investigators have used doses of bevacizumab between 0.25 mg and 0.625 mg.[23] However, these doses are likely much higher that necessary to effectively treat ROP. In addition, intravitreous bevacizumab reaches the systemic circulation, with large and persistent reductions in serum VEGF levels,[33] raising concerns about potential systemic harm. VEGF is necessary for normal development of many tissues, including brain, lungs, bones, kidneys, and retina; thus, blocking VEGF's action could be detrimental to premature infants. In particular, there is concern that anti-VEGF drugs increase the risk for neurodevelopmental disability.[34,35]

To determine whether a much lower dose of bevacizumab than previously used may be effective in treating severe ROP, PEDIG conducted a masked, multicenter, dose de-escalation phase 1 study.[31] Success was defined as improvement in preinjection plus disease or zone I stage 3 ROP by 5 days after injection or sooner, and no recurrence of type 1 ROP or severe neovascularization requiring additional treatment within 4 weeks. One hundred twenty premature infants with type 1 ROP were enrolled and treated, and success was achieved in 11 of 11 eyes at 0.25 mg, 14 of 14 eyes at 0.125 mg, 21 of 24 eyes at 0.063 mg, 9 of 9 eyes at 0.031 mg, 13 of 13 eyes at 0.016 mg, 9 of 9 eyes at 0.008 mg, and 9 of 10 eyes at 0.004 mg; but success was achieved in only 17 of 23 eyes (74%) receiving 0.002 mg.[36] These data were analyzed using a Bayesian statistical model, and it was calculated that there is a 94% probability that 0.063 mg is at least 95% effective in treating type 1 ROP. The probability that 0.125mg is at least 95% successful is 99%.

1.2 Study objectives

The primary objective of the current protocol is to evaluate whether doses of 0.063 mg and 0.25mg are effective as treatment for type 1 ROP, with ROP and retinal vessels all in zone I. Secondary objectives are to compare 1) safety and efficacy, 2) refractive outcomes, and 3) the extent of retinal vascularization at 4 months post-injection between the two dose groups.

1.3 Rational for the Current Study

Type 1 retinopathy of prematurity in zone I represents the most severe type of ROP and has the worst prognosis. Most of these cases occur in very young infants, and many have a rapid onset of plus disease. Some of these infants meet criteria for APROP (Aggressive Posterior ROP). It is unknown whether low-dose bevacizumab will be successful in these severe cases. Also unknown is the timing and extent of peripheral retinal vascularization after low-dose bevacizumab compared with the standard dose. The ROP1 study had an insufficient number of zone I cases to evaluate these questions.

1.4 Potential Risks and Benefits of Bevacizumab

1.4.1 Known Potential Risks of Bevacizumab

Little is known about the safety of using bevacizumab for ROP,[37] 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.[23] 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.[38] Angiogenesis is an important process in the normal development of other organ systems such as the lungs, kidneys, brain, and bones. Intravitreous bevacizumab reaches the systemic circulation, so there is 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.[23] Reducing the dose of bevacizumab to 0.063 mg is expected to reduce the likelihood of negative systemic side effects.[39]

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 package insert for intravenously administered bevacizumab include 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.[40]

The safety and pharmacokinetics of bevacizumab in pediatric patients have not been established,[40] although there is potential for similar adverse events in infants as in adults. Doses administered to infants for treatment of ROP (typically 0.125 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 found serum concentrations of bevacizumab following a 0.5 mg intravitreous injection for ROP were well above concentrations required to completely block in vitro VEGF activities in human umbilical vein endothelial cells.[41] 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.[41] Others have reported similar effects with a lower dose (0.375 mg) of bevacizumab.[42] 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 intravitreous injections of bevacizumab. In some cases, retinal traction may be worsened by injection of bevacizumab, leading to retinal detachment,[43] particularly if fibrovascular tissue has already begun to form.[44] 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.[45] Other complications observed

following bevacizumab treatment for ROP include cataract,[46] retinal hemorrhage,[25,26] transient vascular sheathing,[26] choroidal ischemia,[47] and 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 unknown if these changes will have an effect on vision.[48]

It is unclear whether the mortality rate of premature infants receiving intravitreous 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 intravitreous bevacizumab, 2 after laser).[23] 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 ETROP study by 9 months at an alpha of 0.05 and 80% power).[17] 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.[49] However, it is unclear whether systemic bevacizumab may have exacerbated the existing conditions, particularly in cases of multiple organ failure or lung failure. In the dose de-escalation phase 1 study conducted by PEDIG[31] in which 120 infants were treated with low doses of bevacizumab ranging from 0.625 mg to 0.002 mg, 8 infants passed away during the study due to reasons judged by the Data Safety and Monitoring Committee to be unrelated to intravitreous bevacizumab. Additional dosing studies and long-term follow-up studies with a large number of infants are required to determine the safety of intravitreous bevacizumab injection for the treatment of ROP.[23,37,50]

There are potential concerns about inhibiting VEGF in the developing infant, because drugs introduced into the vitreous enter the blood stream and are minimally diluted in the infant's small blood volume. Testing of pharmacokinetics of bevacizumab in the blood stream is difficult even in animal models, because bevacizumab is a mouse monoclonal antibody and is not effective in mice and rats, the most commonly used models in ROP. VEGF is neuroprotective and its inhibition may adversely affect the developing organs, such as lung, kidney and brain.[34,35] Therefore, long-term safety data on neural structure and cognitive development are very important.

Bevacizumab package inserts warn of the potential risk of ovarian failure and infertility in females with reproductive potential,[40] and it is unclear if this same risk applies to female neonates.

1.4.2 Potential Adverse Effects of Intravitreous 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 intravitreous 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. Indirect ophthalmoscopy is performed immediately post-injection to ensure adequate posterior circulation.

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 intravitreous injection of antibiotics, and there is a risk of permanent severe loss of vision. A meta-analysis of 24 studies in adults reporting endophthalmitis after intravitreous injection of an anti-VEGF agent estimated the risk of endophthalmitis per injection to be 0.049% (95% CI, 0.038% to 0.065%). [51]

Retinal detachment is a rare complication of intravitreous injection. If this occurs, surgery may be needed, which is usually successful at reattaching the retina. However, a retinal detachment can produce permanent and sometimes severe loss of vision. The risk of retinal detachment has been reported to be less than 0.1% in adults.[52]

The risk of vitreous hemorrhage after intravitreous injection has been reported to be less than 1% in adults.[52] 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.

Some infants have oxygen desaturations or bradycardic episodes before, during, or after the injection.

1.4.3 Known Potential Benefits of Bevacizumab

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.[23] Results of the BEAT-ROP trial suggested a benefit of bevacizumab treatment over conventional laser therapy for zone I ROP. In addition, the incidence of high myopia was much less common after bevacizumab compared with laser. The authors reported that the recurrence of ROP was higher in the laser group than the bevacizumab group, but the outcomes from the laser treated group were much poorer than those reported in the Early Treatment For Retinopathy Of Prematurity Cooperative Group (ETROP) study.[17]

Prior to the BEAT-ROP study, Mintz-Hittner and Kuffel reported on a retrospective, consecutive, noncomparative case series of moderate and severe stage 3 ROP in zone I or posterior zone II treated by bilateral intravitreous injections of bevacizumab.[29] Eleven infants

received intravitreous injections of bevacizumab (0.625 mg in 0.025 mL) and never had laser therapy. All 22 eyes were treated successfully (no retinal detachment, macular ectopia, high myopia, anisometropia, or other ocular abnormalities) with only 1 injection. No complications (local or systemic) were encountered. They concluded that intravitreous injection of bevacizumab was safe and effective in treating stage 3 ROP in zone I and posterior zone II in a small series of patients.

In a retrospective chart review of 23 consecutive eyes of 14 patients, Kusaka et al[53] evaluated the short-term efficacy of intravitreous injections of bevacizumab for severe retinopathy of prematurity (ROP). Patients received an intravitreous injection of bevacizumab (0.5 mg), either as the initial treatment (15 eyes) or at the end of vitrectomy (eight eyes). After injection of bevacizumab as the initial treatment, reduced neovascular activity was seen on fluorescein angiography in 14 of 15 eyes. In three eyes, a tractional retinal detachment developed or progressed after bevacizumab injection. No other ocular or systemic adverse effects were identified. These results suggest that intravitreous injection of bevacizumab seems to be associated with reduced neovascularization without apparent ocular or systemic adverse effects, and is beneficial for treating severe ROP.

Dorta and Kychenthal reported on a noncomparative consecutive case series 12 consecutive eyes of 7 premature infants with type 1 retinopathy of prematurity treated with only one intravitreous injection of bevacizumab (0.625mg).[25] All eyes showed regression of the disease with no additional treatment needed. No complications attributable to the bevacizumab were seen in any of their patients. An epiretinal hemorrhage was seen in one eye as a consequence of the injection procedure. The hemorrhage spontaneously reabsorbed without consequence. The authors concluded that intravitreous bevacizumab is a useful therapy for type 1 ROP.

Wu et al[26] investigated the effects and complications of bevacizumab as treatment of ROP in Taiwanese patients. They reported results of a multicenter, retrospective case series study of 27 patients (49 eyes) receiving intravitreous injections of bevacizumab (0.625 mg). Patients were followed for at least 6 months after bevacizumab injection. All of the eyes received only a single injection. A total of 37 of 41 eyes (90%) with stage 3 ROP regressed after bevacizumab injection only. Four eyes (10%) required additional laser treatment. Of 6 eyes (3 patients) with stage 4A ROP, 2 eyes (1 patient; 33%) regressed after bevacizumab injection and 4 eyes (67%) regressed after bevacizumab injection and subsequent vitrectomy. The 2 eyes with stage 5 ROP exhibited decreased vascular tortuosity after bevacizumab injection, but the retina failed to reattach after vitrectomy. Major complications included vitreous or pre-retinal hemorrhage in 4 eyes (8%) and transient vascular sheathing in 2 eyes (4%). The authors concluded that bevacizumab injection seems effective and well tolerated in some cases of ROP, especially in stage 3 ROP.

Law et al[49] reported the results of bevacizumab used in eyes with ROP at high risk for progression. Records of all infants with ROP treated with bevacizumab were reviewed. Bevacizumab was given when conventional laser therapy was not possible in patients with poor pupillary dilation from iris rubeosis, dense vitreous hemorrhage, or increasing vascular activity and vitreoretinal traction despite completed laser therapy. Thirteen eyes of 7 infants were treated with an intravitreous injection of 0.75 mg bevacizumab. Injection was not used as monotherapy in any case. Definitive treatment (laser or vitrectomy) was completed successfully within 72

hours of injection. No systemic complications attributable to bevacizumab treatment were recorded within 2 to 17 months of follow-up. The authors concluded that treatment with bevacizumab may be used to improve visualization for more definitive laser or surgical treatment and may facilitate disease regression without obvious systemic toxicity.

1.4.4 Risks of Examination or Testing Procedures

There is a rare risk of an allergic response to the topical medications used to anesthetize the eye or dilate the pupil. There may be some redness or bleeding on the surface of the eye after the examination. Some infants have oxygen desaturations or bradycardic episodes during or after the eye exams.

There are no known risks associated with OCT or fundus photographs. The bright flashes used to take the photographs may be annoying, but are not painful and cause no damage.

For fluorescein angiography, both the skin and urine are expected to turn yellow/orange for up to 24 hours after the injection of fluorescein dye. There is a small risk of discomfort or phlebitis at the site of the injection. An allergic reaction to the dye used to do the fluorescein angiography imaging is rare. A rash or pruritus (itching) can develop, but the incidence of true anaphylactic reactions is less than 0.1%.[54]

1.5 Risk Assessment

The Sponsor has determined that the protocol's level of risk is consistent with 45 CFR 46.405 and 21 CFR 50.52, which indicates research involving greater than minimal risk but presenting the prospect of direct benefit to the individual child involved in the research**.**

1.6 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 (GCP).

Chapter 2: Study Enrollment and Screening

2.1 Participant Recruitment and Enrollment

Up to 80 participants are expected to be enrolled and have treatment randomly assigned. As the enrollment goal approaches, sites will be notified of the end date for recruitment. Study participants whose parents have signed an informed consent form can be randomized (or enrolled) up until the end date, which means the recruitment goal might be exceeded.

Study participants will be recruited from approximately 40 clinical centers in North America. All eligible participants will be included without regard to gender, race, or ethnicity. There is no restriction on the number of participants to be enrolled or randomized by each site toward the overall recruitment goal.

2.1.1 Informed Consent and Authorization Procedures

A participant will be considered for the study after undergoing a routine examination (as part of standard care) that identifies type 1 ROP in one or both eyes 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)).

Potential eligibility may be assessed as part of a routine-care examination. Before completing any procedures or collecting any data that are not part of usual care, written or electronic informed consent will be obtained.

A parent/legal guardian will be provided with the Informed Consent Form to read and will be given the opportunity to ask questions. If the parent is interested in the study, the investigator will meet with the parent to discuss the study, and if the parent agrees to participate, the Informed Consent Form will be signed. A copy of the signed consent form will be provided to the parent and the original signed informed consent will be added to the participant's study record.

As part of the informed consent process, the parent will be asked to sign an authorization for release of personal information. The authorization form may be combined with the consent or may be on a separate form if the site requires their own process. The investigator, or his or her designee, will review the study-specific information that will be collected and to whom that information will be disclosed. After speaking with the parent, questions will be answered about the details regarding authorization.

A participant is considered enrolled when the informed consent form has been signed and HIPAA authorization has been provided.

2.2 Participant Inclusion Criteria

The study participant must have at least one eye meeting all of the inclusion criteria in order to be eligible to participate.

1. Birth weight < 1251 grams
2. Newly diagnosed (within 2 days) type 1 ROP (as defined in section 2.4.2), with ROP and retinal vessels all in zone I, in one or both eyes

3. Parent understands the protocol and is willing to provide consent.

If both eyes are eligible, then both are included. If one eye is eligible and the other eye has type 1 ROP with ROP or vessels in zone II, then both eyes will receive the treatment randomly assigned. If one eye is eligible and the fellow eye later develops type 1 ROP in zone I (i.e. any ROP in zone I) within 4 weeks of injection in the first eye, then the fellow eye will also receive the treatment randomly assigned to the first 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 eye procedures are designed to minimize bias comparing the study eyes.
If the fellow eye does not develop type 1 ROP in zone I, then treatment and follow-up for the fellow eye is at investigator discretion.

2.3 Participant Exclusion Criteria

Participants meeting any of the following exclusion criteria will be excluded from study participation.

1. Previous treatment for ROP
2. Stage 4 or 5 ROP in either eye
3. Treatment could not be done within 2 days of diagnosis of type 1 ROP
4. Investigator unwilling to randomize or parent unwilling to accept randomized assignment to either treatment
5. Transfer to another hospital anticipated within the next 4 weeks where exams by study-certified examiners are not available. If hospital discharge is anticipated within the next 4 weeks, parents unable or unwilling to return to the PEDIG site for outpatient follow-up visits.
6. Active ocular infection or purulent nasolacrimal duct obstruction in either eye

One eye will be excluded, and other eye may be eligible, if either of the following are present:

- Visually significant ocular anomaly (e.g., cataract, coloboma)
- Opacity that precludes an adequate view of the retina

2.4 Data Collection and Examination at Enrollment

2.4.1 Historical Information

Historical information recorded at enrollment will include gestational age at birth, birth weight, current weight, head circumference, gender, race, ethnicity, concurrent medical conditions (e.g. intraventricular hemorrhage, periventricular leukomalacia, hydrocephalus), and current medications.

2.4.2 ROP Classification

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.[55,56]

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
- **Zone III**: residual crescent of retina anterior to zone II

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 (not eligible):
  - **Stage 4A**: Extrafoveal
  - **Stage 4B**: Foveal
- **Stage 5**: Total retinal detachment (not eligible)

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

Extent of Retinal Vascularization: The extent of retinal vascularization in each quadrant will be classified as:

- Posterior Zone I - when optic nerve is centered using a 28D lens, no retinal vessels extend even to the edge of the lens view
- Anterior Zone I - when optic nerve is centered using a 28D lens, retinal vessels extend to the edge of the lens view or beyond (when placing the optic nerve at the edge of the view with a 28D lens, and the other lens edge approximates the zone I – zone II border)
- Posterior Zone II - posterior one-third of zone II
- Mid Zone II - mid one-third of zone II
- Anterior Zone II - anterior one-third of zone II
- Zone III - vascularized to within one-disc diameter of the ora serrata nasally, but not temporally
- Full Vascularization - within one-disc diameter of the ora serrata

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.[56,57]

Pre-plus Disease: A diagnosis of pre-plus disease will be made when there is abnormal dilation and tortuosity, but it is insufficient for 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 (zone II is not eligible for this study)

2.4.3 Retinal Images

Wide-angle retinal images will be obtained after enrollment and prior to any additional treatment. Imaging will be done at 4 months post-injection if no additional treatment is planned, such as prophylactic laser. Six images will be obtained for each eye: anterior segment, posterior pole, superior, temporal, inferior, and nasal as described in the *ROP Procedures Manual*. These images will later be reviewed by masked expert readers for purposes of quality control. Investigators will be informed throughout the study if their clinical diagnoses disagree with those of expert readers.

If a participating site or location does not have a retinal camera, a second examination will be done by a study-certified examiner after enrollment and if there is treatment failure to assess the location, extent, and stage of disease, as well as presence of pre-plus or plus disease. Results of this exam will only be used for quality control, similar to review of retinal images.

2.4.4 Optional Imaging

Optical Coherence Tomography (OCT) and Fluorescein Angiography (FA) will be offered as an optional part of the study at sites with those imaging capabilities. OCT and/or FA imaging will occur at enrollment and prior to any additional treatment as described in the ROP Procedures Manual. OCT and/or FA will also be done at 4 months post-injection if no additional treatment is planned, such as prophylactic laser.

2.4.5 Optional Blood Collection

The parents of each infant enrolled in the study will be given the option to participate in a study to measure plasma levels of VEGF for both groups. Participants in this optional study will have blood collected at enrollment, 2 weeks, 4 weeks, and 4 months post-injection. Scavenged blood may be used when feasible. Parents will be asked for permission to use these blood samples for future biomarker studies. A single, centralized laboratory will be used for VEGF measurements, and details of sample management will be included in the manual of procedures.

2.5 Randomization

Eligible participants will be randomly assigned with equal probability to receive either:

- Intravitreous bevacizumab 0.063 mg, or
- Intravitreous bevacizumab 0.25 mg

If both eyes are eligible, then both will receive the assigned treatment.
Randomization will be stratified by gestational age (≤ 24 weeks vs > 24 weeks).

Once a study participant is randomized, that participant will be included regardless of whether the assigned treatment is received. Thus, the investigator must not proceed to randomize an individual until they confirm that the individual is eligible and the parent(s) will accept whichever treatment group is assigned through randomization.

Chapter 3: Randomized Trial Procedures

3.1 Bevacizumab Dose and Injection

The study is being conducted under Investigational New Drug Application (IND-122552) as intravitreal injection of bevacizumab for ROP in children is not FDA approved.

All participants will receive a single intravitreal injection of bevacizumab in one or both eyes following enrollment into the study. The injection/s should be given as soon as possible but no later than 2 calendar days after the diagnosis of type 1 ROP meeting all of the inclusion criteria and none of the exclusion criteria.

Eyes meeting eligibility criteria will receive a single dose of 0.063 mg or 0.25mg bevacizumab provided by the pharmacy at the investigator's institution. A coordinator at each site will be unmasked to dosage as they 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 789, 797, and 800 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 other than the coordinator/s at the site will be masked to the dosage level.

3.1.1 Bevacizumab Injection

The bevacizumab injection(s) will be given preferably within 24 hours, but no later than 2 days, after the diagnosis of type 1 ROP. The ophthalmologist may choose to give the intravitreal injection(s) 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 will be available, and the pupils will be dilated.

If only one eye is 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.

Prior to injection, it should be confirmed that the label information on the study syringe to be used for injection matches the information on the Bevacizumab Study Syringe Preparation Form.

The investigator who gives the injection must have previous experience giving intravitreal injections.

The injection will be done as outlined in the *ROP Procedures Manual*.

3.2 Treatment for ROP

If both eyes are eligible, then both are included. If one eye is eligible and the other eye has type 1 ROP with ROP or vessels in zone II, then both eyes will receive the treatment randomly assigned. If one eye is eligible and the fellow eye later develops type 1 ROP in zone I (i.e. any ROP in zone I) within 4 weeks of injection in the first eye, then the fellow eye will also receive the treatment randomly assigned to the first 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 eye procedures are designed to minimize bias comparing the study eyes.
If the fellow eye does not develop type 1 ROP in zone I, then treatment and follow-up for the fellow eye is at investigator discretion.

After 4 weeks, treatment will be at investigator discretion. Examinations in addition to those listed in 3.3 will be at investigator discretion.

3.3 Exam Schedule

The exam schedule is as follows:

Table 1: Visit Schedule

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	0 days	0 to 3 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	39 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).

596 Additional office visits may occur as needed.

Chapter 4: Study Drug

4.1 Study Agent(s) and Control Description

4.1.1 Acquisition

Intravitreous bevacizumab will be provided by the pharmacy at the investigator's institution.

4.1.2 Formulation, Appearance, Packaging, and Labeling

Syringes containing bevacizumab at the appropriate study concentration will be prepared by the pharmacy at the investigator's institution. If one eye is injected, then two syringes will be prepared. If two eyes are injected, then four syringes will be prepared. Two syringes (one for each planned injection) will serve as backups and will only be used if the other syringes are compromised for any reason; otherwise, the backup syringes will be discarded if unused. All syringes will be packaged and labeled as outlined in the *ROP Procedures Manual.*

4.1.3 Product Storage and Stability

Bevacizumab used in the study will be provided by the study pharmacy at the investigator's institution and diluted to the appropriate concentration by the institution per the procedures outlined in the *ROP Procedures Manual.* The institution pharmacy will prepare the bevacizumab syringes in a sterile manner, adhering to USP standards 789, 797, and 800. Syringes must be refrigerated at 2 to 8° Celsius and protected from light and must be used within 8 hours once prepared.

4.1.4 Preparation

Bevacizumab used in the study will be diluted to the appropriate concentration by the study pharmacy at the investigator's institution per the procedures outlined in the *ROP Procedures Manual.* The pharmacy will prepare the bevacizumab in a sterile manner, adhering to USP standards 789, 797, and 800.

4.1.5 Dosing and Administration

Bevacizumab at the proper study concentration and dose will be administered by intravitreous injection as outlined in the *ROP Procedures Manual.*

4.1.6 Route of Administration

Bevacizumab will be administered by intravitreous injection as outlined in the *ROP Procedures Manual.*

4.1.7 Dose Adjustments/Modifications/Delays

The dose should not be adjusted for any reason without first consulting with the protocol chair. For infants randomized to bevacizumab, the injection will be given preferably within 24 hours, but no later than 2 days, after the diagnosis of type 1 ROP. If injection is delayed beyond 2 days after diagnosis for any reason, the injection may still be done after consultation with the protocol chair. For analysis purposes, even if the dose is delayed, participants will be counted towards the randomly assigned treatment arm.

4.1.8 Duration of Therapy

After initial injection, additional treatment is described in Chapter 5.

4.1.9 Tracking of Dose

All syringes will be packaged and labeled by the study pharmacy at the investigator's institution and diluted to the appropriate concentration by the institution per the procedures outlined in the *ROP Procedures Manual.* The pharmacy will prepare the bevacizumab in a sterile manner, adhering to USP standards 789, 797, and 800. A second trained and delegated pharmacist will check that the preparation was compounded correctly and release syringes for use.

4.2 Study Agent Accountability Procedures

Drug accountability procedures will be detailed in the *ROP Procedures Manual.* If only one eye is to be treated, the treating investigator and a second person will review the Bevacizumab Study Syringe Label to confirm which eye will receive the intravitreous injection.

The investigator who gives the injection must have previous experience giving intravitreous 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 intravitreous injections for ROP.

Chapter 5: Testing Procedures

The following procedures will be performed in both groups at each visit as defined in the *ROP Procedures Manual:*

5.1 Procedures at Study Visits

Classification of ROP will be determined at each follow-up exam prior to 6 months corrected age as described in *section 2.4.2.* Data will be collected for both eyes.

At 2 and 4 months post-injection, and 6 and 12 months corrected age, data will be collected on additional treatment(s) for ROP and complications since the last study exam.

At 2- and 4-months post-injection, investigators will use scleral depression to determine extent of retinal vascularization in each quadrant, classified as:

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

Additional participant level data collected at 6 and 12 months corrected age will include the following:

- Date of initial hospital discharge
- Number of times re-hospitalized by 12 months corrected age
- 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)

Additional ocular data collected at 6 and 12 months adusted age will include the following (in both eyes where applicable):

- Ocular Alignment:
    - Alignment will be assessed by the cover test. If a tropia or intermittent tropia is detected, then it will be quantified by the prism and alternate cover test (PACT), if able, or by the Krimsky test if unable to do PACT.
- Assessment of Vision and Amblyopia:

  - Vision will be assessed in each eye by fixing and following.
  - For infants and young children with a manifest tropia, amblyopia will be assessed using binocular fixation patterns in best spectacle correction (if worn). For those without strabismus or with a microtropia, fixation preference will be assessed using the induced tropia test.
- Cycloplegic Refraction
- Ocular Exam:
  - Assessment of cornea, anterior segment, lens, optic nerve and retina.

Additional data will be collected at the time points specified:

- Retinal imaging/ Second exam:

Wide-angle retinal images will be obtained at the time of any additional treatment (laser or bevacizumab). Imaging will be done at 4-months post-injection if no additional treatment is planned such as prophylactic laser. Six images should be obtained for each eye: anterior segment, posterior pole, superior, temporal, inferior, and nasal. These images will later be reviewed by masked expert readers for purposes of quality control. Investigators will be informed throughout the study if their clinical diagnoses disagree with those of expert readers. If a participating site or location does not have a retinal camera, a second examination will be done by a study-certified examiner after enrollment and if there is treatment failure to assess the location, extent, and stage of disease, as well as presence of pre-plus or plus disease. Results of this exam will only be used for quality control, similar to review of retinal images.

- OCT and FA:
  - At sites with imaging capabilities, OCT and/or FA imaging will be obtained at the time of any additional treatment. OCT and/or FA will also be done at 4 months post-injection if no additional treatment is planned, such as prophylactic laser.
- Plasma levels of VEGF:
  - For infants participating in the optional study of plasma VEGF level, their blood will be collected at 2 weeks, 4 weeks, and 4 months post-injection. Scavenged blood may be used when feasible.
- Adverse Events:
  - After the 4-week ocular exam or hospital discharge (whichever is later), only serious adverse events (*see section 6.2.1),* ocular adverse events, and any events judged by the investigator to be related to injection and/or treatment will be recorded.

5.2 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,[55] upon which exam findings and failure criteria will be based.

Success is defined as (1) improvement* by the 4-day exam (3 to 5 days), and (2) no recurrence of type 1 ROP within 4 weeks of injection, and (3) no severe neovascularization requiring additional treatment within 4 weeks of injection. If any of these criteria are not met, then a retinal image or second examination will be done by a study-certified examiner. 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.

*Improvement by the 4-day exam is defined as follows:

- For eyes with pre-treatment plus disease, improvement by the 4-day post-injection exam is defined as plus disease no longer being present.
- For eyes 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 eyes 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.

5.3 Side Effects of Bevacizumab

Data will be collected at each follow-up visit to evaluate potential adverse effects of injection as described in *section 6.2*.

5.4 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.

5.5 Additional Treatment

Treatment is at investigator discretion after the 4-week outcome examination, or sooner if an eye meets criteria for failure. Investigators should not treat the 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 an 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.

Chapter 6: Unanticipated Problem and Adverse Event Reporting

6.1 Unanticipated Problems

Site investigators will promptly report to the Coordinating Center on an eCRF all unanticipated problems meeting the criteria below. Sites must report Unanticipated Problems to the IRB within seven (7) calendar days of recognition. For this protocol, an unanticipated problem is an incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places participants or others at a greater risk of harm than was previously known or recognized **(**including physical, psychological, economic, or social harm)

The Coordinating Center also will report to the IRB all unanticipated problems not directly involving a specific site such as unanticipated problems that occur at the Coordinating Center or at another participating entity such as a pharmacy or laboratory. These instances must be reported to the JCHR IRB within seven calendar days of recognition. The Director of the Human Research Protection Program will report to the appropriate regulatory authorities if the IRB determines that the event indeed meets the criteria of an Unanticipated Problem that requires further reporting.

6.2 Adverse Events

6.2.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a study participant, irrespective of the relationship between the adverse event and the drug(s) under investigation.

Serious Adverse Event (SAE): Any untoward medical 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 a congenital anomaly or birth defect.
- 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).

6.2.2 Reportable Adverse Events

For this protocol, all adverse events between the time of study eye treatment until 4 weeks will be recorded.[58] Any events between randomization and initial treatment will be entered as prior medical conditions.

After 4 weeks, only serious adverse events (*see section 6.2.1),* ocular adverse events, and any events judged by the investigator to be related to study injection and/or study treatment will be recorded. There will be no imputation of data for participants who die or are lost to follow-up prior to the six-month corrected age outcome visit.

All reportable Adverse Events whether volunteered by the participant's parents, discovered by study personnel during questioning, 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 Medical Monitor to verify the coding and the reporting that is required.

6.2.3 Relationship of Adverse Event to Study Treatment or Study Procedure

The study investigator will assess the relationship of each adverse event to be *related* or *unrelated* by deciding if there is a reasonable possibility that the adverse event may have been caused by the treatment or study procedure. The investigator brochure will list potential adverse events that may be expected.

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

> <u>Yes</u>
> 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 participant's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study treatment.
>
> <u>No</u>
> 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.

6.2.4 Severity (Intensity) of Adverse Event

The severity (intensity) of an adverse event will be rated on a three point scale: (1) mild, (2) moderate, or (3) severe. A severity assessment is a clinical determination of the intensity of an event. 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.

- MILD: Usually transient, requires no special treatment, and does not interfere with the participant's daily activities.

- MODERATE: Usually causes a low level of inconvenience, discomfort or concern to the participant and may interfere with daily activities, but is usually ameliorated by simple therapeutic measures and participant is able to continue in study.
- SEVERE: Interrupts a participant's usual daily activities, causes severe discomfort, may cause discontinuation of study drug, and generally requires systemic drug therapy or other treatment.

6.2.5 Expectedness

For a serious adverse event that is considered possibly related to study drug, the Medical Monitor will classify the event as unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described below.

6.2.6 Coding of Adverse Events

Adverse events will be coded using the MedDRA dictionary. To facilitate coding, the site will enter a preliminary MedDRA code which the Medical Monitor may accept or change (the Medical Monitor's MedDRA coding will be used for all reporting). The Medical Monitor will review the investigator's assessment of causality and may agree or disagree. Both the investigator's and Medical Monitor's assessments will be recorded. The Medical Monitor will have the final say in determining the causality as well as whether an event is classified as a serious adverse event and as a serious unanticipated event.

6.2.7 Outcome of Adverse Event

The outcome of each reportable adverse event will be classified by the investigator as follows:

- RECOVERED/RESOLVED: The participant recovered from the AE/SAE without sequelae. Record the AE/SAE stop date.

- RECOVERED/RESOLVED WITH SEQUELAE: The event persisted and had stabilized without change in the event anticipated. Record the AE/SAE stop date.

- FATAL: A fatal outcome is defined as the SAE that resulted in death. Only the event that was the cause of death should be reported as fatal. AEs/SAEs that were ongoing at the time of death; however, were not the cause of death, will be recorded as "resolved" at the time of death. Primary and secondary (if applicable) causes of death will be recorded.

- NOT RECOVERED/NOT RESOLVED (ONGOING): An ongoing AE/SAE is defined as the event was ongoing with an undetermined outcome.
    - An ongoing outcome will require follow-up by the site in order to determine the final outcome of the AE/SAE.
    - The outcome of an ongoing event at the time of death that was not the cause of death, will be updated and recorded as "resolved" with the date of death recorded as the stop date.

- UNKNOWN: An unknown outcome is defined as an inability to access the participant or the participant's records to determine the outcome (for example, a participant that was lost to follow-up).

If any reported adverse events are ongoing when a participant completes the study (or withdraws), adverse events classified as suspected, unexpected serious adverse reactions (SUSARs) will be followed until they are either resolved, or have no prospect of improvement or change, even after the participant has completed all applicable study visits/contacts. For all other adverse events, data collection will end at the time the participant completes the study. Note: participants should continue to receive appropriate medical care for an adverse event after their participation in the study ends.

6.3 Timing of Event Reporting

Serious or unexpected adverse events must be reported to the Coordinating Center within 24 hours via completion of the online serious adverse event form.

Other reportable adverse events will be reported within 3 days of the investigator becoming aware of the event by completion of an electronic case report form.

The Coordinating Center will notify all participating investigators of any adverse event that is serious, related, and unexpected. Notification will be made no later than 15 calendar days after the Coordinating Center becomes aware of the event, and no later than seven calendar days where the event is fatal or life-threatening.

Each principal investigator is responsible for reporting serious study-related adverse events and abiding by any other reporting requirements specific to his/her Institutional Review Board or Ethics Committee. Where the JCHR IRB is the overseeing IRB, sites must report all serious, related adverse events regardless of whether they are expected/anticipated and regardless of whether they are fatal or life-threatening to the JCHR IRB within seven (7) calendar days.

The Coordinating Center will be responsible for notifying the FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than seven (7) calendar days after initial receipt of the information. In addition, the Coordinating Center will notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

6.4 Safety Oversight

The study Medical Monitor will review all adverse events that are reported during the study. SAEs related to study treatment typically will be reviewed within 24 hours of reporting. Other AEs typically will be reviewed on a weekly basis. Additionally, the Medical Monitor will review compiled safety data at periodic intervals (generally timed to the review of compiled safety data by the DSMC).

A Data and Safety Monitoring Committee (DSMC) will review compiled safety data at periodic intervals, with a frequency of no less than twice a year. The DSMC can request modifications to the study protocol or suspension or outright stoppage of the study if deemed necessary based on the totality of safety data available. Details regarding DSMC review will be documented in a separate DSMC charter.

6.5 Stopping Criteria

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

6.5.1 Participant Discontinuation of Study Drug

Rules for discontinuing study drug use are described below.

- The investigator believes it is unsafe for the participant to continue to receive the drug. This could be due to the development of a potential side effect of the drug, a new medical condition or worsening of an existing condition; or participant behavior contrary to the indications for use of the drug that imposes on the participant's safety
- The participant requests that the treatment be stopped

Even if the study drug is discontinued, the participant will be encouraged to remain in the study through the final study visit.

6.5.2 Criteria for Suspending or Stopping Overall Study

The objective of the safety review is to decide whether the study (or study agent for an individual or study cohort) should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment (for a particular group, a particular study site or for the entire study) is a potential outcome of a safety review. Subsequent review of serious, unexpected, and related AEs by the Medical Monitor, DSMB, IRB, the sponsor(s), or the FDA or relevant local regulatory authorities may also result in suspension of further study agent administration at a site. The FDA and study sponsor(s) retain the authority to suspend additional enrollment and study agent for the entire study, as applicable.

6.6 Unmasking due to Adverse Event

Parents, investigators, and examiners will be masked to randomized treatment group. If a parent or investigator believes they need to be unmasked to randomized dosage, the investigator should contact the protocol chair first to discuss the case if possible.

Chapter 7: Miscellaneous Considerations

7.1 Collection of Medical Conditions and Medications

A pre-existing condition is defined as any medical condition that is either present at screening, a chronic disease, or a prior condition that could impact the participant's health during the course of the study (e.g., prior myocardial infarction or stroke). Pre-existing conditions will be recorded.

All medications that the participant is currently taking at enrollment or during the course of the study will be recorded. Preventative treatment also will be recorded (nutraceuticals will not be recorded).

7.2 Prohibited Medications, Treatments, and Procedures

There are no prohibited medications, treatments, or procedures.

7.3 Precautionary Medications, Treatments, and Procedures

There are no study defined rescue medications, treatments, or procedures.

7.4 Participant Compensation

Participant compensation will be specified in the informed consent form.

7.5 Participant Withdrawal

Participation in the study is voluntary, and a parent may withdraw at any time.
If a parent is considering withdrawal from the study, the principal investigator should personally speak to the individual about the reasons, and every effort should be made to accommodate the parent to allow continued participation if possible.

The goal for the study is to have as few losses to follow-up as possible. The Coordinating Center will assist in the tracking of study participants who cannot be contacted by the site. The Coordinating Center will be responsible for classifying a study participant as lost to follow-up. Study participants who have an adverse event attributable to a study treatment or procedure at the time of withdrawal will be asked to continue in follow-up until the adverse event has resolved or stabilized.

For participants who withdraw, their data will be used up until the time of withdrawal. Those that receive off-protocol treatment will be encouraged to remain in the study.

7.6 Confidentiality

For security and confidentiality purposes, participants will be assigned an identifier that will be used instead of their name. Protected health information gathered for this study will be shared with the coordinating center, the Jaeb Center for Health Research in Tampa, FL. De-identified participant information may also be provided to research sites involved in the study.

7.7 Contact Information Provided to the Coordinating Center

The Coordinating Center will be provided with contact information for each study participant. Permission to obtain such information will be included in the Informed Consent Form. The contact information will be maintained in a secure database and will be maintained separately from the

study data.

Phone contact or mailings from the Coordinating Center may be made with each study participant to facilitate the scheduling of the study participant for follow-up visits. A study participant-oriented newsletter may be sent once a year. A study logo item may be sent once a year.

Chapter 8: Statistical Considerations

8.1 Statistical and Analytical Plans

The approach to sample size and statistical analyses are summarized below.

8.2 Study Objectives and Hypothesis

8.2.1 Classification of Success/Failure by 4 Weeks Post-Injection

8.2.1.1 Within Dose Groups

The number of eyes with type 1 ROP injected with each dose (0.063 mg or 0.25 mg) of bevacizumab within four weeks of enrollment and proportion of eyes meeting success criteria as described in *section 5.2* will be evaluated along with a 95% confidence interval (CI) for the proportion. The point estimate and CI will be adjusted to control for any correlation arising from participants contributing two eyes to the analysis.

As an estimate of precision, the potential half-widths for an uncorrected 95% CI for various success proportions and various sample sizes are summarized in Table 2 below:

Table 2: Expected Half-width 95% Confidence Interval for Various Within Dose Group Success Proportions and Sample Sizes

	Observed Success Rate Within Dose Group		
Sample Size	**70%**	**80%**	**90%**
20	20%	18%	13%
25	18%	16%	12%
30	16%	14%	11%
35	15%	13%	10%
40	14%	12%	9%

8.2.1.2 Between Dose Groups

The difference between dose groups (0.25 mg vs 0.063 mg within study eyes only) with respect to the proportion of eyes meeting success criteria as described in *section 5.2* will be evaluated along with a 95% CI for the difference between groups.

For study eyes, the point estimate and CI will be adjusted to control for any correlation arising from participants contributing two eyes to the analysis (by definition, only one fellow eye with type 1 ROP and ROP and vessels in zone II can be treated per infant). To use the information of these participants as much as possible, a time to event analysis will be performed.

The estimated power to reject a two-sided null hypothesis of no difference between dose groups in favor of the alternative that they are different is given in Table 3 below for various effect sizes and sample sizes:

Table 3: Estimated Power for Various Effect Sizes and Sample Sizes

True Difference Between Dose Groups	Sample Size Each Dose Group				
	20	25	30	35	40
90% vs 70% (20%)	23%	30%	38%	45%	52%
90% vs 75% (15%)	13%	18%	23%	27%	32%
90% vs 80% (10%)	7%	9%	11%	13%	16%

8.2.2 Additional Analyses Within Each Dose Cohort

The following tabulations will be done within each dose cohort:

8.2.2.1 Baseline Characteristics

Participant 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.

8.2.2.2 At 2 and 4 Months Post-injection, and 6 and 12 Months Corrected Age

The following will be tabulated:

- The number of and types of additional treatment/s for ROP
- The number of and types of ocular complications
- The proportion of eyes with extent of retinal vascularization (4-months post-injection only) in the eye classified by investigator as:
    - Posterior Zone I
    - Anterior Zone I
    - Posterior Zone II
    - Mid Zone II
    - Anterior Zone II
    - Zone III
    - Full Vascularization

    - In addition, we will calculate the proportion and 95% confidence interval for treated eyes that achieve either Zone III or full vascularization.

Additional participant level data collected at 6 and 12 months will be tabulated:

- Distribution of time from enrollment to initial hospital discharge
- The number of times re-hospitalized
- The most recent head circumference (in centimeters)
    - The median, quartiles, and range will be calculated
- The most recent weight (in grams)
    - The median, quartiles, and range will be calculated
- Whether or not current supplemental oxygen required (yes/no)
- Proportion of participants died; cause of death

- Proportion of participants with the presence or history of systemic co-morbidities including:
  - Periventricular leukomalacia
  - Hydrocephalus (with shunt placement)

Additional ocular data collected at 6 and 12 months will be tabulated as follows:

- Ocular Alignment:
  - If able to test ocular alignment at distance, the following will be tabulated:
    - Number (%) with intermittent tropia or constant tropia
  - If able to test ocular alignment at near, 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
  - The % of eyes able to fix and follow
- Assessment of Amblyopia at participant level:
  - The distribution of responses by fixation preference testing will be tabulated:
    - 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
- Cycloplegic Refraction in each eye:
  - Spherical equivalent cycloplegic refractive error (distribution)
  - Proportion of eyes and 95% CI with Myopia? <-5 vs >=-5 spherical equivalent
  - Proportion of eyes and 95% CI with Hyperopia? >+5 vs <=+5 spherical equivalent
- Ocular Exam:
  - The number and proportion of children with each of the following will be tabulated if known for eyes injected:
    - Most severe abnormality in the eye based upon clinical exam:
      - Essentially normal
      - Straightening 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 for each as present vs (absent or questionable), not including view obscured

8.2.2.3 Safety

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% 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
- Complications after the four-week exam will be listed.

8.2.2.4 Power for Analysis of Adverse Effects

For rare side effects, Table 4 below specifies the chance of not observing at least 1 adverse event in a sample of 30 children for various event rates in the population.

Table 4: Chance of Not Observing at Least One Event in a Sample of 30 Participants

Actual Probability of an Event	Chance of Not Observing at Least One Event for a Given Dosage N=30 Participants
1%	74%
2%	55%
3%	40%
4%	29%
5%	21%

Hence, with the proposed sample size of 30 participants the study has a 21% probability for not observing at least one event for adverse events with 5% occurrence.

8.2.2.5 Plasma Levels of VEGF

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. 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% confidence interval calculated for the change.

8.2.3 Additional Analyses Between Dose Cohorts

The following analyses will be done between dose cohorts

8.2.3.1 Baseline Characteristics

Participant level and eye level characteristics will be visually not statistically compared between treatment dose groups. Analyses may evaluate confounding if sample size allows.

8.2.3.2 At 2 and 4 Months Post-Injection, and 6 and 12 Months Corrected age

Estimates and 95% confidence intervals within each treatment group will be calculated for the outcomes below. These point estimates and CI's will be adjusted to control for any correlation arising from participants contributing two eyes to the analyses. There will be no adjustment of the type 1 error rate as these analyses are exploratory.

At 4 months post-injection:

- The proportion of treated eyes that achieve either Zone III or full vascularization.
  - For this secondary outcome, an estimate and 95% confidence interval for the difference between groups will also be calculated

At 6- and 12 months Corrected Age:

- Cycloplegic Refraction in each eye:
  - Spherical equivalent cycloplegic refractive error (distribution)
  - Proportion of eyes and 95% CI with Myopia? <-5 vs >=-5 spherical equivalent
  - Proportion of eyes and 95% CI with Hyperopia? >+5 vs <=+5 spherical equivalent
- The proportion of participants with the presence or history of systemic co-morbidities including:
  - Periventricular leukomalacia
  - Hydrocephalus (with shunt placement)

At any time during the study:

- Proportion of infants for whom at least one adverse event was reported
- Proportion of infants with an adverse event thought by investigator to be related to study drug or injection
- Proportion of infants for whom at least one serious adverse event was reported
- Proportion of infant deaths

8.2.3.3 Plasma Levels of VEGF:

The distribution of VEGF levels and change in VEGF levels from pre-injection and 95% CI's at 2 weeks, 4 weeks, and 4 months post-injection.

8.3 Sample Size

The objective of the current protocol is to obtain estimates of treatment effect in eyes treated with 0.063mg and 0.25mg bevacizumab; and to explore whether the treatment effect differs between the two dose groups. Effectiveness for the purpose of this study is defined using the study's definition of success (as defined in *section 5.2*).

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. Tables 2, 3, and 4 above indicate the level of statistical power/precision the study would have for various sample sizes.

It is estimated that 80% of participants will have type 1 ROP with ROP and retinal vessels all in zone I in both eyes at the time of enrollment. An estimated 10% may have bilateral ROP at time of enrollment (or within 4 weeks of enrollment) where the fellow eye has retinal vessels in zone II; and 10% may be unilateral with only one eye with ROP and retinal vessels all in zone I during the study.

8.4 Sensitivity Analyses for Primary Outcome

8.4.1 Tipping point analysis for Imaging / second ocular exam

Wide-angle retinal images will be obtained after enrollment and prior to any additional treatment. Imaging will be done at 4 months post-injection if no additional treatment is planned, such as prophylactic laser. Six images will be obtained for each eye: anterior segment, posterior pole, superior, temporal, inferior, and nasal as described in the *ROP Procedures Manual*. These images will later be reviewed by masked expert readers for purposes of quality control. Investigators will be informed throughout the study if their clinical diagnoses disagree with those of expert readers.

If a participating site or location does not have a retinal camera, a second examination will be done by a study-certified examiner after enrollment and if there is treatment failure to assess the location, extent, and stage of disease, as well as presence of pre-plus or plus disease. Results of this exam will only be used for quality control, similar to review of retinal images.

The number of discrepancies between the site diagnosis and the retinal image reviewer diagnosis OR diagnosis by second study certified ROP examiner will be calculated at each visit. If there are cases where the investigator declared failure and masked review of the images OR second study certified examiner 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. If there are discrepancies at enrollment, a sensitivity analysis excluding those participants will be performed.

8.4.2 Deaths as Failures

A sensitivity analysis will be performed where deaths before failure are counted as failures.

8.5 Planned Interim Analyses

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

Chapter 9: Data Collection and Monitoring

9.1 Case Report Forms and Data Collection

The main study data will be collected through electronic case report forms (CRFs). These electronic CRFs from the study website are considered the primary source documentation. When data are directly collected in electronic case report forms, this will be considered the source data. Each participating site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of participants.

9.2 Study Records Retention

Study documents will be retained for a minimum of 3 years following the submission of the final financial report for the last grant cycle for which the study is conducted or 2 years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or if not approved, 2 years after the IND is withdrawn, whichever is later. No records will be destroyed without the written consent of the sponsor. It is the responsibility of the sponsor to inform the investigators when study documents no longer need to be retained.

9.3 Quality Assurance and Monitoring

Designated personnel from the Coordinating Center will be responsible for maintaining quality assurance (QA) and quality control (QC) systems to ensure that the clinical portion of the trial is conducted and data are generated, documented and reported in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements. Adverse events will be prioritized for monitoring.

A risk-based monitoring (RBM) plan will be developed and revised as needed during the course of the study, consistent with the FDA "Guidance for Industry Oversight of Clinical Investigations — A Risk-Based Approach to Monitoring" (August 2013). Study conduct and monitoring will conform with 21 Code of Federal Regulations (CFR) 312.

The data of most importance for monitoring at the site are participant eligibility and adverse events. Therefore, the RBM plan will focus on these areas. As much as possible, remote monitoring will be performed in real-time with on-site monitoring performed to evaluate the verity and completeness of the key site data. Elements of the RBM may include:

- Qualification assessment, training, and certification for sites and site personnel
- Oversight of Institutional Review Board (IRB) coverage and informed consent procedures
- Central (remote) data monitoring: validation of data entry, data edits/audit trail, protocol review of entered data and edits, statistical monitoring, study closeout
- On-site monitoring (site visits): source data verification, site visit report
- Communications with site staff
- Patient retention and visit completion
- Quality control reports
- Management of noncompliance
- Documenting monitoring activities
- Adverse event reporting and monitoring

Coordinating Center representatives or their designees may visit the study facilities at any time in order to maintain current and personal knowledge of the study through review of the records, comparison with source documents, observation and discussion of the conduct and progress of the study.

9.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or procedure requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

The site PI/study staff is responsible for knowing and adhering to the JCHR IRB requirements (i.e., reporting qualifying deviations to the JCHR IRB within seven (7) calendar days of identification). Further details about the handling of protocol deviations will be included in the monitoring plan.

Chapter 10: Ethics/Protection of Human Participants

10.1 Ethical Standard

The investigator will ensure that this study is conducted in full conformity with Regulations for the Protection of Human Participants of Research codified in 45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the ICH E6.

10.2 Institutional Review Boards

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

10.3 Informed Consent Process

10.3.1 Consent Procedures and Documentation

Informed consent is a process that is initiated prior to the parent agreeing to have their child participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the parent will be asked to read and review the document. The investigator will explain the research study to the parent and answer any questions that may arise. All parent(s) will receive a verbal explanation in-person or via phone/video conference in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their child's rights as research participants. Parent(s) will have the opportunity to carefully review the consent form and ask questions prior to signing.

The parent(s) should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The parent will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. The parent will be provided with a copy of the informed consent document for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

10.3.2 Participant and Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and imaging in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor, other authorized representatives of the Jaeb Center for Health Research, or representatives of the IRB may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and

pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by local IRB and Institutional regulations. Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the Jaeb Center for Health Research. Individual participants and their research data will be identified by a unique study identification number.

To further protect the privacy of study participants, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants

The study data entry and study management systems used by clinical sites and by the Jaeb Center for Health Research Coordinating Center research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the Jaeb Center for Health Research and made available to the public.

10.3.3 Future Use of Stored Specimens and Data

After testing for VEGF levels, any remaining blood or serum samples will be saved in a de-identified manner and possibly used for other studies in the future, but will not be used for whole genome sequencing or other such genetic testing that could reidentify the participants. All samples will be destroyed once all analyses are completed, results and published, and it is determined samples no longer need to be saved for future studies.

Chapter 11: References

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