

# Protocol

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Protocol for: Mintz-Hittner HA, Kennedy KA, Chuang AZ. Efficacy of intravitreal bevacizumab for stage 3+ retinopathy of prematurity. *N Engl J Med* 2011;364:603-15.

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TITLE: Intravitreal bevacizumab (Avastin™) injections versus conventional Laser surgery for vision-threatening retinopathy of prematurity: a prospective, randomized, non-blinded, controlled, multi-center, clinical trial

DRUG: Bevacizumab (Avastin™) for intravitreal injection

IND Number: 101,578

INDICATION: Anti-vascular endothelial growth factor to treat vision-threatening retinopathy of prematurity in neonates

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SPONSOR: Department of Pediatrics, The University of Texas Health Science Center-Houston Medical School

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DURATION OF FOLLOW-UP: 60 months (5 years) for each patient

MEDICAL MONITOR: Committee for the Protection of Human Subjects at The University of Texas Health Science Center-Houston Medical School

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**INTRAVITREAL BEVACIZUMAB (AVASTIN™) INJECTIONS  
VERSUS CONVENTIONAL LASER SURGERY FOR  
VISION-THREATENING RETINOPATHY OF PREMATURETY:  
A PROSPECTIVE, RANDOMIZED, NON-BLINDED,  
CONTROLLED, MULTI-CENTER, CLINICAL TRIAL**

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## 1. Abstract:

Protocol Number: HSC-MS-08-0036

Protocol Title: Intravitreal bevacizumab (Avastin™) injections versus conventional Laser surgery for vision-threatening retinopathy of prematurity: a prospective, randomized, non-blinded, controlled, multi-center, clinical trial.

Study Drug Name: Bevacizumab (Avastin™)

Route of Administration: Intravitreal injection

Drug Dose 0.025 ml (0.625 mg) of bevacizumab (Avastin™) in each eye [half of the adult intravitreal injection which is 0.05 ml (1.25 mg)]

Study Phase Phase 2

Study Design: In a prospective, randomized, non-blinded, controlled, multi-center, clinical trial, we will compare intravitreal bevacizumab (Avastin™) injections versus conventional Laser surgery to improve structural and functional outcomes of vision-threatening retinopathy of prematurity.

Duration of enrollment: Completed

Study Population: Neonates  $\leq 1500$  grams or  $\leq 30$  weeks gestational age at birth identified by indirect ophthalmoscopy to have vision-threatening retinopathy of prematurity.

Evaluation Infants would have evaluations with RetCam photographs scheduled 1 week and 1 month following any treatment. Other RetCam photographs would be obtained at any evaluation when the examiner believes that neovascularization has recurred and for all infants at the visit scheduled to be as close to 54 weeks post menstrual age as feasible (between 50 and 70 weeks post menstrual age).

Duration of follow-up: 60 months (5 years) for each patient.

Primary Outcome: An unfavorable outcome is defined as recurrence of neovascularization arising from the inner retinal vessels

needing re-treatment in either eye (based on RetCam photographs) identified at any time before data lock (after the last child enrolled reached 60 weeks postmenstrual age).

The primary outcome would be considered unknown for infants who did not have neovascularization at or before 50 weeks postmenstrual age but were not examined after that age.

**Secondary Outcomes:**

In blinded assessments of cropped RetCam photographs at 54 weeks post menstrual age (or between 50 and 70 weeks post menstrual age), structural outcomes which are associated with unfavorable functional outcomes including dragging, distortion, or detachment of the macula. In non-blinded assessments of RetCam photographs at 54 weeks post menstrual age (or between 50 and 70 weeks post menstrual age), the amount of retina judged by the horizontal extent of the retina (through the optic disc and macula) will be measured.

Amount of myopia (nearsightedness) judged by refraction at 54 weeks post menstrual age (or between 50 weeks post menstrual age and 5 years of age).

Presence of strabismus judged by measurements at 54 weeks post menstrual age (or between 50 weeks post menstrual age and 5 years of age).

The horizontal extent of the retina usually remains unchanged; however, dragging or distortion of the macula and eventually retinal detachment can occur or become more severe as time progresses. Also, myopia and strabismus can occur or become more severe as time progresses. Therefore, information will continue to be gathered at 80 weeks post menstrual age (or after 75 weeks post menstrual age but before 100 weeks post menstrual age) (approximately 1 year post natal age), and at ages 2, 3, 4, and 5 years. Eventually, visual acuity will be obtained in those patients whose central nervous system function permits. These later time points are not necessary to determine which treatment is more beneficial.

**Safety Criteria:**

Incidence and types of adverse events reported.  
Unfavorable outcomes per treatment documented.

## **2. Abbreviations:**

AAO	American Academy of Ophthalmology
AAP	American Academy of Pediatrics
APROP	Aggressive Posterior Retinopathy of Prematurity
CRYO-ROP	Cryotherapy for Retinopathy of Prematurity
ETROP	Early Treatment for Retinopathy of Prematurity
ICROP-R	International Classification for Retinopathy of Prematurity-Revisited
ROP	Retinopathy of Prematurity
VEGF	Vascular Endothelial Growth Factor

### **3. Co-Investigators:**

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Clinical Research Unit/ Center for Clinical and Translational Sciences	University of Texas Health Science Center- Houston Medical School Clinical Coordinator, Health Informatics (Electronic Case Report Form), and Statistical Assistance as needed
BEAT-ROP Reading Center	Three ROP experts to read RetCams

(Thirteen additional centers are participating in this clinical trial and are following the identical protocol—see list at end of protocol.)

#### 4. Introduction:

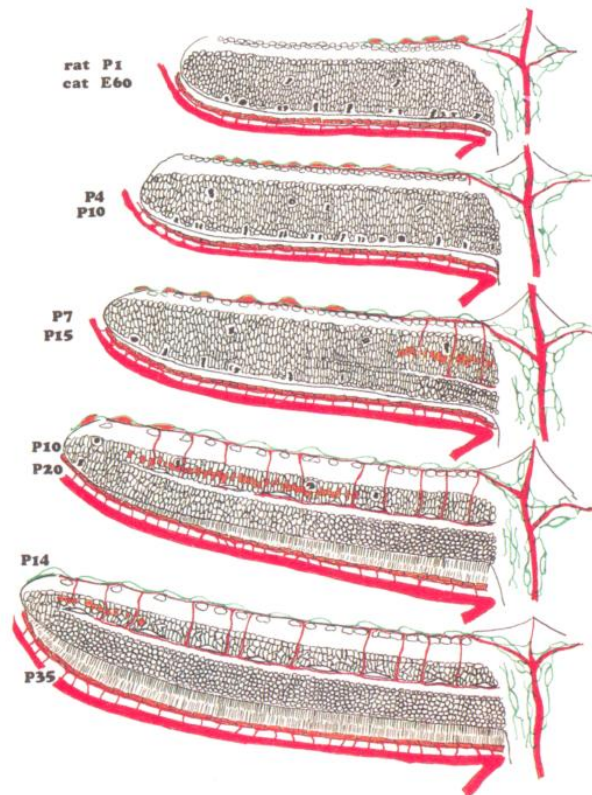
**Background:** Retinopathy of prematurity (ROP) is a common cause of blindness in children in the United States today. Currently, the preferred treatment utilizes ablation of the avascular retina with laser (or cryotherapy) according to the Early Treatment of Retinopathy of Prematurity study (ETROP).<sup>1</sup> Treatment may not reduce the incidence of vision loss especially in patients with acute ROP in Zone I or Posterior Zone II. Failure of three laser surgeries to induce ROP regression, usually predicts the need for additional laser ablations and, ultimately, vitrectomy. Patients with Zone I and posterior Zone II [Zone II (Small)] ROP still have a very high incidence of unfavorable outcomes following laser(s)<sup>2-8</sup> even with vitrectomy (including lensectomy)<sup>9</sup> or (sparing the lens).<sup>10</sup> Better treatment options are needed to prevent progressive loss of vision in these severe cases.

The visual outcome of Zone I or Posterior Zone II ROP patients after treatment with laser or cryotherapy often includes substantial visual field loss.<sup>9-11</sup> Significant early complications of these procedures include cataracts (clouding of the lens), iris synechiae, angle-closure glaucoma (increased intraocular pressure), phthisis (decreased intraocular pressure), hemorrhage (bleeding in the anterior chamber, vitreous, or retina), ROP recurrence, and retinal detachment. Late complications include dragged maculae, severe myopia (nearsightedness), anisometropia (unequal refraction), amblyopia (unilateral decreased vision), strabismus (eye turning inward or outward), cataracts, angle-closure glaucoma, and retinal detachment. Laser therapy is generally considered the preferred ablation treatment; however, neither laser nor cryotherapy is an optimal primary treatment modality for acute ROP. Further, vitrectomy is not an optimal rescue therapy. A less invasive medical therapy, with fewer complications, and with direct regulation of the angiogenic ROP mediator vascular endothelial growth factor (VEGF) is desirable.

VEGF has an important role in the pathogenesis of ROP. As demonstrated in studies of ablation of the avascular retina in severe ROP, down-regulation of VEGF expression, before tractional forces produce retinal detachment, reduces the destructive process of ROP. Retinal VEGF down-regulation appears to be the predominant mechanism behind laser and cryotherapy efficacy. We suspect that direct blockade of VEGF not only will promote ROP regression, but also will allow for further normal retinal vascular growth. Enhancement of normal retinal vascularization is of paramount importance for normal central and peripheral visual development. Tight titration of VEGF blockade prevents complete interruption of inner retinal vascularization. Investigators from many countries including the have reported recently on small number of infants treated for ROP with intravitreal bevacizumab (Avastin<sup>TM</sup>) in combination with laser and/or vitrectomy in Stages 3 and 4a with favorable outcomes.<sup>11-22</sup> Three countries have reported small case series treated with bevacizumab (Avastin<sup>TM</sup>) as monotherapy (no laser and/or vitrectomy) in ROP Stage 3 with favorable outcomes.<sup>22-24</sup> Only when bevacizumab (Avastin<sup>TM</sup>) is given too late in ROP Stages 4b and 5 is the complication of acute contraction of neovascular membranes observed.<sup>22, 25, 26</sup> A clinical trial to study

bevacizumab (Avastin™) safety in premature infants has completed enrollment (without complications due to the drug administration) but a clinical trial to study efficacy of bevacizumab (Avastin™) versus lens-sparing vitrectomy in premature infants who have had laser therapy is currently in progress in the United States was terminated due to lack of enrollment.<sup>27</sup> Safety results from the adult population indicate low rates of adverse effects with intravitreal bevacizumab (Avastin™) injections for a variety of diseases.<sup>28</sup> Pathologic examination of the eyes of an infant (350 gram birth weight; 22 week gestational age) who received intravitreal bevacizumab (Avastin™) injections at ages 9 and 20 weeks of life and expired at 29 weeks of life (51 weeks corrected age) demonstrates no toxicity.<sup>29</sup>

**Normal Vascularization of the Retina:** The outer layers of the retina get blood supply from the choroidal vascular system, which lies beneath the outermost layers of the retina and the retinal pigment epithelium (RPE). The inner layers get blood supply from a superficial plexus of inner retinal vessels located just beneath the inner limiting membrane and by a deep plexus of inner retinal vessels located in the inner nuclear layer. VEGF is secreted by astrocytes in the inner retinal surface, in response to hypoxia. It is also secreted by the Müller cells in the inner nuclear layer, in response to hypoxia. Physiologic VEGF secretion precedes the growing vascular front of the inner retinal vessels.<sup>30</sup>

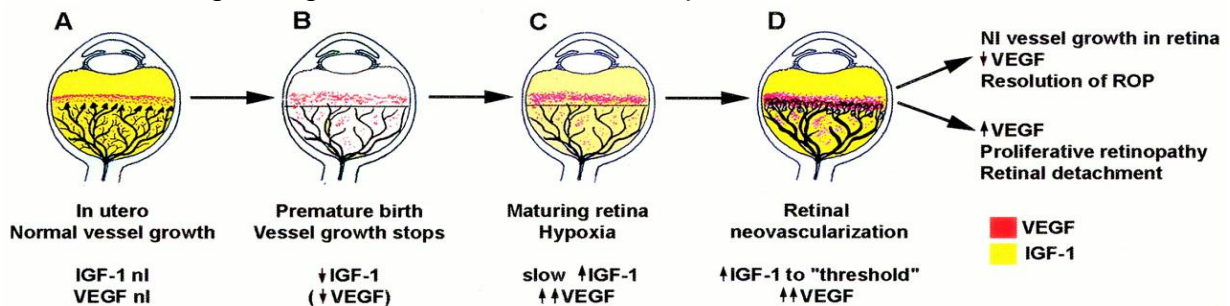


Adapted from Stone, et al. (1995)<sup>30</sup>

Although there is considerable variation in the time course of retinal vascularization among infants, approximately 70% of the retina is vascularized at 27 weeks gestation. Typically, completion of retinal vascularization occurs by 36 weeks gestation on the nasal side and by 40 weeks gestation on the temporal side. It has been shown that vascularization may not proceed normally if the infant is born prematurely. In fact, if an infant is born  $\leq 28$  weeks gestation (even if no treatment is performed for ROP), vascularization may never extend to the ora serrata (Mintz-Hittner and Kretzer, 1994)<sup>31</sup> and the foveal avascular zone may never form (Mintz-Hittner, et al., 1999).<sup>32</sup> The excessive area of avascular retina is responsible for the susceptibility of premature babies to ROP.

### Abnormal Vascularization of the Retina: Retinopathy of Prematurity:

The pathogenesis of retinopathy of prematurity is explained by abnormal levels of vascular endothelial growth factor and insulin-like growth factors,<sup>33</sup> as depicted in the following diagram. A disruption of the physiological VEGF wave anterior to the growing vascular front is noted in premature neonates.



Adapted from Hellstrom, et al. (2001)<sup>33</sup>

Supplemental oxygen to premature infants can further disrupt VEGF-regulated vascular development. The initial phase of ROP (Phase I) is a result of a hyperoxia-induced loss of the physiological VEGF wave anterior to the growing vascular front. VEGF down-regulation leads to cessation of normal vascular growth. Subsequent regression of existing retinal vessels occurs due to apoptosis of vascular endothelial cells. (Approximately 22-30 weeks post menstrual age.)

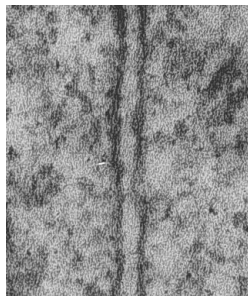
The next phase of ROP (Phase II) coincides with the time of the photoreceptor retinal layer development, as postnatal age advances. Photoreceptors have high metabolic demands due to their continuous need for photosensitive pigment synthesis, cilia rebuilding, and maintenance of membrane integrity for neural signal transmission. Thus, photoreceptor development greatly increases the retinal metabolic demands and oxygen requirement. This process in premature neonates coincides with a time of retinal vaso-oblivation and relative retinal hypoxia. The inability of the existing vasculature to meet the metabolic demands of the retina, results in the increased release of VEGF, in an attempt to enhance retinal blood supply via neo-vascularization. However, this exuberant blood vessel growth is pathologic. These blood vessels tend to be tortuous, leaky

and friable, resulting in hemorrhage and exudation. (Approximately 31-42 weeks post menstrual age.)

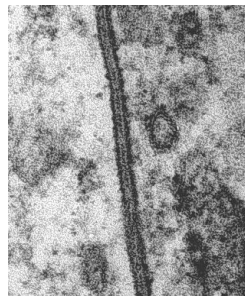
The next phase of ROP (Phase III) is the formation of scar tissue, i.e., fibrosis associated with the up-regulation of TGF- $\beta$ . Scar tissue formation ultimately leads to retinal detachment and loss of vision.<sup>34</sup>

**Vascular Endothelial Growth Factor:** VEGF is the key growth factor driving ocular blood vessel growth. Both Michaelson in 1948<sup>35</sup> and Ashton in 1970<sup>36</sup> had proposed that an angiogenic factor was released from the retina causing retinopathy of prematurity. However, it was not until 1986 that Kretzer, et al. reported an angiogenic factor in the avascular retina in preterm infants and quantified this factor at different time points following birth in infants with and without retinopathy of prematurity.<sup>37-39</sup> The presence of an angiogenic factor was indicated by an increase in the gap junction area between astrocytes of the avascular retina, by an increased surface of rough endoplasmic reticulum in these astrocytes, and by the ability of avascular retina homogenates to induce neo-vascularization in chorio-allantoic membrane substrates.<sup>37-39</sup>

### Astrocyte Gap Junction Surface Area Increased

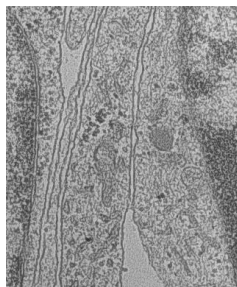


Normal

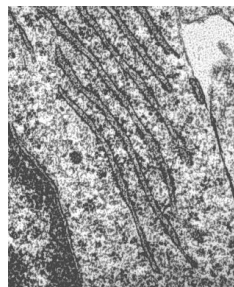


Abnormal

### Astrocyte Rough Endoplasmic Reticulum Increased

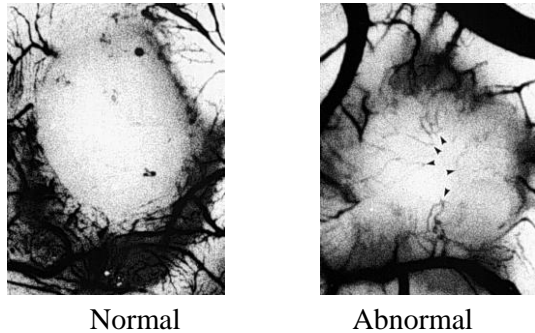


Normal



Abnormal

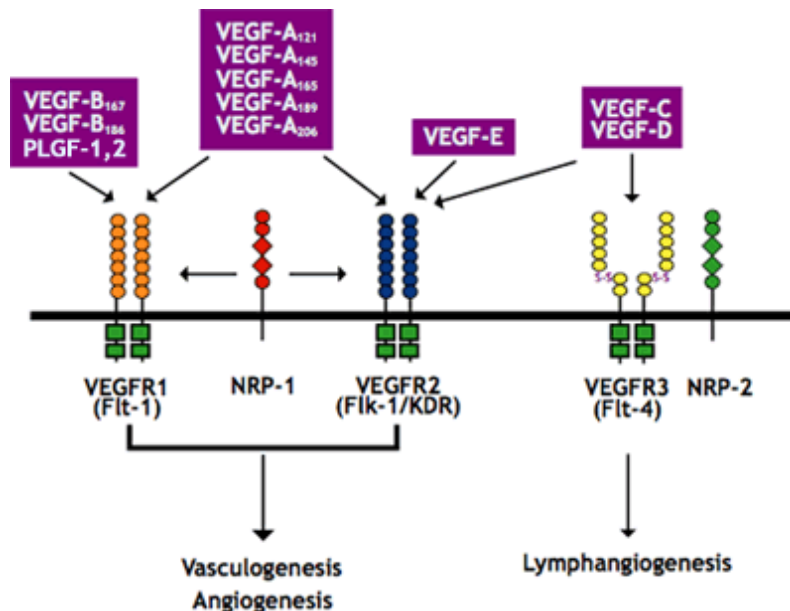
## Angiogenic Factor of Avascular Retinal Extracts Increased



Adapted from Kretzer, et al. (1984<sup>39</sup>, 1986<sup>38</sup>, 1988<sup>37</sup>, personal communication)

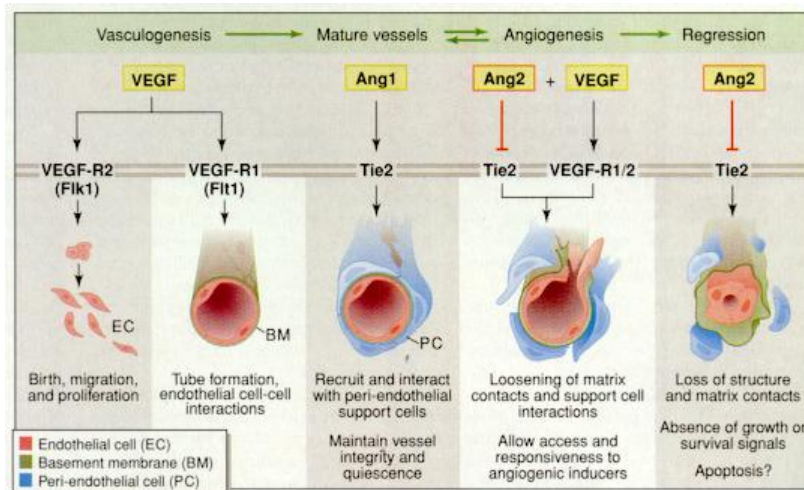
This angiogenic substance was subsequently identified as VEGF, now known to be the substance responsible for the development of ROP.

VEGF exists in several iso-forms: VEGF-A, VEGF-B, VEGF-C, VEGF-D, and VEGF-E.<sup>40</sup> VEGF-A is the form which is predominantly responsible for angiogenesis. It exists in at least four different isoforms: VEGF 121, VEGF 165, VEGF 189, and VEGF 206 that are produced by alternate splicing of the VEGF mRNA. VEGF-A<sub>164(165)</sub> is the pathologic form of VEGF.<sup>41</sup>



Adapted from Hicklin and Ellis (2005)<sup>40</sup>

# Signaling Vascular Morphogenesis and Maintenance



Adapted from Hanahan (1997)<sup>42</sup>

VEGF binds to tyrosine kinase cell surface receptors, VEGF receptor-1 or Flt-1, and VEGF receptor-2 or Flk-1. Neuropilin-1, a neuronal cell receptor that mediates neuronal guidance, has been identified as a co-receptor for VEGF and is critically involved in vascular development. The distribution of Flt-1 and Flk-1 receptors differs markedly during normal retinal development and the receptors have different roles in endothelial cell proliferation and differentiation. In situ hybridization and immunohistochemistry experiments in newborn mice, have shown that Flt-1 protein localizes within retinal vessels, whereas Flk-1 is only detectable in the neural retina. A sixty-fold increase of Flt-1 from postnatal day 3 to day 26, and no significant change in Flk-1 expression were demonstrated. Mice deficient in Flt-1 and Flk-1 died in utero between postnatal days 8.5 and 9.5. Flt-1 knockout mice developed disordered vascularity due to overgrowth of endothelial cells. Mice with targeted disruptions of the Flk-1 gene failed to develop a vasculature and had very few endothelial cells.<sup>42</sup>

VEGF gene expression is regulated by oxygen tension. Expression increases in response to hypoxia and is down regulated by hyperoxia. The mechanism of hypoxia-inducible expression is mediated at least partially via hypoxia inducible factor-1-alpha (HIF-1-alpha), a transcription factor that trans-activates several hypoxia inducible genes.

The increase in VEGF expression and neovascularization seen in retinopathy of prematurity can be inhibited in the mouse model by the use of antisense oligonucleotides, receptor binding chimerical proteins, and monoclonal antibodies (bevacizumab, Avastin<sup>TM</sup>). Clinically, intravitreal bevacizumab (Avastin<sup>TM</sup>) has been used extensively in adult patients with neovascular ocular diseases, such as age-related macular degeneration, neovascularization of proliferative diabetic

retinopathy, macular edema due to diabetes, idiopathic juxtafoveal telangiectasia, retinal vein occlusion, and radiation retinopathy.

Several case series have been reported specifically for the treatment of retinopathy of prematurity, with reduction of the neovascularization after intravitreal injection of bevacizumab (Avastin™) and with no secondary effects related to the treatment.<sup>11-21, 23, 24</sup> No photoreceptor toxicity due to bevacizumab (Avastin™) has been found by electrophysiology studies on adult human retinas.<sup>43</sup> Ultrastructural analyses of the eyes of monkeys given intravitreal bevacizumab (Avastin™) have been reported with no indication of toxicity.<sup>44, 45</sup> An intact retina has been demonstrated by light microscopy and ultrastructural analysis in a preterm infant treated with intravitreal bevacizumab (Avastin™) for Zone I retinopathy of prematurity.<sup>29</sup>

**Bevacizumab (Avastin™) versus Laser for ROP:** Currently, the preferred treatment modality for ROP utilizes ablation of the avascular retina with laser according to the Early Treatment of Retinopathy of Prematurity study [ETROP].<sup>1</sup> Laser has several potential disadvantages as compared to intravitreal Bevacizumab (Avastin™). Some of the differences are indicated bellow.

- 1) Laser surgery is usually performed under general anesthesia, and often thus requires re-intubation of any patient who has been extubated.
- 2) Laser surgery creates inflammation in the eyes that requires several weeks of dilating and anti-inflammatory ophthalmic drops. The pupil often becomes consistently hard to dilate. Only a short course of antibiotic drops will be required after intravitreal Avastin™ injections and the pupil dilates fully on each subsequent examination.
- 3) Laser surgery destroys the peripheral retina which means that:
  - a) The child's visual field will be smaller than normal.
  - b) The child may have less "overlapping field" between the two eyes which may make the child lose binocular vision and develop strabismus.
  - c) After multiple Laser surgeries, the child is likely to develop significant myopia (near-sightedness) due to weakening of the sclera, and severe recurrent ROP disease.
- 4) Laser surgery may cause cataracts (clouding of the lens of the eye), peripheral anterior synechiae (adherence of the iris to the lens of the eye), angle closure glaucoma (increased pressure in the eye, due to block the outflow of fluid from the eye caused by anterior synechiae), hemorrhage in the anterior chamber, vitreous, or retina, or phthisis (ocular death and shrinkage of the eye due to reduced intra-ocular pressure).
- 5) Laser surgery allows the VEGF already in the vitreous to continue to stimulate the growth of blood vessels for at least another week after Laser surgery. This means that the ROP will worsen, before it gets better, when Laser surgery is performed. In contrast, VEGF action is blocked immediately, thus halting ROP progression due to VEGF already in the vitreous, as well as, that being produced in the peripheral retina, after intravitreal bevacizumab (Avastin™) injections. Recurrences generally occur before 54 weeks post menstrual age (or between 50 and 70 weeks post menstrual age). An unfavorable outcome is defined as

recurrence of neovascularization arising from the inner retinal vessels needing re-treatment in either eye (based on RetCam photographs as assessed by three unblinded but independent ROP experts) identified at any time before data lock (after the last child enrolled reached 60 weeks postmenstrual age). (The primary outcome would be considered unknown for infants who did not have neovascularization at or before 50 weeks post menstrual age but were not examined after that age.)

- 6) Additional examinations will be required at 80 weeks post menstrual age (or between 75 and 100 weeks post menstrual age) (approximately age 1 year), and at ages 2, 3, 4, and 5 years to detect progressive macular distortion, dragging, or detachment and myopia and strabismus.

The above differences would make intravitreal Bevacizumab (Avastin™) appealing for the treatment of vulnerable premature babies with ROP, should it prove to offer superior visual outcomes, and no significant systemic severe side effects.

**Information on Bevacizumab (Avastin™) (Genentech, Inc):** Bevacizumab (Avastin™) is a recombinant humanized monoclonal IgG-1 antibody that blocks and inhibits the biologic activity of human vascular endothelial growth factor (VEGF) in vitro and in vivo. It contains human framework regions and the complementary determining regions of a murine antibody that binds to VEGF. It is produced by Chinese-hamster ovarian cells and has a molecular weight of approximately 150 kilo Daltons. Avastin™ is a clear to slightly opalescent, and colorless to pale brown, sterile solution. It is supplied in 100 mg and 400 mg protein-free, single-use vials at a concentration of 25 mg/ml. The 100 mg is formulated in 240 mg alfa, alfa-trehalose dehydrate, 23.2 mg sodium phosphate (monobasic monohydrate), 4.8 mg sodium phosphate (dibasic anhydrous), 1.6 mg polysorbate 20, and water for injection, USP. The 400 mg product is formulated in 960 mg alfa, alfa-trehalose dehydrate, 92.8 mg sodium phosphate (monobasic, monohydrate), (19.2 mg sodium phosphate dibasic anhydrous), 6.4 mg polysorbate 20 and water for injection, USP. The carrier solutions are deemed safe for intraocular use.

**Pharmacokinetics of intravitreal Bevacizumab (Avastin™):** From reported pharmacokinetic studies in animals.<sup>46</sup> we know that systemic absorption of bevacizumab (Avastin™) is small, and the resulting concentrations of the medication to the serum and the contralateral eye are small (1:1,000). This is probably due to the large molecular weight (about 150 kilo Daltons) of bevacizumab (Avastin™). This finding indicates that systemic absorption of bevacizumab (Avastin™) in preterm babies will be low, as compared to other currently available, lower molecular weight (about 50 kilo Daltons) anti-VEGF drugs [such as ranibizumab (Lucentis™) or pegaptanib (Macugen™)]. Further, the half-life of bevacizumab is approximately three weeks (compared to approximately one day for other currently available anti-VEGF drugs). This would allow completion of retinal vascularization In most cases of retinopathy of prematurity.

**Approved Uses:** The FDA approved bevacizumab (Avastin™) in February 2004 for use as a treatment for patients with metastatic cancer of the colon or rectum.<sup>47</sup> In October 2006, the FDA approved bevacizumab (Avastin™) as a treatment for patients with metastatic cancer of the lung.<sup>48</sup> Off-label use of drugs by physicians is widespread. It is legal but individual physicians take responsibility for their use. It is estimated that half of all the drugs used in pediatric setting are off label.

**Consensus Opinion:** A survey by the American Society of Retinal Specialists and its members was completed in March 2006. It found that 98% of the respondents felt intravitreal bevacizumab (Avastin™) was superior to other approved therapies for age related macular degeneration. Further, 96% thought that Avastin™ was the same or better in terms of overall safety compared to other FDA approved therapies. The American Academy of Ophthalmology wrote to Medicare and Medicaid services supporting the efforts for reimbursement for intravitreal injections of Avastin™ in patients with age-related macular degeneration (AMD) that failed photodynamic therapy (PDT) with verteporfin or intravitreal pegaptanib. The Academy has supported the efforts for reimbursement to patients who are deemed by their treating physician to have failed FDA approved therapies or, in the judgment of the physician, based on his/her experience, are likely to have a greater benefit from the use of intravitreal bevacizumab (Avastin™). Bevacizumab (Avastin™) has been successfully used off-label around the world in sporadic babies with severe retinopathy of prematurity that failed Laser treatment.

**Complications of Intravenous Injections of Bevacizumab (Avastin™) in Oncology Patients:** Some patients with metastatic colorectal cancer experience serious complications when Bevacizumab (Avastin™) is given. These complications include gastrointestinal perforations, wound healing complications, hemorrhage, arterial thrombo-embolic events such as stroke or myocardial infarction, hypertension, proteinuria, and congestive heart failure. Patients who experienced these complications not only had metastatic colon cancer, but the dosage utilized was 400 times greater in addition to an increased dosing frequency and an intravenous administration. These patients were also receiving other cancer drugs with inherent complications. The infants in this proposed study would be exposed to smaller doses of intravitreal medication only.

**Complications of Intravitreal Injections of Bevacizumab (Avastin™) in Patients with Eye Disease:** Patients receiving bevacizumab (Avastin™) for eye conditions are, in general, healthier than cancer patients and receive a significantly smaller dose and only into the vitreous cavity of the eye. While there are no FDA-approved studies about the use of bevacizumab (Avastin™) in the eye that prove it is safe and effective, a recent international survey regarding the safety of bevacizumab (Avastin™) has been published.<sup>49</sup> It indicated very low rates of complications after more than 7000 injections of the medication. Lucentis™, a drug with the same active region, but of smaller molecular weight, was approved for intravitreal treatment of wet age related macular degeneration (AMD) in 2006 by the FDA.

**Known Risks of Intravitreal Eye Injections:** Possible complications and side effects of any intravitreal injection include, but are not limited to: endophthalmitis and ocular trauma (cataract formation, lens dislocation with iridodonesis, hemorrhage in the anterior chamber, vitreous, or retina, retinal tears, and retinal detachment).<sup>50</sup> Patients receiving an injection of Avastin™ may experience less severe side effects associated with the pre-injection preparation procedures associated with all ophthalmic examinations (eyelid speculum, anesthetic drops, dilating drops, antibiotic drops, and providone-iodine drops). These side effects may include eye pain, subconjunctival hemorrhage, corneal edema, and corneal epithelial defect.

**Methods-Study Inclusion Criteria:** Entry into this clinical protocol will be offered to the parents or legal guardians of eligible neonates and informed consent will be sought. All premature infants with the following characteristics will be screened for ROP as per established NICU protocols:<sup>51, 52</sup>

- 1) Birth weight  $\leq$ 1500 grams;
- 2) Gestational age  $\leq$ 30 weeks; or
- 3) Infants deemed at risk for ROP due to an unstable general medical course, use of supplemental oxygen, or other medical stressors as determined by the attending neonatologist.

Neonates eligible for enrollment will be diagnosed by indirect ophthalmoscopy and documented with the RetCam photographic system to have:

- 1) ROP for which treatment is advisable according the Early Treatment of Retinopathy of Prematurity study (ETROP) (i.e. any stage ROP in Zone I or Stage 2 or 3 ROP in small Zone II).
- 2) Aggressive Posterior ROP (APROP) (formerly known as RUSH disease) or ROP more advanced than ETROP.

**Methods-Study Exclusion Criteria:** Entry into this clinical protocol will not be offered to the parents or legal guardians of neonates who have:

- 1) Unilateral or Bilateral ROP Stage 1.
- 2) Unilateral or Bilateral ROP Stage 4 or 5.
- 3) Extreme discrepancy of the ROP between the 2 eyes [more than 1 Stage difference in ROP between the two eyes].

**Control Selection:** Because there is an established treatment for ROP (Laser followed by lens-sparing Vitrectomy when Laser fails), and because of the blinding potential of untreated severe posterior ROP, the use of placebo controls would be unethical and will not be used in this study.

Laser causes an intense vitreo-retinal inflammation, whereas Avastin™ does not. Therefore, randomization of one eye to Avastin™ injections and of the second eye to Laser treatment would predictably lead to poor functional outcomes, due to severe amblyopia in the Laser-treated eye. Additionally, VEGF of ocular origin, as regulated by different treatment modalities, circulates in the systemic circulation, and affects the degree of ROP disease or remission in the contralateral eye. Therefore, for the purposes of the study, both eyes of each patient will receive the

same treatment, and each enrolled patient will be randomized to one of the study groups.

Due to the constantly changing variables in the NICU, including the resuscitation and survival of smaller and sicker premature babies, a potential comparison of Avastin™-treated subjects to historic Laser-treated controls would be systematically biased. To avoid such bias in our study, we elected to use concurrent randomized controls.

**Methods-Study Protocol:** The study subjects will be randomized to either the treatment group, which will receive intravitreal injections of bevacizumab (Avastin™) may receive conscious sedation, or the control group, which will undergo Laser ablation of their avascular retina under conscious sedation or general anesthesia. The enrolled patients will be randomized equally to the two arms of the study, by a computer generated system.

An unfavorable outcome is defined as recurrence of neovascularization arising from the inner retinal vessels needing re-treatment in either eyes (based on RetCam photographs as assessed by three unblinded but independent ROP experts) identified at any time before data lock (after the last child enrolled reached 60 weeks post menstrual age). (The primary outcome would be considered unknown for infants who did not have neovascularization at or before 50 weeks post menstrual age but were not examined after that age.)

Patients who have been randomized to either the Avastin group or the Laser group will not be allowed to switch groups so that visual and anatomic outcomes will not reflect a treatment bias on the part of the investigators. All recurrences will be confirmed by the BEAT-ROP Reading Center consisting of three ROP experts not participating in the clinical trial.

**Stratification:** In preterm babies with ROP, the best predictor of treatment success and of future functional and anatomic outcomes is the ROP Zone, rather than birth weight, gestational age, or other risk factors. ROP in Zone I (two times the distance between the center of the optic disc and the center of the macula on the temporal side) or ROP in Posterior Zone II (three times the distance between the center of the optic disc and the center of the macula on the temporal side) have the highest rates of treatment failure and poor structural and functional outcomes. The enrolled patients will be stratified according to the ROP Zone. The first stratum will include patients with ROP in Zone I, and the second stratum will include patients with ROP in Posterior Zone II.

The age of the patient precludes the ability to obtain reasonable short-term visual acuity (VA) and peripheral visual field (VF) data. This necessitates the use of electrophysiologic and anatomic criteria to determine if there has been an adequate response to treatment. The appearance of the retina on fundoscopic examination will be recorded repeatedly by the ophthalmologist before and after bevacizumab (Avastin™) or Laser treatment. Fundus photography with a RetCam™ photographic system (Clarity Medical Systems, Inc.) will be documented before treatment, and categorized by the Revisited International Classification of Retinopathy of Prematurity.<sup>53, 54</sup> An independent ophthalmologist, along with the

treating physician, will review the RetCam fundus photographs (.jpg files transferred electronically) to confirm the need for therapy for ROP and to determine the need for re-treatment. Utilizing the same .jpg files transferred electronically, The BEAT-ROP Reading Center will confirm (unblinded) the need for re-treatment and will evaluate (blinded) the outcome at 54 weeks post menstrual age (or between 50 and 70 weeks post menstrual age) of the posterior segment with out laser marks visible. The cropped photograph would of course be assessed prior to the full photograph. The BEAT-ROP Reading Center will also evaluate (blinded) the posterior segment without laser marks at later time points (80 weeks post menstrual age (or 75 to 100 weeks post menstrual age), and 2, 3, 4, and 5 years) to grade increasing macular dragging, myopia, etc.

**Methods-Screening of Patients:** All premature infants with the following characteristics will be screened for ROP as per established NICU protocols:<sup>51</sup>

- 4) Birth weight  $\leq$ 1500 grams;
- 5) Gestational age  $\leq$ 30 weeks; or
- 6) Infants deemed at risk for ROP due to an unstable general medical course, use of supplemental oxygen, or other medical stressors as determined by the attending neonatologist.

The screening examinations will begin according to published consensus screening guidelines for ROP. The guidelines mandate the first ophthalmologic evaluation for ROP to be done at a corrected gestational age of  $\geq$ 31 weeks and a chronologic age of no less than four weeks of life.<sup>51</sup> Screening will be recorded according to the Revisited International Classification for ROP.

**TABLE 1** Timing of First Eye Examination Based on Gestational Age at Birth

Gestational Age at Birth, wk	Age at Initial Examination, wk	
	Postmenstrual	Chronologic
22 <sup>a</sup>	31	9
23 <sup>a</sup>	31	8
24	31	7
25	31	6
26	31	5
27	31	4
28	32	4
29	33	4
30	34	4
31 <sup>b</sup>	35	4
32 <sup>b</sup>	36	4

Shown is a schedule for detecting prethreshold ROP with 99% confidence, usually well before any required treatment.

<sup>a</sup> This guideline should be considered tentative rather than evidence-based for infants with a gestational age of 22 to 23 weeks because of the small number of survivors in these gestational-age categories.

<sup>b</sup> If necessary.

**Methods – Informed Consent:** Entry into this clinical protocol will be offered to the Mother or legal guardian of eligible neonates and informed consent will be sought. The informed consent will be explained to the parents by one of the study

investigators. Signatures will be required on the UTHSC-Houston Informed Consents (Section 16) and on the UTHSC-Houston HIPAA forms (Section 17). Both will be provided to Children's Memorial Hermann Hospital and Memorial Hermann Southwest Hospital in both English and Spanish.

**Methods - Bevacizumab (Avastin™) Injections:** The following protocol will be followed for all intra-vitreous Bevacizumab injections:

- 1) The patient will be attached to a continuous cardio-respiratory monitor and continuous pulse oximeter, with specialized NRP-certified personnel in attendance at all times.
- 2) The infant will be premedicated with an oral, intramuscular, or intravenous sedative drug of choice, as determined by the attending neonatologist.
- 3) A drop of tetracaine hydrochloride 0.5% or proparacaine hydrochloride 0.5% ophthalmic solution will be placed into the conjunctival sac between the lids of the infant for local analgesia.
- 4) A sterile speculum for use in preterm infants will be placed between the lids.
- 5) Antisepsis will be achieved with a drop of povidone iodine (Betadine™) 5% ophthalmic solution placed between the lids of the infant, into the conjunctival sac. Excessive Betadine will be removed with a sterile cotton tip applicator from the temporal lid margin.
- 6) The infant's eye will be stabilized with a sterile toothed forceps, cotton tip applicator, or scleral depressor.
- 7) A unit dose Bevacizumab (Avastin™) of 0.025 ml (0.625 mg) will be injected behind the lens into the vitreous, in a sterile manner. The medication will be supplied by the compounding pharmacy in single-use syringes with a 5/16<sup>th</sup> inch, 31 gauge needles attached.
- 8) Povidone iodine (Betadine™) antisepsis will again be applied to the eye.
- 9) The sterile speculum will be removed from between the lids.
- 10) The same procedure will be followed, by repeating steps 3 to 9 for the contralateral eye injection.
- 11) An ophthalmic antibiotic drop [gatifloxacin (0.35%) (Zymar™) or moxifloxacin (0.5%) (Vigamox™) ophthalmic solution] will be prescribed for both eyes, and will be used every 6 hours for 7 days post-operatively.
- 12) Re-injection for recurrent disease in the bevacizumab (Avastin™) group will depend on the ophthalmologist's findings on fundoscopic examination and on retinal photographs by the RetCam photographic system. It will be performed at 6 to 10 weeks intervals as needed, up to 2 additional times.

**Methods -- Acquisition and Payment for the Bevacizumab (Avastin™) and Electro-retinograms – Funding for the Study:**

Bevacizumab (Avastin™) will be obtained from the Greenpark Pharmacy via Genentec Pharmaceuticals. The medication will be supplied by the compounding pharmacy in sterile unit doses of 0.025ml (0.625mg), in single-use syringes, with a 5/16<sup>th</sup> inch, 31 gauge needle attached. Any participating hospital may inspect the product or decide to compound equivalent unit doses of the medication in their

hospital pharmacy. A list of lot numbers must be kept by each participating hospital pharmacy.

The study medication will be free of charge for the patients.

The cost for the study medication will be covered by the Alfred W. Lasher, III, Professorship in Ophthalmology Research Funds of the University of Texas-Houston Medical School.

**Methods – Monitoring for Side Effects:** We will observe for systemic complications from Avastin, such as hypertension [requiring treatment with anti-hypertensive medications], GI bleeding or intestinal perforation [requiring surgery], renal dysfunction [causing proteinuria] or periventricular leukomalacia development [diagnosed by brain MRI or ultrasonography]. We will also observe for local complications from Avastin injections, including hemorrhage, endophthalmitis, retinal detachment, or cataracts. Laser treatment complications, such as intraocular inflammation, cataracts, hemorrhage, and increased or decreased intraocular pressure will be recorded.

To document any complication of the bevacizumab (Avastin™) injection, an indirect ophthalmoscopic examination will be done by the ophthalmologist immediately after and 24 to 48 hours after the procedure. The patients will undergo weekly fundoscopic examinations for the first four weeks. They will then have examinations of the retinas every two weeks for the following eight weeks, and monthly thereafter, until inner retinal vascularization is complete. Some variation may occur based on the experience of the ophthalmologist and the availability of the patient.

Retinal photographs with the RetCam photographic system will be performed before, one week, and one month after each enrollment procedure [bevacizumab (Avastin™) injection or laser treatment], and at 54 weeks (or between 50 and 70 weeks post menstrual age) (approximately age 6 months) and 80 weeks (or between 75 and 100 weeks post menstrual age) (approximately age 12 months) post menstrual age. Some variation may occur based on the experience of the ophthalmologist and the availability of the patient. If recurrence (the primary end point) occurs, retinal photographs will be performed before, one week, and one month after each recurrence procedure [bevacizumab (Avastin™) injection or laser treatment].

All adverse and unexpected events due to or temporally associated with the intravitreal administration of bevacizumab (Avastin™) or with Laser treatment will be monitored and reported to CPHS, the FDA, and the Data and Safety Monitoring Committee. (See UTHSC-Houston Adverse Event Form-Section 18.)

**Methods – Discontinuation of the Study:** Intravitreal bevacizumab (Avastin™) therapy may be stopped for suspected allergic reactions, for systemic adverse effects, such as hypertension, proteinuria, thrombotic events, and gastric perforation, or for local reactions, such as endophthalmitis. The patients may also drop out of the study after parental withdrawal of consent.

**Methods - Data Safety Monitoring Board:** A Data and Safety Monitoring Board will oversee the study. Analyses of the results will be performed when 50% (75

patients) and 100% (150 patients) of patients have reached 60 weeks post menstrual age (the time when the primary hypothesis will be determined). Based on the occurrence of unexpected adverse events the DSMB may recommend discontinuing enrollment based on the first set of analyses. Enrollment would not be discontinued based on evidence of short term benefit because the sample size is relatively small, later outcomes will be clinically more important, and the full sample size is needed to help assess the safety as well as the effectiveness of Avastin therapy.

## **5. Overview:**

### **5.1 Primary Hypothesis**

Inhibition of the intraocular action of VEGF with intravitreal bevacizumab (Avastin™) will induce regression of ROP with fewer treatment failures than with laser treatment. Intravitreal bevacizumab (Avastin™) injections are indicated only for vision threatening ROP (i.e., meeting inclusion criteria) or for recurrence of neovascularization (i.e., return of plus disease and formation of extraretinal fibrovascular proliferation). A greater number of treatment failures are anticipated in patients with Zone I ROP than in patients with Posterior Zone II ROP.

### **5.2 Secondary Hypotheses**

Intravitreal bevacizumab (Avastin™) should allow normal inner retinal vascularization to proceed toward the peripheral retina. Current Laser therapy destroys the peripheral retina and normal inner retinal vascularization never occurs. Less severe myopia and fewer cases of strabismus may occur in infants treated with intravitreal bevacizumab (Avastin™) than in infants treated by conventional Laser surgery. These clinical outcomes will be assessed at 54 (or between 50 and 70 weeks post menstrual age) and 80 weeks post menstrual age (or between 75 and 100 weeks post menstrual age) and at ages 2, 3, 4, and 5 years.

These differences may be greater in patients with Zone I ROP than in patients with Posterior Zone II ROP.

### **5.3 Experimental Design**

This study will enroll only patients with severe ROP in Zone I or Posterior Zone II. These patients will be referred from the general population of neonates who undergo routine screening for ROP as required by the AAO and the AAP. These patients have the worst outcome when current Laser treatments and vitrectomy are performed. They also suffer more complications of Laser therapy because these eyes are generally smaller and more vulnerable to the energy generated by the Laser. The two groups will not be combined at any point in their treatment regimen.

### **5.4 Data Collection**

Important baseline data will be collected on an Electronic Case Report Form that has been designed by Health Informatics (CRU) and the data set will be locked. (See Electronic Case Report Form-Section 19.) All data will be backed up appropriately. This data will include basic information on the infant, known ROP risk factors, type and frequency of treatment, recurrences, and type and frequency of re-treatment. The BEAT-ROP Reading Center consisting of three ROP experts not participating in the clinical trial will confirm all recurrences (necessarily not blinded) and evaluate (blinded) posterior segment images including the optic disc and macula (without laser marks). These ultimate structural outcomes will utilize the RetCam images that will be an integral part of the study. Functional outcomes will be determined by evaluation of infant fixation, and, ultimately, by determination of visual acuity by independent ophthalmologists.

### **5.5 Independent BEAT-ROP Reading Center**

Three ROP experts not involved in the clinical trial will confirm all recurrences (unblinded) after first evaluating (blinded) the cropped 54 week post menstrual age (or between 50 and 70 weeks post menstrual age) RetCam photographs showing the optic nerve and macula

## **6. Subjects:**

### **6.1 Inclusion criteria**

Infants who have been screened by the AAO/AAP guidelines ( $\leq 1500$  grams at birth and  $\leq 30$  weeks gestation)<sup>51, 52</sup> who develop severe ROP:

1. ROP for which treatment is advisable according to the results of the Early Treatment for Retinopathy of Prematurity randomized trial (ETROP)<sup>1</sup> will be randomized equally between bevacizumab (Avastin<sup>TM</sup>) treatment and Laser treatment.
2. Aggressive Posterior ROP (APROP) (formerly known as RUSH disease)<sup>53</sup> or ROP more advanced than ETROP would be randomized equally between bevacizumab (Avastin<sup>TM</sup>) treatment and Laser treatment.
3. Informed Consent from a parent or guardian.

### **6.2 Exclusion criteria**

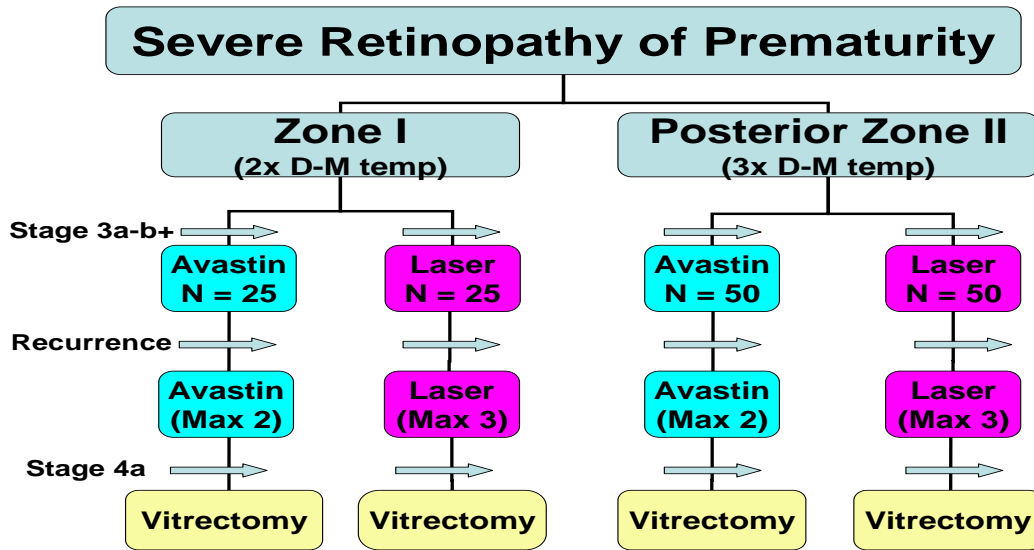
1. Infants who have a congenital systemic anomaly or have a congenital ocular abnormality.
2. Infants who cannot be treated by conventional Laser therapy because of problems with media clarity. Generally, blind external cryotherapy would be utilized as an initial therapy and the infant would be excluded from the study even if the media clear subsequently.

3. Informed Consent from a parent or guardian refused. This will mean that an infant automatically will receive Laser therapy, i.e., bevacizumab (Avastin™) treatment cannot be given outside of the protocol. No data will be used from an infant without Informed Consent including a separate HIPAA form for all infants enrolled at Memorial Hermann Hospitals.

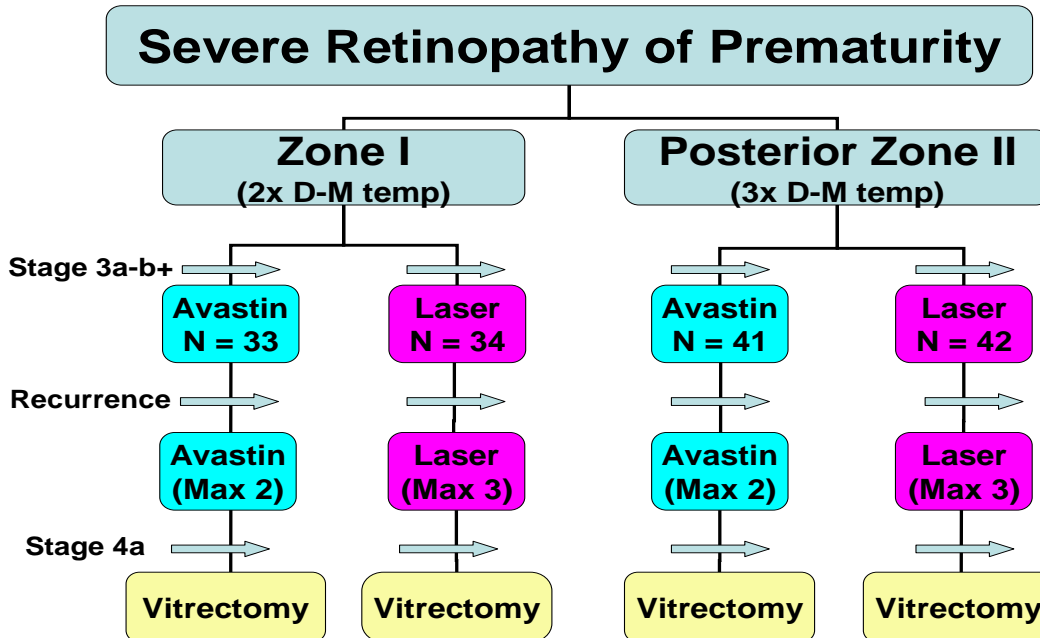
## 7. Treatment Groups:

### a. Randomization:

#### DESIGN:

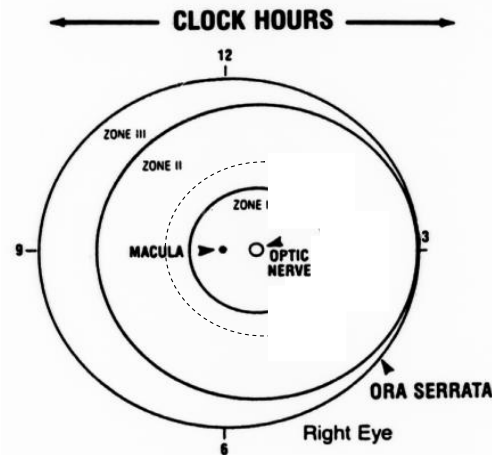


#### ACTUAL:



# Randomization by Zone (Revisited 05/2005) with Stratification

## Zone I and Posterior Zone II: Measured Temporally



### **b. Treatment (Avastin) Groups:**

There will be two treatment groups receiving bevacizumab (Avastin™) injections:

1. Zone I ROP which is twice the distance from the optic disc to the macula measured temporally.
2. Posterior Zone II ROP which is three times the distance from the optic disc to the macula measured temporally.

### **c. Control (Current Standard Surgery) Groups:**

There will be two control groups receiving Laser treatments and possibly lens-sparing vitrectomy:

1. Zone I ROP which is twice the distance from the optic disc to the macula measured temporally.
2. Posterior Zone II ROP which is three times the distance from the optic disc to the macula measured temporally.

## **8. Study Endpoints:**

**A. Primary Endpoint:**

An unfavorable outcome is defined as recurrence of neovascularization arising from the inner retinal vessels needing re-treatment in either eye (based on RetCam photographs as assessed by three unblinded but independent ROP experts) identified at any time before data lock (after the last child enrolled reached 60 weeks post menstrual age). (The primary outcome would be considered unknown for infants who did not have neovascularization at or before 50 weeks post menstrual age but were not examined after that age.)

**B. Secondary Endpoints:**

Ultimate end points will include: cropped RetCam photographs at 54 weeks post menstrual age (or between 50 and 70 weeks post menstrual age), structural outcomes which are associated with unfavorable functional outcomes including dragging, distortion, or detachment of the macula; the amount of retina on RetCam photographs at 54 weeks post menstrual age (or between 50 and 70 weeks post menstrual age) measured through the horizontal extent of the retina (through the optic disc and macula); the amount of myopia (nearsightedness) judged by refraction at 54 weeks post menstrual age (or between 50 weeks post menstrual age and 5 years of age); and presence of strabismus judged by measurements at 54 weeks post menstrual age (or between 50 weeks post menstrual age and 5 years of age). The determination of visual acuity, refraction, motility examination, and other parameters that might be compromised by severe ROP or its treatment will continue to be assessed at 80 weeks post menstrual age and at ages 2, 3, 4, and 5 years.

**9. Drug Accountability:**

A letter from Greenpark Pharmacy is Appendix 1 (See Section 20). This letter describes in detail the preparation of the sterile syringes that contain exactly 0.025 cc (0.625 mg) of the treatment drug. One syringe will be used on each eye when treatments are given. A central randomization list will be maintained in the Department of Pediatrics of The University of Texas-Houston Medical School. Bevacizumab (Avastin™) will be obtained from Greenpark Pharmacy. The Dispensing Log is Appendix 2 (See Section 21).

**10. Sample Size Estimate:**

**Methods - End of Enrollment:** We pre-determined that the study will end when we enroll **150** patients and at least 50 of them are in Stratum 1. The study may also terminate if the interim analyses so dictate.

**Methods – Data Management:** Data collection and the protocol management will be performed by the study investigators. An Electronic Case Report Form designed by CRU will be utilized for data locking and all data will be backed up.

## 2. Sample Size Estimate:

**Methods - Sample size calculations:** All sample size calculations were performed in PASS. Sample size was calculated to permit analysis of the differences of ROP treatment failure in infants treated with either Avastin or Laser. For the calculations, we assumed different treatment effect size in the different strata, as dictated by the clinical data available to us at the time of study design. Infants who die will be considered in the analysis as their appearance at last examination (failure or success) or they will be dropped from the analysis.

Preliminary calculations were performed for each stratum because the proportions that would be enrolled in each stratum were unknown.

-Stratum 1: Using calculations in PASS, and assuming an Avastin success rate of 95%, a Laser success rate of 50%, an allocation ratio of 1:1 between the study groups, and a 10% rate of death or loss to follow-up, we determined that a sample size of **19** patients per group to be necessary to detect the effect difference with an  $\alpha=0.05$ ,  $\beta=0.20$ , and a power=0.80.

-Stratum 2: Using calculations in PASS, and assuming an Avastin success rate of 99%, a Laser success rate of 80%, an allocation ratio of 1:1 between the study groups, and a 10% rate of death or loss to follow-up, we determined that a sample size of **49** patients per group to be necessary to detect the effect difference with an  $\alpha=0.05$ ,  $\beta=0.20$ , and a power=0.80.

The primary analysis will be done on the entire population; the primary analysis will be stratified for enrollment stratum only.

**Methods - End of Enrollment:** We pre-determined that the study will end when we enroll **150** patients and at least **50** of them are in Stratum 1. The study may also terminate if the interim analyses so dictate.

The primary analysis will be powered as follows with variations in projected outcomes and enrollment in each stratum (assuming 10% rate of death or loss in each group):

Stratum 1		Stratum 2		Total		Power ( $\alpha=0.05$ )
n	% success	n	% success	n	% success	
50	95% vs 50%	100	99% vs 80%	150	98% vs 70%	0.99
75	95% vs 50%	75	99% vs 80%	150	97% vs 65%	0.99
50	90% vs 60%	100	99% vs 95%	150	96% vs 83%	0.62

75	90% vs 60%	75	99% vs 95%	150	94% vs 78%	0.78
50	90% vs 60%	100	99% vs 90%	150	96% vs 80%	0.78
75	90% vs 60%	75	99% vs 90%	150	94% vs 75%	0.87

**Methods – Data Management:** Data collection and the protocol management will be performed by the study investigators. An Electronic Case Report Form designed by CRU will be utilized for data locking and all data will be backed up.

## **11. Monitoring Board:**

After 75 infants (one half of the study group) have reached 54 weeks post menstrual age (or between 50 and 70 weeks post menstrual age), the Data Safety Monitoring Board (DSMB) will review submitted data. Discharge data will be reviewed as it becomes available. If at any point, the DSMB finds it necessary to suspend enrollment or halt the trial, they will prepare a statement and provide it to the principal investigator for immediate distribution.

## **12. Statistical Analysis and Interpretation:**

**Statistical Analysis:** Descriptive statistics will be used to evaluate whether the Avastin and laser treatment groups are comparable at baseline with respect to demographic and clinical variables. Analyses evaluating the study hypotheses will be by intention to treat, i.e., treatment groups will be analyzed as randomized. The infant rather than the eye was defined as the unit of analysis. Newborns will be enrolled at 18 different centers. Only a few patients are expected to be contributed by each center. Clustered sandwich estimators of parameter standard errors will be calculated to address center related correlations in the data.

The primary outcome, ROP recurrence (as judged by 3 independent, expert ophthalmologists using RetCam photographs of the retina) needing re-treatment, is a three valued ordinal variable (success in both eyes, unilateral recurrence needing re-treatment, and bilateral recurrences needing re-treatment). The primary hypothesis that Avastin treatment is more effective than laser treatment in preventing the recurrence of ROP will be evaluated by an ordered logistic regression model. The unadjusted analysis will include treatment and the stratifying variable, retinal Zone, in the regression model. The treatment X Zone interaction will also be included to evaluate whether the effects of treatment on recurrence differed in newborns with Zones 1 and 2 ROP. Diagnostics will be conducted to assure that model assumptions (including proportional odds) are met and outliers are not unduly affecting the results. Adjusted analyses will also be performed to assure that imbalances due to randomization in risk factors related to ROP recurrence do not explain the observed treatment effects. Potential confounders at the time of enrollment (birth weight, gestational age, ethnicity, and Grade III/IV intracranial hemorrhage, necrotizing enterocolitis, treatment with oxygen at randomization, and treatment with mechanical ventilation at randomization) will be added to the ordered logistic regression with

treatment, Zone, and the treatment X Zone interaction. Sensitivity analyses will be conducted to evaluate the effects of missing outcomes (no recurrence by last evaluation for newborns lost to follow-up prior to 50 weeks) and timing of recurrence. If unilateral recurrences are few in number, this study will not be adequately powered to evaluate whether treatment effects are similar for unilateral and bilateral recurrences. In that case, the ordinal primary outcome will be collapsed to a binary outcome. Unilateral recurrences will be combined with bilateral recurrences as bad outcomes and contrasted with the good outcome of no recurrence in either eye. In addition, unilateral recurrences will be combined with no recurrence in either eye. Both of these re-codings of the primary outcome have merit clinically. We will calculate separate binary logistic regressions to model these two different interpretations.

Although there are compelling clinical reasons that the infant is the unit of analysis, there are also reasons (including precedence in the ophthalmology literature) that eye should be considered the unit of analysis. Therefore, we will conduct secondary analyses considering each eye nested within newborn as the unit of analysis. The primary outcome, ROP recurrence will be assessed in each eye of the study newborns. The primary hypothesis that Avastin treatment is more effective than laser treatment in preventing the recurrence of ROP will be evaluated and a 2 level binary logistic regression mixed model to account for the correlation in outcomes of the two eyes from each study newborn. The unadjusted analysis will include treatment, the stratifying variable, retinal Zone, and eye (best eye, worst eye) in the regression model. The treatment X Zone, treatment X eye, and treatment X Zone X eye interactions will also be included to evaluate whether the effects of treatment on recurrence differed in newborns with Zones 1 and 2 ROP and as a function of unilateral and bilateral recurrence. The same potential confounders adjusted for in the ordered logistic regression addressing the primary hypothesis will be added to the mixed model with treatment, Zone, eye and the treatment X Zone, treatment X eye, and treatment X Zone X eye interactions.

Based partly on the findings and recommendations by Wijeyesundera, et al.<sup>55</sup> we will perform Bayesian as well as frequent analyses and assess the posterior probability that Avastin and also laser prevents ROP recurrence in preterm newborns. We will adopt skeptical, neutral, and optimistic priors to bracket the range of opinions currently held by clinicians.

An analytic approach similar to the approach adopted to evaluate the primary study hypothesis will evaluate the secondary study hypotheses. The regression models (binary, multinomial, or ordered logistic and linear regressions) will depend on the scaling of the secondary outcomes. As follow-up continues mixed models will be used to account for repeated measurements over time.

**Interpretation: The trial is powered to assess a large treatment effect of Avastin on the primary outcome. If a statistically significant benefit is not found, the data would not necessarily exclude a lesser but still clinically important effect. Providing the direction of the difference in the primary outcome and subsequent visual outcomes do not favor the Laser group, consideration may be given to recommending Avastin if analyses of adverse events indicate that Avastin administration is better tolerated than Laser therapy.**

### **13. Trial Organization:**

Helen A, Mintz-Hittner, M.D. is the principal investigator and will decide authorship on any publications. She is responsible for: finalizing the clinical protocol following the investigators meeting; finalizing the case report form, helping recruit patients for participation in this clinical trial; and completing a manuscript for publication within two (2) months of locking the dataset.

### **14. Adverse Event Reporting:**

Protocol violations and adverse events will all be reported to the institution's IRB, to the FDA, and to the data and safety monitoring board.

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**15. Consent Forms:**

(See PDFs in iRIS)

Children's Memorial Hermann-English  
Children's Memorial Hermann-Spanish  
Memorial Hermann Southwest-English  
Memorial Hermann Southwest-Spanish  
Avastin Pharmacokinetic Study-English

**16. UTHSC-Houston HIPAA form:**

(See PDFs in iRIS) (English) (Spanish)

**17. UTHSC-Houston Adverse Event Form:**

(See PDF in iRIS)

**18. Electronic Case Report Form:**

(See Attached PDF: NEW FORM)

**19. Appendix 1: Preparation of Bevacizumab (Avastin™):**

May 30, 2007

Dr. Helen Hittner  
6410 Fannin # 920  
Houston, TX 77030

Dr. Hittner:

We purchase Avastin from Genentech, the manufacture. We transfer the sterile solution into unit dose syringes for many ophthalmologist throughout Texas and the United States.

We process our sterile products in an aseptic ISO 6 controlled clean room environment with the critical site manipulations being done inside an ISO 5 certified vertical laminar flow hood and follow USP <797> guidelines. Our process is outlined below:

1. Prepare the Avastin bottle for our clean room sterile area by spraying and wiping the bottle with 70% Isopropyl Alcohol.
2. In the ISO 5 environment, aseptically remove the top from the Avastin with decrimper pliers.
3. Aseptically transfer the proper amount of Avastin into a BD 0.5ml syringe/30 gauge needle. Recap syringe with the same syringe cap.
4. Syringes are removed from the clean room environment for proper labeling and distribution to physician office, clinic or hospital.

The prescription is now labeled with the generic name Bevacizumab instead of Avastin because the solution is no longer packaged by Genentech. The Bevacizumab syringe should be refrigerated and the solution is stable in plastic syringes for 3 months.

Please let me know if you need more information,



Ken Hughes, RPh

**Greenpark Compounding Pharmacy**  
4061-F Bellaire Blvd, Houston, TX 77025  
713.432.9855 Ken@greenparkrx.com

**20. Appendix 2: Dispensing Log:**

This list will be kept in the Children's Memorial Hermann Hospital Pharmacy:

## 21. Participating Sites (15):

### United States, California

Huntington Memorial Hospital  
Pasadena, California, United States, 91109

Active,  
Not recruiting

### United States, Colorado

Presbyterian-St. Luke's Hospital  
Denver, Colorado, United States, 80218

Active,  
Not recruiting

### United States, Illinois

OSF St. Francis Medical Center-Children's Hospital of Illinois  
Peoria, Illinois, United States, 61637

Active,  
Not recruiting

### United States, South Carolina

Palmetto Health Richland Hospital  
Columbia, South Carolina, United States, 29203

Active,  
Not recruiting

Palmetto Health Baptist Hospital  
Columbia, South Carolina, United States, 29223

Active,  
Not recruiting

### United States, Texas

Driscoll Children's Hospital  
Corpus Christi, Texas, United States, 78411

Active,  
Not recruiting

Baylor University Medical Center  
Dallas, Texas, United States, 75346

Active,  
Not recruiting

R.E. Thomason Hospital  
El Paso, Texas, United States, 79905

Active,  
Not recruiting

Del Sol Medical Center  
El Paso, Texas, United States, 79925

Active,  
Not recruiting

Las Palmas Medical Center  
El Paso, Texas, United States, 79902

Active,  
Not recruiting

Cook Children's Medical Center

Active,  
Not recruiting

Fort Worth, Texas, United States, 76104

Memorial Hermann Southwest Hospital

Houston, Texas, United States, 77074

**Active,  
Not recruiting**

Children's Memorial Hermann Hospital

Houston, Texas, United States, 77030

**Active,  
Not recruiting**

St. Joseph Medical Center

Houston, Texas, United States, 77002

**Active,  
Not recruiting**

Clear Lake Regional Medical Center

Webster, Texas, United States, 77598

**Active,  
Not recruiting**