A RANDOMIZED TRIAL OF LOW-DOSE BEVACIZUMAB VERSUS LASER FOR TYPE 1 RETINOPATHY OF PREMATURITY

Protocol Identifying Number: ROP3

IND Sponsor: Jaeb Center for Health Research

Funded by: National Eye Institute, National Institutes of Health

NCT04634604

Version Number: 1.3

28 April 2022

PROTOCOL AMENDMENT #2

11 February 2022

This amendment provides for the following protocol changes:

Protocol Change # 1:

Current Protocol

For unilateral cases with type 1 ROP in one eye at the time of enrollment, the current protocol does not specify treatment options for the fellow eye unless it meets criteria for type 1 ROP at a later time. If the fellow eye becomes eligible for treatment by developing type 1 ROP after enrollment, the eye will receive the randomized treatment assigned to the first eligible eye (the study eye). If the study eye has failed, then then the investigator may choose to treat the fellow eye with either laser or bevacizumab 0.25 mg.

Proposed Change

Sections 2.5 and 3.1 have been updated to specify treatment options for fellow eyes. The investigator is not limited to treatment options by eligibility of the fellow eye. At investigator discretion, the fellow eye may be treated at the same time as the eligible eye using the same randomized treatment assigned to the eligible eye. The investigator may also choose to treat the fellow eye at a later time using the same randomized treatment assigned to the eligible eye. However, if the eligible eye has non-response to the initial treatment, then the fellow eye may be treated with either laser or bevacizumab. Also, if the infant is $\geq$ 45 weeks PMA or it has been $\geq$ 4 weeks since treatment of the eligible eye with bevacizumab, then the fellow eye may be treated with either laser or bevacizumab.

Rationale for Change

It is in the participant's best interest to have a fellow eye treated at investigator discretion. For example, if one eye is type 1 ROP and the other eye has impending type 1 ROP, and the infant is randomized to laser, some centers perform laser under general anesthesia. In such a case, the investigator may want to treat both eyes at the same time. Also, if the first eye has non-response to one treatment, it will be in the infant's best interest if the investigator has the option to use a different treatment for the 2nd eye.

Additional Protocol Changes

In addition to the change above, the following clarifications have been made to the protocol:

1. The schedule of study visits and procedures has been updated:
    a. To reflect that information related to medical and ocular history will be collected at every protocol visit; and
    b. To reflect systemic outcome collection as specified in protocol at the time of enrollment.
2. Section 3.8 has been added to describe non-case report form data access.

3. The IQ and Neuropsychiatric tests referenced throughout the protocol has been updated to reflect the current versions (Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV) and Beery–Buktenic Developmental Test of Visual-Motor Integration, 6th edition (VMI-6))
4. Section 8.8 Safety Analyses has been updated to reflect a comparison of mean blood pressure measurements between treatment groups. Furthermore, adverse events in untreated fellow eyes will be tabulated separately. Adverse events in treated fellow eyes without type 1 ROP will be tabulated separately.
5. Gestational age stratification factor for randomization has been clarified as <=25 weeks and zero days versus >= 25 weeks and one day.
6. The definition of plus disease has been updated to be consistent with the ICROP3 classification.
7. The timing of retinal imaging has been made consistent throughout the protocol.

PROTOCOL AMENDMENT #1

26 August 2021

This amendment provides for the following protocol change:

Minor changes to the visit windows for the one, two, and four-week post-treatment exams. These changes will affect the definition of the primary outcome

Protocol Version 1.1

Visit	Target Day	Target Window (Around Target Day)	Allowable Window (Around Target Day)
1 week post-treatment*	Treatment Date + 1 week	6 to 8 days	3 to 10 days
2 weeks post-treatment*	Treatment Date + 2 weeks	11 to 17 days	11 to 17 days
4 weeks post-treatment*	Treatment Date + 4 weeks	25 to 31 days	18 to 38 days

Treatment success, determined at 6 months corrected age, and meeting all the following criteria:

- Five to 13 days after treatment (or re-treatment if indicated), there is no increase in the extent or severity of stage 3, or development of new plus disease.
- Two weeks after treatment (or re-treatment if indicated) to 6 months corrected age, there is no plus disease or severe neovascularization.
- At 6 months corrected age, there is no unfavorable structural outcome, defined as (1) macular ectopia, (2) a posterior retinal fold involving the macula, (3) a retinal detachment involving the macula, or (4) retrolental tissue or mass obscuring the view of the posterior pole.
- No scleral buckle or vitrectomy has been done by 6 months corrected age.

Protocol Version 1.2

Visit	Target Day	Target Window (Around Target Day)	Allowable Window (Around Target Day)
1 week post-treatment*	Treatment Date + 1 week	5 to 9 days	5 to 11 days
2 weeks post-treatment*	Treatment Date + 2 weeks	12 to 16 days	12 to 16 days
4 weeks post-treatment*	Treatment Date + 4 weeks	25 to 31 days	17 to 38 days

Treatment success, determined at 6 months corrected age, and meeting all the following criteria:

- Five to 11 days after treatment (or re-treatment if indicated), there is no increase in the extent or severity of stage 3, or development of new plus disease.

- Twelve to 16 days after treatment (or re-treatment if indicated) to 6 months corrected age, there is no plus disease or severe neovascularization.
- At 6 months corrected age, there is no unfavorable structural outcome, defined as (1) macular ectopia, (2) a posterior retinal fold involving the macula, (3) a retinal detachment involving the macula, or (4) retrolental tissue or mass obscuring the view of the posterior pole.
- No scleral buckle or vitrectomy has been done by 6 months corrected age.

Rationale for the change

The rationale for changing the one-week exam window from 5-13 days to 5-11 days is because the routine 2-week follow-up exam will occur at 12, 13 or 14 days after treatment. ROP rounds are typically done on the same day each week, and treatment may occur on the day that type 1 ROP is diagnosed, or 1 to 2 days after that exam. E.g. Rounds on Tuesday, type 1 diagnosed, treatment Thursday, 1 week exam on Tuesday (5 days post-treatment) and 2-week exam on the following Tuesday (12 days post-treatment). It has always been intended that there must be improvement by the 2-week exam, or non-response would be declared and additional treatment allowed. Since the target for the 2-week exam is being clarified as 12-16 days, it requires a corresponding change in the time period before the 2-week exam from 5-13 days to 5-11 days.

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TABLE OF CONTENTS

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
GMA	General Movements Assessment
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
USA	United States of America
VEGF	Vascular endothelial growth factor

SITE PRINCIPAL INVESTIGATOR STATEMENT OF COMPLIANCE

Protocol Title: A Randomized Trial of Low-Dose Bevacizumab versus Laser for Type 1 Retinopathy of Prematurity (ROP3)

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: _____

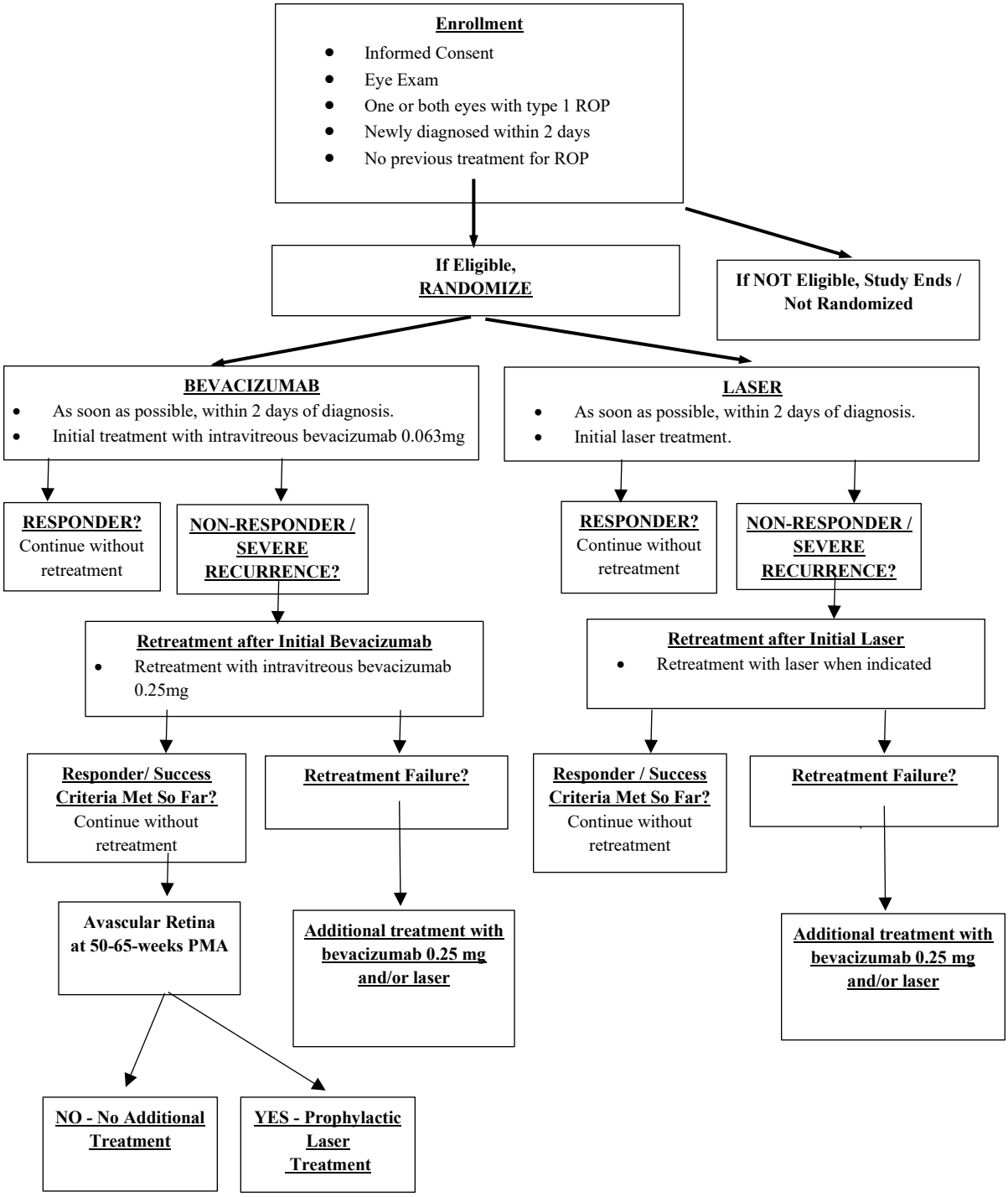
Site Name / Number: _____

PROTOCOL SUMMARY

ITEM	DESCRIPTION
Title	A Randomized Trial of Low-Dose Bevacizumab versus Laser for Type 1 Retinopathy of Prematurity (ROP3)
Précis	This randomized clinical trial will compare retinal outcomes with low-dose intravitreous bevacizumab (0.063 mg) versus laser photocoagulation as treatment for infants with type 1 retinopathy of prematurity (ROP). The study also will assess neurodevelopment, refractive error, visual acuity, and peripheral visual fields.
Investigational Drug	Bevacizumab.
Objectives	The primary objective is to determine if infants with type 1 ROP treated with intravitreous bevacizumab have retinal treatment success rates that are noninferior to that of infants treated with laser photocoagulation. Secondary objectives are to compare the number of re-treatments, extent of retinal vascularization, refractive error, visual acuity, visual fields, neurodevelopment, and systemic morbidities between treatment groups.
Study Design	Multicenter, randomized clinical trial.
Number of Sites	Up to forty (40) clinical sites in North America
Outcomes	**Primary Efficacy Outcome** ***Treatment success***, determined at 6 months corrected age, and meeting all the following criteria: • Five to 11 days after treatment (or re-treatment if indicated), there is no increase in the extent or severity of stage 3, or development of new plus disease. • Two weeks (12-16 days) after treatment (or re-treatment if indicated) to 6 months corrected age, there is no plus disease or severe neovascularization. • At 6 months corrected age, there is no unfavorable structural outcome, defined as (1) macular ectopia, (2) a posterior retinal fold involving the macula, (3) a retinal detachment involving the macula, or (4) retrolental tissue or mass obscuring the view of the posterior pole. • No scleral buckle or vitrectomy has been done by 6 months corrected age. **Secondary Efficacy Outcomes** • Number of re-treatments • Extent of retinal vascularization • Refractive error • Visual acuity • Visual fields • Neurodevelopment, by General Movements Assessment • Neurodevelopment, by the Bayley-4 test • IQ and Neuropsychiatric Testing • Systemic morbidities **Key Safety Outcomes** Systemic • Neurodevelopment, by General Movements Assessment • Neurodevelopment, by the Bayley-4 test • IQ and Neuropsychiatric Testing • A battery of systemic markers, including general, neurological, pulmonary, renal, hepatic, gastrointestinal, and auditory Ocular • Endophthalmitis • Cataract • Retinal detachment

ITEM	DESCRIPTION
Population	**Key Inclusion Criteria:** 1. Birth weight < 1251 grams 2. Type 1 ROP in one or both eyes; meeting the following criteria: • Zone I, any stage ROP with plus disease, with retinal vessels or ROP in Zone II in any quadrant, or • Zone I, stage 3 ROP without plus disease, with retinal vessels or ROP in Zone II in any quadrant, or • Zone II, stage 2 or 3 ROP with plus disease **Key Exclusion Criteria:** 1. Previous treatment for ROP 2. All ROP in zone I in either eye (no retinal vessels or ROP extend into zone II in any quadrant) 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 7. Stage 4 or Stage 5 in either eye Eye excluded (but the other eye may be eligible) if: 8. Visually significant ocular anomaly (e.g., cataract, coloboma) 9. Opacity that precludes an adequate view of the retina
Sample Size	212 participants (106 in each treatment group)
Phase	Phase III 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, re-treatment with 0.25 mg when indicated, and laser treatment later when indicated 2. Retinal laser photocoagulation, and re-treatment with laser when indicated
Participant Duration	Study exams will occur at 1, 2, and 4 weeks, and at 2 and 4-months post-treatment (and re-treatment when indicated). Additional study exams will occur at corrected ages (chronological age minus number of weeks born prematurely) 6 months, 1, 2, 3, 4, 5 and 6 years. 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 peripheral retinal laser ablation. Study exams will be at weeks 1, 2, 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 months, 1 year, and then annually for 5 more years. Non-study examinations will be at clinician discretion and are likely to occur more often. The primary outcome will be treatment success, defined as no worsening of ROP 5-11 days after treatment (or re-treatment if indicated), no plus disease or severe neovascularization 2 weeks to 6 months after treatment (or re-treatment if indicated), and no unfavorable structural outcome (or prior scleral buckle or vitrectomy) at 6 months corrected age. Important secondary outcomes include the number of re-treatments, extent of retinal vascularization, refractive error, neurodevelopment assessed by the Bayley-4 test, IQ and neuropsychiatric testing, visual acuity, visual fields, General Movement Assessment and systemic morbidities.

STUDY SUMMARY FLOWCHART



SCHEDULE OF STUDY VISITS AND PROCEDURES

	Enroll	1w*	2w*	4w*	2m*	4m*	6m†	1y†	2y†	3y†	4y†	5y†	6y†
Informed Consent	X												
Medical/Ocular History[a]	X	X	X	X	X	X	X	X	X	X	X	X	X
Ocular Exam/Classify ROP	X	X	X	X	X	X	X	X	X	X	X	X	X
Retinal Imaging (or Second Ocular Exam)	X[b]												
Cycloplegic Refraction							X	X	X	X	X	X	X
Visual Acuity									X	X	X	X	X
Visual Fields													X
General Movements Assessment						X[c]							
Bayley-4									X				
IQ test + Neuropsychiatric exam													X
Adverse Events		X[d]	X[d]	X[d]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]	X[e]
Systemic Outcomes	X	X		X			X	X	X	X	X	X	X
Quality of Life								X				X	X
Optional:													
Fluorescein Angiography	X[b]												
OCT	X[b]												
Wide-Fundus Photography													X
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 (defined as number of days since the date of birth minus number of days the baby was preterm). If a vitrectomy or scleral buckle is scheduled prior to the 6-month exam, then the 6-month exam will be completed early and prior to vitrectomy or scleral buckle surgery.

[a] Medical and ocular history to include concomitant medications and pre-existing medical conditions at time of enrollment
[b] Done at Enrollment, 40 weeks PMA and will also be collected when there is treatment failure before the 6 month xam. If a site or location does not have a retinal camera, a second examiner will classify ROP at enrollment and if there is treatment failure. OCT and FA are optional.
[c] General Movements Assessment will be recorded at home by parent(s) at 54 (52-56) weeks PMA
[d] All adverse events recorded at each visit until 4 weeks post-treatment
[e] Only serious adverse events and ocular adverse events recorded beyond 4 weeks post-treatment

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 area of retina without blood vessels (i.e. anterior to the vascular/avascular junction) 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 Pathophysiology of ROP

Studies in both humans and in animal models have shown that the pathophysiology of ROP is complex, in that it may be affected by genetic differences[18] as well as environmental risks.[19] ROP also has different characteristics throughout the world that may depend on a number of factors, including prenatal care and the ability to monitor and regulate oxygen levels.[14] In the US, when ROP was first identified in the 1940's, high oxygen at birth was the major risk factor.[20-23] Since then, technologic advancements in oxygen monitoring and regulation in the US reduced the incidence of ROP, but with further improvements in neonatal care and greater ability to save infants of small birth weights and young gestational ages, ROP has re-emerged and additional risk factors are recognized.[24] From these observations it has also been recognized that some ROP is associated with high oxygen (as in ROP of the 1940's in the US and elsewhere now in developing countries) that constricts and attenuates newly developed blood vessels and capillaries in the preterm infant. ROP is also associated with fluctuations in oxygen, oxidative stress, supplemental oxygen, poor postnatal weight gain and intrauterine growth restriction (IUGR),[25,26] which appear to delay normal retinal vascular development.

Because external factors that influence the course of ROP are different throughout the world based on resources for neonatal care, and because neonatal care is constantly changing, understanding the pathophysiology of ROP is challenging. A hypothesis to describe the development of ROP and account for these worldwide differences is that the disease occurs in different phases. Initially, a retinal avascular phase occurs associated with a delay in developmental angiogenesis and/or constriction of newly formed blood vessels and capillaries. Later, as the number of photoreceptors and retinal metabolism increase, the lack of vascular support causes retinal hypoxia and upregulation of angiogenic factors.[27,28] Finally, disordering of physiologic retinal angiogenesis causes vessels to grow into the vitreous (intravitreous neovascularization) rather than continue physiologic intraretinal angiogenesis.[19] This hypothesis has been refined to clarify the pathophysiology of current day ROP and to link the "phases", which described research from animal models in the 1950's to the clinical stages that were developed decades later to describe human ROP.[29,30]

Many angiogenic factors have both physiologic and pathologic effects. Evidence is increasing that there may be a threshold of signaling that, when exceeded, causes normal retinovascular development to become pathologic. The timing of these signaling events may also be important. That is, angiogenesis during a growth-restricted phase can be beneficial to physiologic retinal angiogenesis.[31-36] However, angiogenesis induced during a "vasoproliferative phase" can cause pathologic intravitreous neovascularization.

Vascular endothelial growth factor (VEGF) is a 45 kD protein that is important in vascular permeability and angiogenesis.[37] Since its discovery, VEGF has been found essential to physiologic retinal vascular development, which is ongoing in the preterm infant.[38-41] Other pathways are also important, but VEGF is an important mediator of many of these pathways or of their biologic outcomes.[42-44] However, VEGF is also one of the most important angiogenic factors that cause pathologic choroidal neovascular diseases, such as in age-related macular

degeneration, or retinovascular diseases, such as in diabetic retinopathy and retinal vein occlusion, in which blood vessels grow into the vitreous rather than the retina.[44] Injections of large doses of VEGF into the vitreous in mice cause endothelial cell filopodia to point into the vitreous,[38] promoting retinal vascular pathologic changes.[45] Inhibition of VEGF bioactivity reduces pathology in animal models and in human disease.[46-48]

Long-term clinical evidence for the benefits of anti-VEGF treatment in retinal vascular diseases exists for adults, but not for the preterm infant. In the developing infant, there is concern about inhibiting a factor that is important in physiologic as well as pathologic angiogenesis. There is ample evidence that inducing relative hypoxia in animal models reduces intravitreous endothelial growth,[49] including the Smith and D'Amore model, and the Penn model, of oxygen induced retinopathy.[50] However, there is also evidence suggesting that exceeding a threshold of VEGF signaling, particularly through the angiogenic receptor, VEGF receptor 2 (VEGFR2), may be detrimental to the retinal vasculature and affect the orderliness of developmental angiogenesis.[51] Zeng et al. have used an embryonic stem cell model that allows study of genes that produce nonviable knockouts. One such gene encodes VEGF receptor (R)1 (murine equivalent *flt-1*$^{-/-}$), which traps VEGF during development, thereby regulating the amount of VEGF available to bind and trigger signaling through VEGFR2. Zeng et al. showed that increased VEGF-VEGFR2 signaling caused disoriented cleavage planes in dividing endothelial cells during anaphase in the *flt-1*$^{-/-}$ model. Ordered angiogenesis was restored with a PECAM-sflt-1 transgene, providing evidence that excessive VEGF-VEGFR2 signaling caused disordered developmental angiogenesis. This study also supports the notion that restoring physiologic levels of VEGF can be beneficial to promote developmental angiogenesis.[51]

In a rat model of fluctuating oxygen developed by John Penn, cleavage planes of dividing vascular cells were analyzed *in vivo* in newly formed major retinal vessels at developmental time points when retinal VEGF was increased compared to room air raised rat pups. Increased VEGF-VEGFR2 signaling caused a greater proportion of vascular cleavage planes in veins to be parallel, favoring dilation, rather than perpendicular, which would favor elongation. Tortuosity of the arterioles was also increased. The pattern of dilation and tortuosity was reduced by neutralizing VEGF with intravitreous antibody.[52] The study provides evidence that excessive VEGF signaling may relate to "plus disease" seen in human severe ROP. Using the same model, neutralizing VEGF with antibody or with a VEGFR2 tyrosine kinase inhibitor at certain doses delivered into the vitreous reduced intravitreous neovascularization without interfering with ongoing retinal vascular development.[53] Additional studies showed the importance of specific regulation of VEGFR2 in retinal endothelial cells. Using gene therapy to specifically knockdown and regulate, but not knockout, VEGFR2 in retinal endothelial cells, Simmons et al. showed that VEGFR2 regulation in retinal endothelial cells not only reduced intravitreous neovascularization, but also permitted extension of physiologic retinal vascular development. However, when VEGF was reduced using a different gene therapy approach that knocked down VEGF expressed in Mueller cells, the neural retina appeared to be adversely affected. These findings together suggest that reducing VEGF available to bind other receptors in cells besides retinal endothelial cells had unwanted consequences. However, the authors also addressed the question of dose by knocking down a splice variant of VEGF, VEGF164, which enabled other forms of VEGF to still have access to the retina. Using this approach, the neural retinal appeared less adversely affected than when the full length VEGF was knocked down in Mueller cells.[54] These findings suggested

that a dose of intravitreous anti-VEGF might be found that would regulate VEGFR2 signaling in endothelial cells and be safe. Further evidence was found in a beagle model of ROP, in which a high dose of VEGFtrap virtually stopped pathologic and physiologic angiogenesis, whereas low dose VEGFtrap inhibited pathologic intravitreous neovascularization and permitted physiologic angiogenesis.[55] These studies support the importance of dose and the quandary of determining the right dose for an individual infant. The finding that increased VEGFR2 activation caused venous dilation and arteriolar tortuosity in the rat ROP model suggest that the optimal timing of anti-VEGF treatment may be at the time of onset of human plus disease.[27] Clinical observations also suggest that treatment should be prior to the onset of fibrovascular changes.[31] Thus, a window of treatment for anti-VEGF therapy may be at the time of plus disease and prior to the onset of fibrovascular activity. In addition, evidence from basic science suggests that inhibiting VEGF in an ROP model can lead to compensatory signaling through other angiogenic pathways.[56] Therefore, follow up of preterm infants treated with anti-VEGF agents will need to be longer than proposed in previous clinical studies.

1.1.3 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.[57] 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.[34,58] 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.[59] In the ETROP Study, only about 35% of children had visual acuity better than 20/40, even with earlier treatment.[60] 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.[61-69] 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.[61] 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.[64] Quiroz-Mercado et al observed neovascular regression in 17 of 18 eyes with high-risk prethreshold, threshold, or stage 4 ROP after intravitreous bevacizumab.[66] 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.[68]

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.[70] 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.

1.1.4 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.[61] However, these doses are likely much higher that necessary to effectively treat ROP. In addition, intravitreous bevacizumab reaches the systemic circulation, with large persistent reductions in serum VEGF levels,[71] raising concerns about potential systemic toxicity. 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.[72,73]

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.[69] 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. 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 Public Health Importance

Laser therapy is still considered by most clinicians to be the standard primary treatment for ROP, except for posterior zone I case, although bevacizumab is increasingly used in the US for primary treatment of ROP. Studies published to date report that bevacizumab's effectiveness in

preventing retinal detachment is at least as good as laser. In addition, there are potential advantages of bevacizumab when compared with laser, including ease of administration, less myopia, and better peripheral retinal vascularization that could translate to better visual fields later in life. One disadvantage is that there can be late recurrence of disease that is not observed after laser; therefore, many ophthalmologists now give prophylactic laser treatment several weeks following bevacizumab treatment, after there has been vascular growth toward the retinal periphery. The primary concern with bevacizumab is that sustained VEGF blockage could cause ocular or systemic side effects. There is particular concern about neurodevelopment, and 2 observational studies have reported worse neurological outcomes after bevacizumab compared with laser.[72,73] Other studies have found no difference in neurodevelopment between infants treated with bevacizumab or laser.[74-76] These observational studies are confounded by baseline factors associated with neurodevelopment, such as low birth weight, young gestational age, and systemic illness, because the smallest, sickest infants are more likely be treated with anti-VEGF agents, since the treatment takes less time than laser and is less stressful to the infant. Therefore, a randomized trial is necessary to compare ocular and neurological outcomes after bevacizumab versus laser. Both the BEAT-ROP and RAINBOW studies are randomized trials, but neither will be able to answer the question of whether neurological outcomes are worse after bevacizumab compared with laser. The BEAT-ROP study included neurodevelopmental assessment for only 16 of the participants, so it was underpowered to detect a difference between groups. The RAINBOW study plans neurodevelopmental assessments when the participants are older, but the study evaluated ranibizumab instead of bevacizumab. Ranibizumab has a shorter half-life in the serum than bevacizumab, and the dosage in their study was much higher relative to the adult dose than is planned in the current study, so it will not answer the question of whether low-dose bevacizumab causes neurological morbidity.

If this study shows that bevacizumab is at least as effective as laser, and neurological outcomes are not worse, then it would have an important impact on treatment of infants with ROP, even beyond the US. Because of its advantages over laser, bevacizumab would likely be more widely used for the primary treatment of ROP. Compared with peripheral retinal photocoagulation, bevacizumab injection is less expensive, takes less time to perform, is less stressful for infants, and, in some locations, is more readily available. Also, because there is growth of blood vessels into the previously avascular retinal periphery after bevacizumab treatment, it may possibly result in better peripheral visual fields (and perhaps less myopia) than peripheral laser photocoagulation. Some ophthalmologists currently use ranibizumab instead of bevacizumab because it has a shorter half-life in the bloodstream, so it may have better systemic safety, but it is much more expensive that bevacizumab. If low-dose bevacizumab demonstrates a good safety profile in this study, then these ophthalmologists who use ranibizumab for ROP could feel reassured to switch to bevacizumab. However, if bevacizumab is less effective than peripheral retinal photocoagulation or if it causes worse neurodevelopmental outcomes, then peripheral retinal photocoagulation would remain the standard of care for most infants with severe retinopathy of prematurity.

1.3 Study objectives

The primary objective is to determine if infants with type 1 ROP treated with intravitreous bevacizumab have retinal treatment success rates that are not worse compared with infants treated with laser. Secondary objectives are to compare neurodevelopment, number of re-

treatments, extent of retinal vascularization, refractive error, visual acuity, visual fields, and systemic morbidities between treatment groups.

1.4 Rationale

Peripheral retinal laser is the standard of care for most cases of severe ROP. An alternative for primary treatment is intravitreous bevacizumab, which an increasing number of ophthalmologists are using to treat severe ROP. Bevacizumab has several advantages, but it is unknown whether it causes systemic side effects, such as adverse neurodevelopmental outcomes. No adequately powered randomized trial has been done to compare ocular and neurodevelopmental outcomes in infants treated with bevacizumab versus laser.

Our hypothesis is that infants with type 1 ROP treated with intravitreous bevacizumab have retinal treatment success rates that are not worse compared with infants treated with laser. The study population will include premature infants with zone II or anterior zone I ROP (i.e. "straddlers," with at least some retinal vessels or ROP advanced into zone II). PEDIG recently completed a phase 1 dose de-escalation study to find a low dose of bevacizumab that was effective, and based on these data, a dosage of 0.063 mg was selected to use in the phase 3 trial. Using a Bayesian statistical model, it was estimated that there is a 94% probability that 0.063 mg is at least 95% effective. Even though 0.031 mg, 0.016 mg and 0.008 mg were both successful in most or all eyes in the phase 1 study, their probabilities of 95% effectiveness were estimated to be 84%, 64% and 33%, respectively.

Laser treatment serves as the ideal comparison group, because (1) it is the standard of care, and (2) infants treated with laser will not be exposed to anti-VEGF treatment, allowing for assessment of any effect of anti-VEGF treatment on neurodevelopment or other systemic outcomes. A potential problem when assessing systemic outcomes will be crossover of the laser therapy group. If laser treatment and re-treatment are not successful, then investigators may use bevacizumab as a treatment alternative.

1.5 Potential Risks and Benefits of Bevacizumab

1.5.1 Known Potential Risks of Bevacizumab

Little is known about the safety of using bevacizumab for ROP,[77] 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.[61] 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.[78] 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.[61] Reducing the dose of bevacizumab to 0.063 mg is expected to reduce the likelihood of negative systemic side effects.[79]

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.[80]

The safety and pharmacokinetics of bevacizumab in pediatric patients have not been established,[80] 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.[81] 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.[81] Others have reported similar effects with a lower dose (0.375 mg) of bevacizumab.[82] 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,[83] particularly if fibrovascular tissue has already begun to form.[84] 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.[85] Other complications observed following bevacizumab treatment for ROP include cataract,[86] retinal hemorrhage,[63,64] transient vascular sheathing,[64] choroidal ischemia,[87] 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.[88]

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).[61] 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.[89] 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[69] 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.[61,77,90]

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.[72,73] 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,[80] and it is unclear if this same risk applies to female neonates.

1.5.2 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%). [91]

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.[92]

The risk of vitreous hemorrhage after intravitreous injection has been reported to be less than 1% in adults.[92] 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.

1.5.3 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.[61] 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.[67] 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[93] 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).[63] 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[64] 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[89] 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.

1.5.4 Potential Adverse Effects of Laser Treatment

Laser treatment can be associated with bradycardia, arrhythmia, asystole, hypoventilation, apnea, aspiration, seizures and feeding intolerance. Laser treatment can be complicated by corneal burns, iris burns, cataract, rupture of Bruch's membrane, and accidental photocoagulation of the macula, including the fovea. Post-treatment complications of laser include intraocular inflammation, retinal and/or preretinal hemorrhage, posterior synechiae, and band keratopathy. Rare postoperative complications include serous retinal detachment, choroidal effusion, hypotony and phthisis.

1.5.5 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 do not cause damage to the ocular structures.

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%.[94]

1.6 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**.**

1.7 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

A minimum of 212 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 goals 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

Participants must meet all the following inclusion criteria in order to be eligible to participate in the study.

- Birth weight < 1251 grams

- Newly diagnosed (within 2 days) type 1 ROP in one or both eyes; meeting the following criteria:
    - Zone I, any stage ROP with plus disease, with retinal vessels or ROP in Zone II in any quadrant, or
    - Zone I, stage 3 ROP without plus disease, with retinal vessels or ROP in zone II in any quadrant or
    - Zone II, stage 2 or 3 ROP with plus disease

2.3 Participant Exclusion Criteria

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

- Previous treatment for ROP
- Stage 4 or 5 ROP in either eye
- All ROP in zone I in either eye (no retinal vessels or ROP extend into zone II in any quadrant)
- Either treatment could not be done within 2 days of diagnosis of type 1 ROP
- Investigator unwilling to randomize or parent unwilling to accept random assignment to either treatment
- Transfer to another hospital not covered by study-certified examiners anticipated within the next 4 weeks
- 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, blood pressure, gender, race, ethnicity, concurrent medical conditions (e.g. intraventricular hemorrhage (IVH), periventricular leukomalacia (PVL), hydrocephalus bronchopulmonary dysplasia, sepsis), and current medications.

2.4.2 ROP Classification

ROP will be classified by the investigator using the revised International Classification of Retinopathy of Prematurity (ICROP) criteria. Location, extent, and stage of disease, as well as presence of pre-plus or plus disease, 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: Stage will be recorded as follows:

- **Stage 0**: Incomplete retinal vascularization without demarcation line or ridge
- **Stage 1**: Demarcation line
- **Stage 2**: Ridge
- **Stage 3**: Extraretinal fibrovascular proliferation
- **Stage 4**: Partial retinal detachment. Stage 4 will be further classified based on location of the partial retinal detachment:
  - **Stage 4A**: Extrafoveal
  - **Stage 4B**: Involving the fovea
- **Stage 5**: Total retinal detachment

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

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

- Posterior Zone I - when optic nerve is centered using a 28D lens, no vessels extend even to the edge of the lens view
- Anterior Zone I - when optic nerve is centered using a 28D lens, vessels extend to the edge of the lens view or beyond, but not to zone II (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 is made when there is abnormal dilation and tortuosity of retinal blood vessels in zone I, and the degree of abnormality meets or exceeds that shown in representative photographs of plus disease from ICROP3.[95]

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

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

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

2.4.3 Retinal Images

Wide-angle retinal images will be obtained after enrollment, at 40 weeks PMA, and if there is failure prior to the 6 month exam. Six images will be obtained for each eye: anterior segment, posterior pole, superior, temporal, inferior, and nasal as described in the *ROP Procedures Manual*. To mitigate unmasked assessment bias, 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 as described in the ROP Procedures Manual. OCT and/or FA will also be done at 40 weeks PMA post-treatment and if there is failure prior to the 6-month exam.

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 (laser group VEGF levels will serve as a control). Participants in this optional study will have blood collected at enrollment, 2 weeks, 4 weeks, and 4 months post-treatment. 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 lab manual of procedures.

2.5 Randomization

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

- Intravitreous bevacizumab 0.063 mg, re-treatment with 0.25 mg when indicated, and prophylactic laser treatment later when indicated
- Retinal laser photocoagulation, and re-treatment with laser when indicated

If both eyes are eligible, then both eyes will receive the assigned treatment. If one eye is eligible and the fellow eye is not, then the eligible eye will receive the randomized treatment. If the fellow eye requires treatment (i.e., has type 1 ROP, or, in the investigator's judgment, requires treatment for ROP), then the fellow eye will receive the same randomized treatment assigned to the first eye. In the following scenarios, the fellow eye may receive a different treatment. (1)I If the eligible eye has non-response to the initial treatment, then the fellow eye may be treated with either laser or bevacizumab. (2) If treatment of the eligible eye was with bevacizumab, and the infant is now $\geq$ 45 weeks PMA or it has been $\geq$ 4 weeks since treatment of the eligible eye, then the fellow eye may be treated with either laser or bevacizumab. In these asymmetric cases, the eye treated later will not be included in the primary analysis.

Randomization will be stratified by zone (I vs II) for the most severe eye and by gestational age ($\leq$ 25 weeks and zero days vs $\geq$ 25 weeks and one day).

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.

Treatment of eligible eye/s by injection or with laser must be given within 2 calendar days of the diagnosis of type 1 ROP.

Chapter 3: Randomized Trial Procedures

3.1 Treatment

Treatment of eligible eye/s should be given as soon as possible but no later than 2 calendar days after the diagnosis of type 1 ROP. If both eyes are eligible then both eyes will receive the assigned treatment. If one eye is eligible and the fellow eye is not, then the eligible eye will receive the randomized treatment. If the fellow eye requires treatment at the same time or later (based on investigator judgment), then the fellow eye will receive the same randomized treatment assignment to the first eye. However, if the eligible eye has non-response to the initial treatment, then the fellow eye may be treated with either laser or bevacizumab. Also, if the infant is $\geq$ 45 weeks PMA or it has been $\geq$ 4 weeks since treatment of the eligible eye with bevacizumab, then the fellow eye may be treated with either laser or bevacizumab. In these asymmetric cases, the eye treated later will not be included in the primary analysis.

3.1.1 Bevacizumab Injection

For infants randomized to bevacizumab, the injection will be given no later than 2 days after the diagnosis of type 1 ROP. The ophthalmologist may choose to give the intravitreous injection in the operating room or at the bedside, with or without anesthesia, after consultation with the attending neonatologist. A binocular indirect ophthalmoscope with an appropriate condensing lens will be available, and the pupils will be dilated.

Intravitreous bevacizumab 0.063 mg will be provided by the research pharmacy at the investigator's institution. The pharmacy will prepare the bevacizumab in a sterile manner, adhering to USP standards 789, 797, and 800. Syringes containing bevacizumab at the appropriate study concentration will be prepared. If one eye is to be injected, then two syringes will be prepared. If two eyes are to be 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.

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.

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 will be certified for the study and must have previous experience giving intravitreous injections.

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

3.1.1.1 Treatment of Non-response / Severe Recurrence after Initial Bevacizumab

If there is inadequate improvement after initial treatment (non-response) or severe recurrence (see definition *section 3.2*), then the investigator will re-treat using bevacizumab at a dose of 0.25 mg. If the infant is $\geq$ 45 weeks PMA or it has been $\geq$ 4 weeks since the initial bevacizumab injection, then the investigator may choose to treat with laser instead of a 2nd bevacizumab injection. If there is fibrovascular or vitreous organization, or retinal dragging, then

bevacizumab should be avoided at any age, and re-treatment should instead be with laser, unless under the direct recommendation of a vitreoretinal specialist with ROP surgical experience.

3.1.1.2 Treatment of Non-response / Severe Recurrence after Re-treatment with Bevacizumab

If there is inadequate improvement after re-treatment (non-response) or severe recurrence, then failure is declared and the investigator may give laser treatment and/or bevacizumab 0.25 mg. If there is fibrovascular or vitreous organization, or retinal dragging, then bevacizumab should be avoided, unless under the direct recommendation of a vitreoretinal specialist with ROP surgical experience. Off-protocol treatment should first be discussed with the protocol chair.

3.1.1.3 Prophylactic Laser Treatment after Bevacizumab

Prophylactic laser to avascular retina will be done at 50-65 weeks PMA, or sooner if an infant is being transferred to another hospital, or is being discharged to home and arranging for outpatient laser would be difficult and/or not in the infant's best interest. If retina vessels/mild ROP are in zone III, or if retina vascularization is full or within 2 DD of the ora serrata both nasally and temporally by 65 weeks PMA, then laser is not required and is at investigator discretion. Investigators may also choose to give prophylactic laser at 50-65 PMA weeks at their discretion for reasons other than avascular retina, such as a worrisome appearance of ROP at the vascular-avascular junction, even if it does not meet criteria for severe recurrence. Laser treatment should not be given sooner than 4 weeks after bevacizumab unless failure has occurred.

3.1.2 Laser Treatment

For infants randomized to laser treatment, it will be given in conjunction with a binocular indirect ophthalmoscope and an appropriate condensing lens, by a study-certified ophthalmologist experienced in the use of this equipment. The treating investigator will be certified as having sufficient experience with laser for ROP, and adequacy of laser treatment will be confirmed by expert review of photographs. Special laser precautions, as mandated by OSHA and facility standards, will be followed. The procedure will be done as outlined in the *ROP Procedures Manual*.

Note: Re-treatment with laser may also be done in the absence of criteria for non-response / severe recurrence, at investigator discretion, to fill in lightly treated or skip areas.

3.1.2.1 Treatment of Non-response / Severe Recurrence after Initial Laser Treatment

If there is inadequate improvement after initial laser treatment (non-response) or severe recurrence (see definition *section 3.2*), then the investigator will re-treat using laser when indicated. Re-treatment will be indicated if, in the judgment of the investigator, there is opportunity to enhance the initial treatment by filling in lightly treated or untreated (skip) areas. If there is no such opportunity, then failure will be declared and treatment with bevacizumab 0.25 mg may be given instead.

3.1.2.2 Treatment of Non-response / Severe Recurrence after Re-treatment with Laser

If there is inadequate improvement after re-treatment (i.e. non-responder) or severe recurrence, then failure is declared and the investigator may give laser treatment and/or bevacizumab 0.25 mg. If there is fibrovascular or vitreous organization, or retinal dragging, then bevacizumab

should be avoided, unless under the direct recommendation of a vitreoretinal specialist with ROP surgical experience. Off-protocol treatment should first be discussed with the protocol chair.

3.1.2.3 Re-treatment of Fellow Eyes in Asymmetric Cases

If the fellow eye is treated for type 1 ROP, then additional treatment will be guided by the same non-response and failure criteria as the study eye. If the fellow eye did not have type 1 ROP when treated, then the investigator will decide if and when to re-treat, based on their clinical judgment. If re-treatment is needed, choice of treatment modality will follow the same guidelines as described above for the study eye.

3.2 Definition of Non-responder / Severe Recurrence

An eligible eye will be classified as non-response / severe recurrence when any of the following are present:

- Worse ROP 5 to 11 days after treatment, defined as an increase in the extent or severity of stage 3, or development of new plus disease
- No improvement* 12-16 days after treatment (two-week exam)
- Beyond the 2-week exam, recurrence of severe neovascularization** or recurrence of plus disease***

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

** Severe neovascularization is defined as at least one clock hour of extraretinal vascularization that, in the judgment of the investigator, poses a significant risk for retinal detachment and/or dragging.

*** Recurrent plus disease is defined as *both dilation and tortuosity* meeting or exceeding the degree of abnormality represented by the standard photograph of plus disease. Tortuosity without significant dilation is common after bevacizumab and does not meet criteria for recurrent plus disease.

3.3 Definition of Success

The primary efficacy outcome will be treatment success at the eligible eye level determined at 6 months corrected age, where success is defined as meeting all the following criteria as determined by the investigator:

- Five to 11 days after treatment (or re-treatment if indicated), there is no increase in the extent or severity of stage 3, or development of new plus disease
- Two weeks (12-16 days) after treatment (or re-treatment if indicated) to 6 months corrected age, there is no plus disease or severe neovascularization
- No unfavorable structural outcome at 6 months corrected age defined as (1) macular ectopia*, (2) a posterior retinal fold involving the macula, (3) a retinal detachment involving the macula, or (4) retrolental tissue or mass obscuring the view of the posterior pole.
- No scleral buckle or vitrectomy by 6 months corrected age.

* Macular ectopia must be definite to meet criteria for an unfavorable outcome. Questionable ectopia or vessel straightening alone are not considered unfavorable.

3.4 Study Visits and Phone Contacts

Data will be collected on the day of treatment with injection or laser. Post-treatment study exams will occur at 1, 2, and 4 weeks, and at 2 and 4 months after initial treatment (and re-treatment if indicated).

Additional study exams will occur at corrected ages 6 months, 1, 2, 3, 4, 5 and 6 years (calculated as chronological age minus number of weeks born prematurely). It is anticipated that non-study visits will occur more frequently, and the timing of these will be at clinician discretion.

Protocol-specified study visits (and target windows) are 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 (with Injection or Laser)	Day of Randomization	+ 1 day	+ 2 days
1 week post-treatment*	Treatment Date + 1 week	5 to 9 days	5 to 11 days
2 weeks post-treatment*	Treatment Date + 2 weeks	12 to 16 days	12 to 16 days
4 weeks post-treatment*	Treatment Date + 4 weeks	25 to 31 days	17 to 38 days
2 months post-treatment*	Treatment Date + 2 months	54 to 68 days	39 to 83 days
4 months post-treatment*	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
1 year corrected age	EDC + 1 year	335 to 395 days	243 to 547 days
2 years corrected age	EDC + 2 years	700 to 760 days	548 to 912 days
3 years corrected age	EDC + 3 years	1065 to 1125 days	913 to 1277 days
4 years corrected age	EDC + 4 years	1431 to 1491 days	1278 to 1643 days
5 years corrected age	EDC + 5 years	1796 to 1856 days	1644 to 2008 days
6 years corrected age	EDC + 6 years	2162 to 2222 days	2009 to 2375 days

* Post-initial treatment. If re-treatment is indicated and performed, exams will be repeated after re-treatment.

Due to retreatments, it is possible that retreatment windows will overlap with the corrected age visits. In these cases, the corrected age visits will take priority.

The goal is 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 as many study visits as possible 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).

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 within the allowable window.

3.4.1 Procedures at Study Visits

The following procedures will be performed in both groups at each visit as defined in Chapter 5 and the *ROP Procedures Manual*:

1) Classification of ROP: Will be determined at each follow-up exam as described in *section 2.4.2.* It is not expected that scleral depression will be done routinely after 50 weeks postmenstrual age, or after the 4-month post-treatment exam. Extent of retinal vascularization will be assessed for each quadrant as posterior or anterior zone I, posterior/mid/anterior zone II, zone III, or complete vascularization (as defined in *ROP Procedures Manual*). If a vitrectomy or scleral buckle is scheduled prior to the 6-month exam, then the 6-month exam will be completed early prior to vitrectomy or scleral buckle surgery.
2) Assessment of Amblyopia: Beginning at 6 months corrected age (when able). For infants and young children with a manifest tropia, binocular fixation patterns in best spectacle correction (if worn) will be used. For those without strabismus or with a microtropia, the induced tropia test will be done.
3) Ocular Alignment: Will be assessed by the cover test beginning at 6 months corrected age (when able). 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.
4) Cycloplegic Refraction: Will be done at 6 months corrected age and every study visit thereafter.
5) Visual Acuity Assessment: Recognition visual acuity will be done (when able) beginning at 2 years corrected age and every year thereafter using ATS-HOTV.
6) Assessment of Visual Fields: Will be measured using kinetic sphere perimetry at the 6-year corrected age exam.
7) General Movements Assessment: Will be administered at 54 weeks PMA by the parent(s)
8) Bayley-4 Test: Will be administered at 2 years corrected age.
9) IQ and Neuropsychiatric Testing: Will occur at age 6 years corrected age. Testing will include:
    a. Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV)
    b. Beery–Buktenic Developmental Test of Visual-Motor Integration, 6th edition (VMI-6)
    c. A pediatric neurologist and/or a clinical nurse specialist in child neurology will conduct a standard neurologic examination and give their overall impression using the following disability scale:
        i. 1 point indicating a normal result,
        ii. 2 points normal or questionable results,
        iii. 3 points abnormal results with minimal functional disability,
        iv. 4 points abnormal results with moderate functional disability, and
        v. 5 points abnormal results with severe functional disability
10) PedEyeQ Quality of Life Questionnaires at 1, 5, and 6 years corrected age:

a. A child questionnaire will be administered by study personnel to children at 5 and 6 years corrected age.
b. Parent and proxy questionnaires will be completed by the parent at 1, 5, and 6 years corrected age.

11) Retinal imaging:
a. At sites with a retinal camera, wide-angle retinal images will be obtained after enrollment and at 40 weeks PMA. Images will also be collected when treatment failure is declared before the 6 month exam. 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.
b. 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.

12) Plasma levels of VEGF: For infants participating in the optional study of plasma VEGF level, their blood will be collected at baseline, 2 weeks, 4 weeks, and 4 months post-treatment. Scavenged blood may be used when feasible.
13) OCT (+/- OCT-A) and FA: Will be offered as an optional part of the study at sites with those imaging capabilities. OCT (+/- OCT-A) and/or FA imaging will occur at enrollment, at 40 weeks PMA, and if there is failure prior to the 6-month exam.
14) Wide-Fundus Photographs (e.g. Optos): Will be offered as an optional part of the study at sites with imaging capability at 6 years corrected age.

The following information will be collected by interviewing parent(s) and reviewing the medical records:

15) Assessment of Adverse Events: All adverse events between the time of study eye treatment and the 4-week ocular exam will be recorded. After the 4-week ocular exam, only serious adverse events (*see section 6.2),* ocular adverse events, and any events judged by the investigator to be related to injection and/or treatment will be recorded.
16) Blood Pressure: Systolic and diastolic blood pressure measurements will be obtained from chart review at 1, 2, 3, and 4-weeks after treatment from chart reviews at 1, 2, and 4 weeks
17) Assessment of Systemic Outcome Measures as follows:

Table 2: Systemic Outcome Measures and Study Visits at Which They Are Assessed

System	Outcome measure	Study visit				
		Enrollment	1 week after treatment	4 weeks after treatment	6 months corrected age	Annually 1-6 years corrected age
General	Surgical wound infection	x	x	x		
General	Delayed healing of surgical wound	x	x	x		
General	Re-hospitalization (defined as inpatient status)				x	x
General	If yes, number of times re-hospitalized				x	x
General	If yes, primary reason(s) for re-hospitalization (from list)				x	x

General	Age at initial hospital discharge (post-birth)			x	x	
General	Weight	x	x	x	x	x
General	Recumbent length / height				x	x
Neurological	Intraventricular hemorrhage (grade)	x				
Neurological	Periventricular leukomalacia	x			x	
Neurological	Hydrocephalus requiring shunt	x			x	
Neurological	Cerebral palsy (mild, mod, severe)				x	x
Neurological	Seizures, excluding febrile	x	x	x	x	x
Neurological	Autism spectrum disorder - physician diagnosed				x	x
Neurological	Cerebrovascular accident / thrombosis	x	x	x		
Neurological	Occipital-frontal circumference (cm)				x	
Pulmonary	Effective inspired oxygen concentration	x	x	x		
Pulmonary	Increased oxygen requirement after treatment		x	x		
Pulmonary	If yes, days until O2 need returns to baseline		x	x		
Pulmonary	Bronchopulmonary dysplasia	x				
Pulmonary	Asthma diagnosed by a physician					x
Pulmonary	Age at which supplemental oxygen discontinued			x	x	x
Pulmonary	Coughing or vomiting blood				x	x
Pulmonary	Need for re-intubation after treatment		x	x		
Renal	Serum creatinine – most recent	x	x			
Renal	Systemic edema	x	x	x		
Renal	Hypertension requiring daily medication	x	x	x	x	x
Hepatic	Scleral icterus	x	x	x		
Hepatic	Serum alanine aminotransferase (ALT) – most recent	x	x			
Hepatic	Serum aspartate aminotransferase (AST) – most recent	x	x			
Hepatic	Serum bilirubin, total and direct – most recent	x	x			
Hepatic	Serum albumin– most recent	x	x			
Gastrointestinal	After tx, time (hours) until full enteral feeds		x	x		
Gastrointestinal	Necrotizing enterocolitis (surgical / nonsurgical)	x	x	x		
Gastrointestinal	Blood in stool, noted grossly	x	x	x	x	x
Cardiovascular	Cardiac arrest		x	x		
Cardiovascular	Vascular thrombosis, not cerebral	x	x	x		
Auditory	Hearing screen at discharge: AABR or OAE; normal / abnormal				x	
Auditory	Hearing aid requirement (right, left, both)				x	x
Auditory	Cochlear implant (right, left, both)				x	x
	Currently using any of the following:					
Pulmonary	Apnea monitor				x	x
Pulmonary	Oxygen				x	x
Pulmonary	Ventilator/CPAP				x	x
Pulmonary	Tracheostomy				x	x
Pulmonary	Pulse oximeter				x	x
Gastrointestinal	Gastrostomy tube and/or tube feeding				x	x

3.5 Phone Contacts

A phone contact from the site to the parent will be scheduled in between follow-up visits at 18, 30, 42, 54 and 66 months corrected age. The purpose of the phone contact is to remind parents about upcoming study visits and to maximize study retention.

Additional phone contacts may be performed as needed.

3.6 Unscheduled Visits

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

3.7 Participant Access to Study Agent at Study Closure

Participants will be treated or retreated with bevacizumab as defined in the sections above describing non-response / severe recurrence. Bevacizumab will not be offered to any participant at the end of the study.

977
978 **3.8 Non-Case Report Form Data Access**
979 Participants will be able to access GMA, Bayley, and other neurological testing results via
980 reports from the patient menu. Sites can request optional blood test results from the coordinating
981 center.

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 to be injected, then two syringes will be prepared. If two eyes are to be 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 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 Starting Dose and Dose Escalation Schedule

The initial dose of Bevacizumab injected into the study eye (or fellow eye if eligible) will be 0.063 mg; eyes may be retreated with 0.25 mg Bevacizumab after non-response / severe recurrence to initial treatment as defined in Chapter 3.

4.1.8 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.

4.1.9 Duration of Therapy

After initial injection, eyes may be retreated as described in Chapter 3.

4.1.10 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 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:*

1) Classification of ROP: Classification of ROP will be determined at each follow-up exam as described in *section 2.4.2.* It is not expected that scleral depression will be done routinely after 50 weeks postmenstrual age, or the 4-month post-treatment exam. If a vitrectomy or scleral buckle is scheduled prior to the 6-month exam, then the 6-month exam will be completed early prior to vitrectomy or scleral buckle surgery.
   - The exam must be done by a certified examiner. Certification will require attendance on a conference call to review standardized examples with the protocol chair.
2) Assessment of Amblyopia beginning at 6 months (when able). For infants and children unable to do recognition visual acuity, the presence or absence of amblyopia will be determined by tests of fixation preference. For those participants with no strabismus (or microtropia), fixation preference will be assessed using the induced tropia test. For those participants with strabismus, fixation preference will be assessed using binocular fixation patterns. Testing time is typically about one minute. See *ROP Procedures Manual* for details.
3) Ocular Alignment: ocular alignment will be assessed by the cover test beginning at 6 months corrected age (when able). 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. Testing time is typically in the range of 1 to 3 minutes.
4) Cycloplegic Refraction: A cycloplegic refraction will be done for both eyes at 6 months corrected age and every study visit thereafter. After administration of eye drops, it typically takes 30 minutes to achieve cycloplegia, and testing time for refraction of both eyes is in the range of 2 to 10 minutes.
5) Visual acuity will be tested in both eyes (when able) beginning at 2 years corrected age and every year thereafter through age 6, beginning with the ATS HOTV testing protocol on the Electronic Visual Acuity Tester (EVA). Testing time for both eyes typically is in the range of 5 to 15 minutes.
6) Assessment of Visual Fields: Visual fields will be measured using kinetic sphere perimetry at the 6-year exam. Testing time is typically in the range of 25 to 35 minutes.
7) General Movements Assessment: The GMA test will be administered at 54 weeks (52-56) PMA. See *ROP Procedures Manual* for details.
8) Bayley-4 Test: The Bayley-4 test of infant and toddler development will be administered at 2 years corrected age. See *ROP Procedures Manual* for details.
9) IQ and Neuropsychiatric Testing: IQ and neuropsychiatric testing will occur at age 6 years. See *ROP Procedures Manual* for details.
10) PedEyeQ Questionnaires[96-99]: Surveys will be administered at given timepoints to the applicable participants and parents of participants. Testing time is typically in the range of 3 to 5 minutes for the children and the same for their parents.
11) Retinal imaging
    a. Wide-angle retinal images will be obtained after enrollment and at 40 weeks PMA. Images will also be collected when treatment failure is declared before the 6 month exam. 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. If an investigator's clinical diagnoses disagree with that of the expert readers, then the protocol chair or vice-chair will discuss the findings with the investigator. Testing time for both eyes is typically in the range of 5 to 15 minutes. Details are in the *ROP Procedures Manual*.

b. 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. Repeat exam time for both eyes is typically in the range of 1-5 minutes.

12) Plasma levels of VEGF: For infants participating in the optional study of plasma VEGF level, their blood will be collected at baseline, 2 weeks, 4 weeks, and 4 months post-treatment. Scavenged blood may be used when feasible. If needed, time to collect blood is typically in the range of 2 to 10 minutes.

13) OCT (+/- OCT-A) and FA: These tests will be offered as an optional part of the study at sites with those imaging capabilities. Testing time for both eyes is typically in the range of 10 to 20 minutes for each of these procedures. Details are in the *ROP Procedures Manual*.

14) Wide-Fundus Photographs (e.g.Optos): Will be offered as an optional part of the study at sites with imaging capability at 6 years corrected age. Testing time for both eyes is typically in the range of 10-20 minutes for the procedure. Details are in the *ROP Procedures Manual*.

The following information will be collected by interviewing parent(s) and reviewing the medical records:

15) Assessment of Adverse Events: All adverse events between the time of study eye treatment and the 4-week ocular exam will be recorded. After the 4-week ocular exam, only serious adverse events (*see section 6),* ocular adverse events, and any events judged by the investigator to be related to injection and/or treatment will be recorded.

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.[100] 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 adjusted-age outcome visit. Several systemic measures will be monitored throughout the study (*see Table 2, section 3.3.1*).

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:

> Yes
> There is a plausible temporal relationship between the onset of the adverse event and administration of the study treatment and the adverse event cannot be readily explained by the participant's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study treatment.
>
> No
> Evidence exists that the adverse event has an etiology other than the study treatment (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to study treatment administration.

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, coordinators, and examiners will not be masked to randomized treatment group.

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 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 Primary Efficacy Outcome – Treatment Success At 6 Months Corrected age

The primary objective for the randomized trial is to determine if infants with type 1 ROP treated with intravitreal bevacizumab (subsequently referred to as BV) have a treatment success rate determined at 6 months corrected age that is non-inferior compared with infants treated with laser photocoagulation (subsequently referred to as LASER).

The primary efficacy outcome will be treatment success at the eye level (for eyes meeting eligibility criteria at time of enrollment) determined at 6 months corrected age, where success is defined as meeting all the following criteria as determined by the investigator:

- Five to 11 days after treatment (or re-treatment if indicated), there is no increase in the extent or severity of stage 3, or development of new plus disease
- Two weeks (12-16 days) after treatment (or re-treatment if indicated) to 6 months corrected age, there is no plus disease or severe neovascularization
- No unfavorable structural outcome at 6 months corrected age defined as (1) macular ectopia*, (2) a posterior retinal fold involving the macula, (3) a retinal detachment involving the macula, or (4) retrolental tissue or mass obscuring the view of the posterior pole.
- No scleral buckle or vitrectomy by 6 months corrected age.

* Macular ectopia must be definite to meet criteria for an unfavorable outcome. Questionable ectopia or vessel straightening alone are not considered unfavorable.

The study is designed as a non-inferiority study to evaluate a 1-sided null hypothesis that the success probability after six months with BV is inferior to LASER by 5% or more in favor of the alternative hypothesis that the success probability with BV is not inferior to LASER (>-5%).

$\mathbf{H_{null}}$ = $BV_{\text{success\% at 6m}}$ minus $\text{LASER}_{\text{success\% at 6m}}$ <= -5% *(BV inferior to LASER)*

$\mathbf{H_{alternative}}$ = $BV_{\text{success\% at 6m}}$ minus $\text{LASER}_{\text{success\% at 6m}}$ > -5% *(BV not inferior to LASER)*

Non-inferiority of BV compared to LASER will be declared if the LOWER limit of the upper 1-sided 97.5% CI for the difference between treatment groups in success proportions (BV minus LASER) is greater than the non-inferiority limit of -5% favoring laser.

If non-inferiority is declared, a test of no difference (superiority test) for BV compared with laser will be conducted.

8.2.3 Margin Choice

The 5% non-inferiority margin was selected by the planning committee based on clinical judgment. Planning committee members were willing to accept that bevacizumab may be

slightly inferior to laser (up to 5%) because bevacizumab has several other advantages, including ease of administration, less stress to infants during treatment, less myopia, better peripheral vascularization, and possibly better visual fields. Planning committee members were unwilling to accept a margin greater than 5% as "non-inferior," because severe ROP can lead to blindness, and laser is generally successful in preventing blindness.

8.3 Sample Size

The non-inferiority margin was selected based upon clinical judgement. For the primary efficacy outcome, treatment success at six months corrected age, members of the planning committee felt that a difference of 5% or more was a clinically meaningful difference.

It is expected that there will be a high correlation with respect to success between eyes of the same participant in those with bilateral treatment. Therefore, sample size calculations have been calculated based upon the number of participants to be conservative.

Based on previous data analyzed, the treatment success rate was estimated to be 95% for eyes initially treated with BV 0.063 mg (and retreated with BV 0.25 mg if necessary), and 85% for eyes initially treated with LASER (and retreated with LASER if necessary).[17,61,70]

Assuming that the true difference in success rates is 10% favoring BV, a total sample size of 180 participants (90 per group) will have 90% power to reject a 1-sided null hypothesis that the success rate after six months with BV is inferior to LASER by 5% or more in favor of the alternative hypothesis that the success rate with BV is not inferior to LASER (> -5%), with a type 1 error rate of 2.5%.

The percentage of participants lost to follow-up or withdrawn prior to six months corrected age is expected to be no more than 15% and equal between the two treatment groups. After adjusting for 15% lost to follow-up, the total sample size is 212 participants (~106 per group).

8.4 Analysis Dataset

The primary efficacy analysis will follow an intent-to-treat (ITT) principle. Therefore, the primary analysis dataset will include all eligible eyes at time of enrollment for randomized participants.

8.5 Analysis of the Primary Efficacy Outcome

The proportion of eyes eligible at randomization that achieve success will be tabulated by treatment group.

With a short time to primary outcome, it is expected that lost to follow-up to be minimal and non-informative. However, there may be a few participants who die or are lost to follow up before the 6-month visit. To use the information of these participants as much as possible, a time to event analysis will be performed. Censoring will occur at the time of death or lost to follow up before the 6-month outcome. If a participant reaches failure criteria at one of the visits before the censoring event, that participant will be counted as a failure. The protocol visit that reports the failure or censoring event will be used as the timepoint for the analysis: 1-week post-treatment, 2 weeks post-treatment, 4 weeks post-treatment, 2 months post-treatment, 4 months post-treatment,

6 months corrected age. The number of failures, censoring events, and 6-month visits by treatment group will also be reported on an eye level.

The hazard ratio of failure for BV: Laser and a one-sided upper tailed confidence (CI) will be calculated with a Cox model with robust variance estimation to control for correlation arising from participants contributing two study eyes to the analysis. Analyses will be adjusted for the stratification factors of zone (I vs II) for the most severe eye and by gestational age (≤ 25 weeks vs > 25 weeks). An estimate of the success probability difference at 6 months with the upper 1-sided 97.5% CI will be calculated with direct adjustment methods. The proportional hazards assumption will be checked.

8.6 Sensitivity Analyses for Primary Outcome

8.6.1 Tipping Point Analysis for Imaging and Repeat ROP Exam

Wide-angle retinal images will be obtained after enrollment, at 40 weeks PMA, and if there is treatment failure before the 6-month exam. Six images will be obtained for each eye: anterior segment, posterior pole, superior, temporal, inferior, and nasal as described in the ROP Procedures Manual. To mitigate unmasked assessment bias, 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 masked 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.

8.6.2 Deaths as Failures

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

However, if one group's failure rate meaningfully increases compared to the other, this may indicate informative censoring. In this case, the primary analysis may be adjusted to use Fine and Gray's extension of the Cox model to fit deaths as a competing risk to protocol treatment failure.

8.6.3 Primary Outcome Contingency Plan

If Cox model assumptions fail, exact logistic regression with the same adjustments of zone (I vs II) for the most severe eye and by gestational age (≤ 25 weeks vs > 25 weeks) will be attempted. This analysis of success or failure will be on a per-participant basis (if either eye fails, that participant is counted as a failure). Multiple imputation will be used based on baseline information for missing values.

If the model fails to converge, a model will be fit without the adjustments for stratification. If exact logistic regression still fails to converge, Barnard's exact test will be performed.

8.7 Analysis of the Secondary Outcomes

All secondary outcomes will be tabulated and/or summarized with descriptive statistics. There will be additional regression analyses for some outcomes (Table 3).

Multiplicity:

Type I error for the primary outcome analysis will be 5%, as only one analysis is pre-specified as primary. All other outcomes and their analyses will be pre-specified as either secondary or exploratory. Multiplicity in pre-specified secondary outcome analyses will be controlled using the False Discovery Rate (FDR) method. It is planned to report results for secondary outcomes annually; the FDR will be set to 5% for each of the annual reports and will apply to all pre-specified secondary analyses.

For FDR-controlled outcomes, an estimate and FDR-adjusted confidence interval for the difference between treatments will be provided. Estimates and 95% confidence intervals within each treatment also will be provided for all secondary outcomes.

Table 3. Secondary Outcomes

	6-Month	1 year	2 year	3 year	4 year	5 year	6 year	FDR or Exploratory	Outcome type	Additional Analyses
Number of re-treatments	X							Exploratory	Count	
Refractive error	X	X	X	X	X	X	X	FDR	Continuous	Regression
Proportion of study eyes with myopia (spherical equivalent refractive error <= -5.0D)	X	X	X	X	X	X	X	FDR	Binary	Regression
Bayley Neuro-development continuous[1]			X					FDR	Ordinal analyzed as continuous	Regression
Bayley Neuro-development categories[1]			X					Exploratory	Categorical	See section 8.7.1)
Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV), continuous							X	FDR	Ordinal analyzed as continuous	Regression

Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV), categorical								Exploratory	Categorical	See section 8.7.2)
Beery–Buktenic Developmental Test of Visual-Motor Integration, 6th edition (VMI-6)							X	FDR	Ordinal analyzed as continuous	Regression
Visual acuity			X	X	X	X	X	FDR	Continuous	Regression
Visual fields							X	FDR	Continuous	Regression
Systemic morbidities	X	X	X	X	X	X	X	Exploratory	Counts	
Extent of retinal vascularization		X						Exploratory	Categorical	
Proxy PedEyeQ[2]		X				X	X	FDR	Ordinal analyzed as continuous	Regression
Parent PedEyeQ[3]		X				X	X	FDR	Ordinal analyzed as continuous	Regression
Child PedEyeQ[4]						X	X	FDR	Ordinal analyzed as continuous	Regression

1. Contains
    a. Bayley Cognitive subscale
    b. Bayley Language subscale
    c. Bayley Motor subscale
    d. Bayley Social-Emotional subscale
    e. Bayley Adaptive Behavior subscale
2. Contains domains:
    a. Functional Vision
    b. Bothered by Eyes and Vision
    c. Social
    d. Frustration / Worry
    e. Eyecare
3. Contains domains
    a. Impact on Parent and Family
    b. Worry about Child's Eye Condition
    c. Worry about Self-perception and Interactions
    d. Worry about Functional Vision
4. Contains domains:
    a. Functional Vision
    b. Bothered by Eyes and Vision
    c. Social
    d. Frustration / Worry

8.7.1 Bayley Neurodevelopmental Outcomes

Secondary neurodevelopmental outcomes will be determined at 2 years corrected age as measured by the Bayley Scales of Infant and Toddler Development Fourth Edition Test (Bayley-IV, subsequently referred to as Bayley).

The Bayley has five major component scales that are tested for each child: Cognitive, Language, Motor, Social-Emotional, and Adaptive Behavior.

Scores for each scale range from 45 to 155, with a SD of 15. The neurodevelopment analysis will follow a modified intent-to-treat (ITT) principle. Data will be included only from participants who complete the outcome at the time point in question. There will be no imputation of data for participants who are lost to follow-up or who die prior to a secondary outcome. Multiple imputation for missing data (other than death) will be performed as a secondary approach, and results of the analysis with imputation of missing data (other than death) will be assessed for consistency with the primary analysis. If the ITT with no imputation and analyses with imputation give inconsistent results, exploratory analyses will be performed to evaluate possible factors contributing to the difference.

Table 4 gives the expected half-width of a 95% confidence interval around an observed difference for different number of outcomes expected at 1, 2, and 5 years corrected age, and possible standard deviations.

Table 4. Expected Half-width of 95% Confidence Intervals for Various Total Sample Size and Standard Deviations

Total Number of Outcomes	SD = 13	SD = 14	SD = 15	SD = 16	SD = 17
180 (15% lost)	± 3.80	± 4.10	± 4.40	± 4.70	± 5.00
170 (20% lost)	± 3.95	± 4.25	± 4.55	± 4.85	± 5.15
160 (25% lost)	± 4.05	± 4.35	± 4.70	± 5.00	± 5.30

At 2 years corrected age, each of the five Bayley composite subscale scores also will be categorized as normal, slightly impaired, or significantly impaired. Participants with scores 85 or higher are considered normal.[72,75] Participants with scores 70 to 84 are considered slightly impaired; and participants with scores <70 are considered significantly impaired. The proportion of participants within each score category at 2 years corrected age visits will be compared to evaluate whether there is a difference in this outcome between randomized treatment groups. Depending on cell counts in each treatment group and Bayley category, an ordinal logistic regression model will be considered.

8.7.2 General Movements Assessment

A certified general movements assessment (GMA) expert will review the video taken at 52 to 56 weeks PMA and grade the infant's movements as normal, absent, and abnormal as follows:

- Normal: fidgety general movements are intermittently or continuously present
- Absent: fidgety general movements are not observed or are sporadically present
- Abnormal: fidgety general movements are exaggerated in speed and amplitude

The proportion of infants with normal, absent, and abnormal gradings will be tabulated by treatment group.

To explore whether or not the GMA is predictive of neurodevelopment at 2 years corrected age, an ordinal logistic regression model will be considered.

8.7.3 Wechsler Preschool and Primary Scale of Intelligence (WPPSI-IV), categorical.

The overall WPPSI-IV score has cut points that will be tabulated in each treatment group:

- below 70 is Extremely Low
- 70-79 is Borderline
- 80-89 is Low Average
- 90-109 is Average
- 110-119 is High Average
- 120-129 is Superior
- 130+ is Very Superior

8.7.4 PedEyeQ

Rasch scores for each questionnaire item will be obtained from published look-up tables available at www.pedig.net. For each domain below, a score will be calculated for each participant and summarized by treatment group. Scores will also be converted to a 0-100 scale to aid in interpretation.

- Child PedEyeQ:
  - Functional Vision
  - Bothered by Eyes and Vision
  - Social
  - Frustration / Worry
- Proxy PedEyeQ:
  - Functional Vision
  - Bothered by Eyes and Vision
  - Social
  - Frustration / Worry
  - Eyecare
- Parent PedEyeQ:
  - Impact on Parent and Family
  - Worry about Child's Eye Condition
  - Worry about Self-perception and Interactions
  - Worry about Functional Vision

8.8 Safety Analyses

Ocular

Ocular adverse events will be tabulated separately for eligible eyes at the time of enrollment receiving randomized treatment. The total number of adverse ocular events occurring in each randomized group will be counted. For outcomes that are possible in each treatment group, the

percentage of eyes experiencing each outcome at least once will be compared between treatment groups, separately for each outcome. If possible, correlation will be accounted for. Adverse events in fellow eyes will be tabulated separately in groups defined by how they were treated (or not treated) for ROP at investigator discretion.

The following ocular adverse events are of primary interest:

- Endophthalmitis
- Retinal detachment
- Cataract
- Vitreous hemorrhage
- Inflammation
- Neovascular glaucoma
- Iris neovascularization

Systemic

Systemic adverse events will be reported with treatment grouped two ways:

The first grouping will be according to laterality and randomized treatment assignment (ITT groups):

1. Unilateral participants randomized to laser
2. Bilateral participants randomized to laser
3. Unilateral participants randomized to bevacizumab
4. Bilateral participants randomized to bevacizumab

The second grouping will be per protocol, i.e. participants will be classified based upon how they were actually treated (or retreated), with infants who ever received bevacizumab included in one group and infants who never received bevacizumab included in the other, regardless of randomized treatment assignment.

Systemic

The following systemic adverse events are of primary interest:

- Death
- Serious adverse event (at least one)
- Hospitalization (at least one)
- Cardiovascular/cerebrovascular events
  - The change in systolic and diastolic blood pressure measures at 1, 2, 3, and 4-weeks post-treatment between treatment groups will be evaluated.
  - Non-fatal myocardial infarction
  - Non-fatal stroke (counted only if symptoms lasted at least 24 hours)
  - Death of unknown cause
  - Death attributed to cardiac, cerebral, hemorrhagic, embolic, or other vascular cause (does not need to be ischemic in origin)
    Notes: Transient ischemic attacks, angina, and possible myocardial infarction or stroke are not counted. 'Nonfatal' myocardial infarction or stroke require that the participant is alive at the end of the study. If not, only the death is counted.

- Secondary systemic adverse events of interest:
  - For each MedDRA system organ class, percentage of participants with at least one event

A tabulation of all study eye ocular, non-study eye ocular, and systemic adverse events will be tabulated according to treatment groups as described above.

8.9 Protocol Adherence and Retention

Protocol deviations will be tabulated by randomized treatment group.

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

8.10 Baseline Descriptive Statistics

Baseline characteristics will be tabulated according to randomized treatment group, overall, and by completion (vs non-completion) of each follow-up visit. Baseline characteristics will also be tabulated according to mortality.

8.11 Planned Interim Analyses

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

8.12 Sub-Group Analyses

Subgroup analyses/assessments of effect modification (interaction) will be considered exploratory and will be pre-defined in the SAP.

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/videoconference 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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