Antivascular endothelial growth factor for retinopathy of prematurity

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Purpose of review

This review will discuss a potentially more effective treatment for retinopathy of prematurity (ROP) with fewer acute and long-term complications. Avastin (bevacizumab) therapy is a promising anti-vascular endothelial growth factor (anti-VEGF) administered directly into the vitreous.

Recent findings

Recent reports detail the use of Avastin alone, and in combination with light amplification by stimulated emission of radiation (LASER) therapy and vitrectomy, for ROP stages 3, 4, and 5. Currently, one clinical trial is studying Avastin alone for acute vision-threatening ROP stage 3 in zone I and posterior zone II without LASER therapy. Another clinical trial is investigating Avastin following LASER therapy for recurrent ROP stages 4 and 5.

Summary

Treatment for ROP has evolved from later, more destructive (cryotherapy) to earlier, less destructive (LASER therapy) peripheral retinal ablation. If evidence-based data supports early findings, the use of Avastin may be recommended without the need for ablative LASER therapy and before retinal detachment develops. Avastin will be especially useful for ROP stage 3 cases with hemorrhage decreasing retinal visualization, rigid pupils, intravitreal neovascularization with early or developing (minimal) fibrous membranes, or aggressive posterior retinopathy of prematurity (AP-ROP). These cases all continue to have poor outcomes with LASER therapy.

Keywords

intravitreal bevacizumab (Avastin), light amplification by stimulated emission of radiation (LASER) therapy, retinopathy of prematurity (ROP), vascular endothelial growth factor (VEGF), vitrectomy

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Introduction

Retinopathy of prematurity (ROP) remains a significant morbidity in extremely immature infants whose survival rate continues to increase [1–4]. Traditional treatment for acute phase ROP has evolved from cryotherapy (cold probe applied externally to the sclera) in the 1980s to the current standard of light amplification by stimulated emission of radiation (LASER) therapy (LASER beam applied internally to the retina through the dilated pupil) beginning in the 1990s. Efforts to develop more effective, less destructive therapy for ROP persist because of undesirable visual outcomes and lasting side effects, despite appropriate LASER treatment. Anti-vascular endothelial growth factor (anti-VEGF) therapy is currently under investigation to fulfill this objective.

Retinopathy of prematurity classification

In 1984, the International Classification for Retinopathy of Prematurity was published to define ROP in terms of location [zones (I–III)], severity [stages (1–5)], extent [clock hours (1–12)], and vascular dilatation and tortuosity (plus disease). In 2005, this classification was revised to include aggressive posterior ROP (AP-ROP) (formerly called RUSH disease), pre-plus disease, and a practical way to estimate the extent of zone I clinically with the indirect ophthalmoscope [5,6]. Plus disease became a requirement for treatment (except for stage 3 in zone I) and number of clock hours became less important. ROP stage 3 is pathologic new vessel formation between the vascular and avascular interface before retinal detachment occurs. ROP stage 4 is partial retinal detachment and ROP stage 5 is total retinal detachment due to the transformation of extensive overgrowth of abnormal new vessels into fibrotic tractional membranes in the vitreous. Because of the increased importance of plus disease, and the lack of agreement, even among experts, regarding the presence of plus disease, concern has developed that many infants are being treated with LASER unnecessarily. Thus, the latest efforts regarding classification center around a paradigm of plus disease [7–12].

Poor outcomes, especially in conventional zone I and posterior zone II cases, and also in AP–ROP cases, have prompted substantial efforts toward the development of telemedicine screening that is sensitive and specific [7–11,13–16].

Pathogenesis of retinopathy of prematurity and neovascularization: vasculogenesis versus angiogenesis

ROP is the leading cause of blindness in infants in the United States and other developed countries and an increasing cause of blindness in developing countries. Fortunately, its pathogenesis is well understood and described as a two-phase process [17]. Vasculogenesis is the formation of normal inner retinal vessels in utero in an environment of relative hypoxia with physiologic levels of vascular endothelial growth factor (VEGF). At the time of premature birth, the infant retina becomes hyperoxic (even in room air) with decreased levels of VEGF. For a period of time, vessel formation is halted at the interface between the vascular and avascular retina (Phase I, clinically 22–30 weeks postmenstrual age). As the eye grows, the avascular retina continues to increase in size without accompanying inner retinal vessels. This creates a peripheral area of hypoxic retina, resulting in increased levels of VEGF, which stimulates angiogenesis (pathological neovascularization) at the interface between the vascular and avascular retina (Phase II, clinically 31–45 weeks postmenstrual age). This two-phase process leads to vision-threatening ROP most frequently in extremely immature infants with other comorbidities of prematurity (risk factors for ROP). In late Phase II and thereafter, VEGF becomes less important and intravitreal fibrosis with membrane formation and resultant retinal traction becomes the determinant of ROP outcome.

Multiple modulators of pathologic neovascularization

The two-phase process of pathologic neovascularization has been modulated in Phase I (postmenstrual age 22–30 weeks) by increasing ω-3-(omega-3) polyunsaturated fatty acids (Omegaven) [18], erythropoietin (Epogen) [19–20], insulin-like growth factor binding protein-3 (IGFBP3) [21], and VEGF [22]. The emphasis is on regulating and simulating as much as possible the environment that would have occurred had the pregnancy not been interrupted by premature birth. All of these substances would have been provided by the mother *in utero*. In Phase II (postmenstrual age 31–45 weeks), erythropoietin administration should be halted and anti-VEGF drugs can be administered because erythropoietin and VEGF stimulate pathologic neovascularization. In contrast, ω-3-polyunsaturated fatty acids and IGFBP3 supplementation can be maintained as these continue to be beneficial.

Complications and disadvantages of light amplification by stimulated emission of radiation therapy

Even early LASER therapy has significant acute complications including cataract formation, anterior segment and vitreous hemorrhage, iris adhesions to the lens, and increased and decreased intraocular pressure. With both cryotherapy and LASER therapy, the peripheral retina is ablated. Long-term complications, especially in ROP zone I cases, include a significant loss of peripheral vision, often with the development of strabismus with loss of binocular vision, and progression to severe nearsightedness. Studies that demonstrate a minimal effect of LASER on the visual field have been done on children who generally had ROP zone II [23]. Early treatment of ROP with LASER does not decrease the nearsightedness that occurs following conventional LASER for threshold ROP [24]. When treatment fails, with continued progression of ROP or recurrence of ROP despite appropriate timing and placement of LASER therapy, early or late retinal detachment with complete loss of vision may result. Additional disadvantages of LASER therapy are the need for anesthesia with intubation of infants for precise LASER treatment, requirement of dilation and steroid drops following LASER for acute inflammation created by LASER, and the continued progression of ROP for at least a week following LASER that results from VEGF already in the vitreous continuing to stimulate pathologic neovascularization. In the current era, more ROP cases occur in zone I of extremely immature infants. Strict cutaneous monitoring of oxygen (keeping oxygen saturation below 95%) has decreased the number of ROP zone II cases. Thus, there is an increased ratio of zone I to zone II cases.

Antivascular endothelial growth factor therapy

The fact that the endothelial cell is the target of VEGF makes it logical to consider intravitreal anti-VEGF therapy. Therapy for ROP is directed at treating the underlying pathogenesis by decreasing VEGF levels (specifically VEGF-A), either by completely ablating the peripheral avascular retina that produces the VEGF (LASER therapy) or by inactivating VEGF by binding it after its production (anti-VEGF therapy). Avastin (bevacizumab) is an anti-VEGF drug that binds VEGF-A, and is a complete antibody rather than an antibody fragment like Lucentis (ranibizumab). Because of its high molecular weight as a complete antibody, Avastin, of all of the current anti-VEGF drugs, has: i) less ability to penetrate into the retina, which could potentially affect normal

retinal development, perhaps irreparably; and ii) less possibility of escaping from the eye, remaining trapped within the thick vitreous gel of the infant (in contrast to the watery vitreous of the adult). The relatively large molecular size of the Avastin antibody prevents to the greatest extent possible (with currently available anti-VEGF drugs) systemic complications (provided LASER has not breached the normal protective retinal barrier). Avastin is already in common use for adult ocular neovascular diseases, is readily available, and relatively inexpensive. However, there is reluctance to use this drug because of the possibility of ocular or systemic complications in neonates. Avastin is known to have extremely serious systemic complications when administered to terminally ill cancer patients by intravenous injection, repeatedly, in high doses. Fortunately, Avastin given to extremely immature infants by intravitreous injection as a single, low dose has not shown systemic or local toxicity. Current literature includes reports of multiple approaches utilizing anti-VEGF therapy (usually Avastin) at various stages in the course of ROP, with differing dosages, and in combination with LASER and/or vitrectomy or alone.

Combination of light amplification by stimulated emission of radiation and Avastin for retinopathy of prematurity stage 3

There are multiple reports of using Avastin for ROP stage 3 simultaneously with or closely followed by LASER therapy [25•,26•,27•]. LASER therapy is the current standard of care, and without sufficient evidence-based data there is an understandable reluctance to rely upon Avastin alone for treatment. However, this combination therapy introduces the complications of LASER therapy unnecessarily.

Combination of light amplification by stimulated emission of radiation and Avastin for retinopathy of prematurity stages 4 and 5

The current study using pan-VEGF blockade for the treatment of ROP (BLOCK-ROP: ClinicalTrials.gov Identifier: NCT 00702819) [28•], is a Phase I study. Its primary purpose is to determine the safety of a single injection of Avastin (0.75 mg) into the vitreous cavity. The study's secondary purpose is to evaluate the efficacy of this treatment for AP–ROP that has failed appropriate LASER ablation at a minimum of 1 week following LASER. A Phase II clinical trial to compare the safety and efficacy of Avastin versus standard vitrectomy (randomizing eyes of each infant) is planned if safety is established. Other cases have been reported which use Avastin at the same time or closely following LASER for stages 4 and 5 [27•,29•,30,31••]. Unfortunately, the extent of fibrous membranes and retinal detachment that were present at the time of the injection of Avastin were inversely related to the success of this treatment combination. Avastin acts against angiogenesis, not against fibrosis, and does not prevent contraction of fibrous membranes. Thus, it reduces the angiogenic component in the vitreous of a detaching retina and can be helpful in performing a vitrectomy by limiting extensive hemorrhage from these fragile intravitreal pathologic vessels. However, it can accelerate the contraction of fibrous membranes with resultant worsening of retinal detachment [31••].

Avastin alone for retinopathy of prematurity stage 3

The BEAT–ROP study (bevacizumab eliminates the angiogenic threat of ROP: ClinicalTrials.gov Identifier: NCT 00622726) [32•] is a Phase II study (intravitreal bevacizumab injections versus conventional LASER surgery for ROP). The study's primary purpose is to determine the safety and efficacy of Avastin as a single injection (0.625 mg) into the vitreous cavity versus standard LASER therapy (randomizing both eyes of every infant into either the Avastin or the LASER-treated group) with the end point being the development of a primary recurrence of ROP at a minimum of 1 week. The study's secondary purpose is to evaluate the long-term visual acuity, visual field, binocular vision, refraction, and any other appropriate parameters (visual evoked potentials, electroretinogram, fluorescein angiography, foveal optical coherence tomography, etc.). This study is enrolling zone I and posterior zone II cases (including some cases of AP–ROP). The majority of infants with zone I and AP–ROP cases have had moderate to severe intraventricular hemorrhage or periventricular leukomalacia which complicates the evaluation of visual function or central nervous system development. Posterior zone II cases usually occur in larger infants with fewer comorbidities. This design provides an opportunity to compare a population that has received Avastin with a population that has not received Avastin. This will allow evaluation of the visual system and the central nervous system, as well as other organ systems that are undeveloped at the time of administration of intravitreal Avastin. Currently, published reports in neonates include only the plasma levels of VEGF during the development of ROP [22,33], and the vitreous levels of VEGF in ROP stage 4 [30]. To date, serum levels following intravitreal Avastin injection have not been determined in neonates. Avastin levels in serum and in the vitreous of the fellow eye have been determined only in animal models and adults [34,35].

Avastin has been shown in a small case series to temporarily slow vasculogenesis and permanently halt angiogenesis, usually with a single intravitreal injection, when used for vision-threatening ROP stage 3 (acute phase ROP including AP–ROP) [36••]. Eventually, intravitreal pathological neovascularization disappears, leaving only scant fibrous remnants, and normal inner retinal vascularization proceeds toward the ora serrata. The

continued vascularization of the peripheral retina is demonstrated by fluorescein angiography (Fig. 1). Myopia (nearsightedness) is rare. In contrast, the permanent effects of ablation of the peripheral retina have been demonstrated by fluorescein angiography (Fig. 2). Severe myopia often develops. Thus far, Avastin is so effective that it expands the window of treatment, which has been diminished by the current recommendations of early treatment for ROP, and allows telemedicine to be more successful. Avastin also makes it less critical to determine when enough plus disease is present to justify performing ablative LASER therapy to the peripheral retina.

Avastin alone for retinopathy of prematurity stages 4 and 5

Unfortunately, in ROP stages 4 and 5, whereas the drug inhibits angiogenesis, it has no beneficial effect on fibrous membranes and may trigger contraction of these membranes and accelerate retinal detachment [37•]. Thus, it is important to use Avastin before ROP stages 4 and 5 or its benefits will be considerably diminshed.

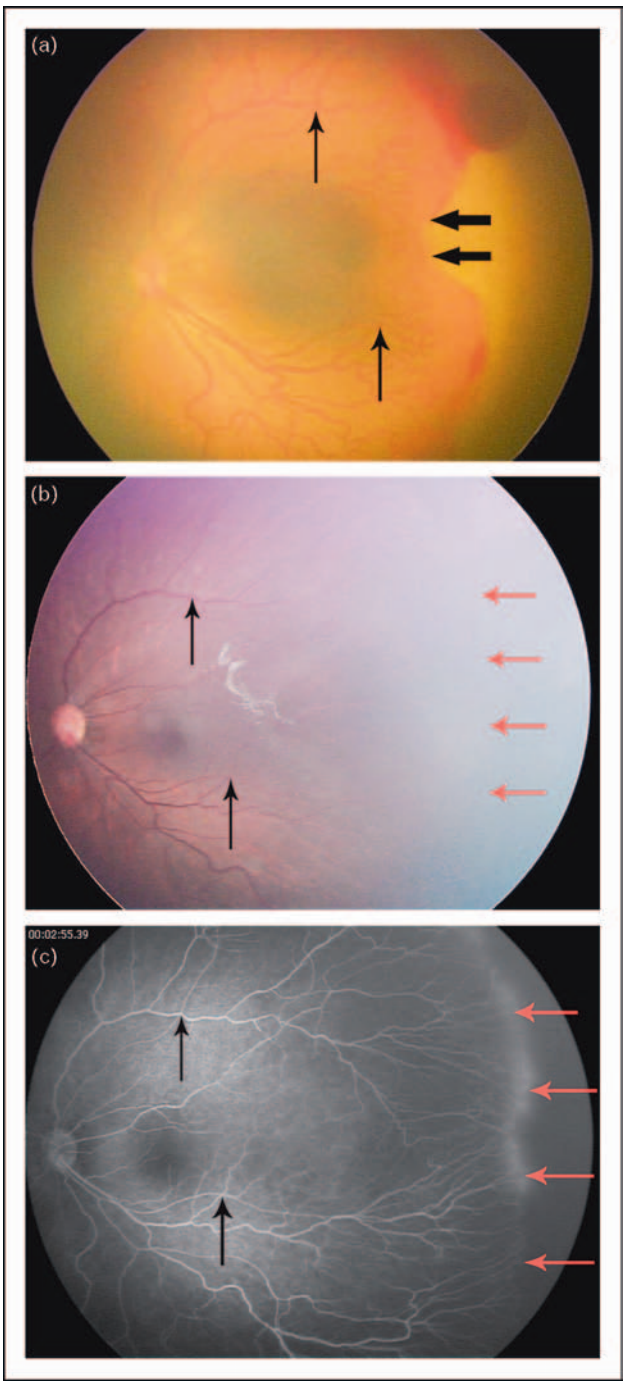
Avastin alone for inflammation, pupillary rigidity, or poor visualization due to hemorrhages in the anterior or posterior segments

Avastin is especially useful in ROP stage 3 cases with rigid pupils due to iris neovascularization, with anterior chamber and intravitreal hemorrhage, with early intravitreal fibrous membrane formation, or with AP–ROP [31••,36••,37•,38,39]. Provided that only ROP stage 3 is concealed behind these deterrents of retinal visualization (which can be verified by B-scan ultrasonography), Avastin is as efficacious for these cases as for cases with ROP stage 3 alone [26•,36••,37•]. Avastin will not prevent the development of complete retinal detachment when diminished visualization of the retina conceals ROP stages 4 and 5. Even though Avastin may decrease the angiogenic (neovascular) component of the disease, the fibrotic (membranous) component of the disease will rapidly advance and make successful vitrectomy unlikely.

Possible complications of Avastin intravitreal injections

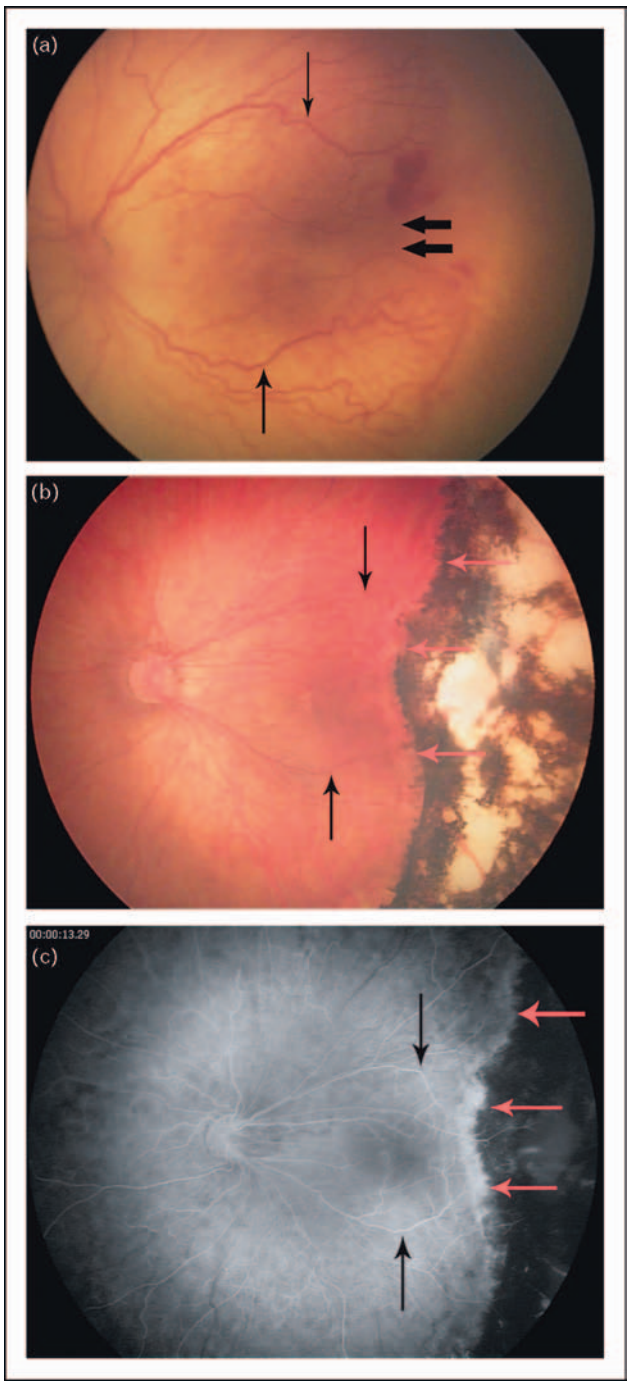
The most likely local complications of Avastin injections are infectious and traumatic. Infections can be avoided by strict sterile technique followed by 1 week of appropriate antibiotic ophthalmic drops. Trauma may occur to the lens because the injection is given too anteriorly. It is preferable to inject through the anterior, undifferentiated, neuroblastic retina, rather than to cause a cataract by contact with the lens, which is extremely large in very immature eyes. Another local concern is that retinal development will be adversely affected. In fact, immunohistopathology on a pair of preterm infant eyes that

Figure 1 Photographs of Avastin treatment for retinopathy of prematurity



Intravitreal Avastin for Zone I ROP on an infant who was 550 g and 24 weeks gestational age at birth: (a) Pre-Avastin – 2.5 months of age (photo); b) Post-Avastin – 15 months of age (photo); c) Post-Avastin – 15 months of age (fluorescein angiogram). Narrow black arrows mark corresponding points along retinal vessels; broad black arrows mark the vascular–avascular interface at the time of initial treatment; red arrows mark the extent of continued retinal vascularization with underlying viable retina and no myopia.

Figure 2 Photographs of LASER treatment for retinopathy of prematurity



LASER therapy for Zone I ROP was required three times because of recurrences on an infant who was 690 g and 23 weeks gestational age at birth: (a) Pre-LASER therapy – 2 months of age (photo); (b) Post-LASER therapy – 35 months of age (photo); (c) Post-LASER therapy – 35 months of age (fluorescein angiogram). Narrow black arrows mark corresponding points along retinal vessels; broad black arrows mark the vascular–avascular interface at the time of initial treatment; red arrows mark the extent of halted retinal vascularization with macular dragging present and 17 diopters of myopia.

were harvested 20 weeks following bilateral intravitreal Avastin injections demonstrated no abnormalities of ocular development [40••]. All layers of the retina had developed normally to the ora serrata and inner retinal vessels had advanced well beyond the vascular and avascular interface at the time of the Avastin injections. These findings were especially impressive because the infant was extremely immature (350 g birth weight; 22 weeks gestational age), and thus had a vulnerable, undifferentiated retina.

Systemic complications are always feared in the preterm neonate. No systemic complications have been encountered to date whether Avastin is given alone, or in combination with LASER surgery (when the retinal barrier has been breached). Long-term systemic complications are considered unlikely following the single injection of a small quantity of a large whole antibody into the vitreous cavity, which is sequestered when LASER therapy has not been performed.

Conclusion

Avastin alone for ROP stage 3 may become not just an adjunct to LASER therapy or vitrectomy, but primary treatment replacing LASER therapy as standard of care if efficacy and safety are validated by evidence-based data. LASER eventually may be contraindicated because it is a very destructive therapy that is never completely without residual effects and targets the same pathogenic substance: VEGF. ROP stages 4 and 5 will continue to occur due to late diagnosis of acute disease from less than adequately screened nurseries, and in these desperate cases vitrectomy will be required, perhaps with Avastin as an adjunctive therapy.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 273).

1 Eliason K, Osborn J, Amsel E, Richards S. Incidence, progression and duration of retinopathy of prematurity in Hispanics and white non-Hispanic infants. Journal of AAPOS 2007; 11:447–451.

2 Field D, Dorling J, Manktelow B, Draper E. Survival of extreme premature babies in a geographically defined population: prospective cohort study of 1994-9 compared with 2000-5. BMJ 2008; 336:1221–1223.

3 Giannantonio C, Papacci P, Molle F, *et al.* An epidemiological analysis of retinopathy of prematurity over ten years. J Pediatr Ophthalmol Strabismus 2008; 45:162–167.

4 Lad E, Nguyen T, Morton J, Moshfeghi D. Retinopathy of prematurity in the United States. Br J Ophthalmol 2008; 92:320–325.

5 An international committee for classification of retinopathy of prematurity. The international classification of retinopathy of prematurity revisited. Arch Ophthalmol 2005; 123:991–999.

6 Capone A, Ells A, Fielder A, *et al.* Standard image of plus disease in retinopathy of prematurity. Arch Ophthalmol 2006; 124:1669–1670.

7 Lajoie A, Koreen S, Wang L, *et al.* Retinopathy of prematurity management using single image vs multiimage telemedicine examinations. Am J Ophthalmol 2008; 146:298–309.

8 Scott K, Kim D, Wang L, *et al.* Telemedical diagnosis of retinopathy of prematurity. Ophthalmology 2008; 115:1222–1228.

9 Silva R, Murakami Y, Jain A, *et al.* Stanford university network for diagnosis of retinopathy of prematurity (SUNDROP): 18-month experience with telemedicine. Graefes Arch Clin Exp Ophthalmol 2009; 247:129–136.

10 Soh Y, Fujino T, Hatsukawa Y. Progression and timing of treatment of zone I retinopathy of prematurity. Am J Ophthalmol 2008; 146:369–374.

11 Wallace D. Computer assisted quantification of vascular tortuosity in retinopathy of prematurity (an American Ophthalmological Society Thesis). Trans Am Ophthalmol Soc 2007; 105:594–615.

12 Wallace D, Quinn G, Freedman S, Chiang M. Agreement among pediatric ophthalmologists in diagnosing plus and preplus disease in retinopathy of prematurity. Journal of AAPOS 2008; 12:352–356.

13 Kemper A, Freedman S, Wallace D. Retinopathy of prematurity care: patterns of care and workforce analysis. Journal of AAPOS 2008; 12:344–348.

14 O'Keefe M, Kirwan C. Screening for retinopathy of prematurity. Early Hum Dev 2008; 84:89–94.

15 Skalet A, Quinn G, Ying G-S, *et al.* Telemedicine screening for retinopathy of prematurity in developing countries using digital retinal images: a feasibility project. Journal of AAPOS 2008; 12:252–258.

16 Wilson C, Cocker K, Moseley M, *et al.* Computerized analysis of retinal vessel width and tortuosity in premature infants. Invest Ophthalmol Vis Sci 2008; 49:3577–3585.

17 Chen J, Smith L. Retinopathy of Prematurity. Angiogenesis 2007; 10:133–140.

18 Connor K, SanGiovanni J, Lofqvist C, *et al.* Increased dietary intake of ω-3-polyunsaturated fatty acids reduces pathological angiogenesis. Nat Med 2007; 13:868–873.

19 Suk K, Dunbar J, Liu A, *et al.* Human recombinant erythropoietin and the incidence of retinopathy of prematurity: a multiple regression model. Journal of AAPOS 2008; 12:233–238.

20 Fauchere J, Dame C, Vonthein R, *et al.* An approach to using recombinant erythropoietin for neuroprotection in very preterm infants. Pediatrics 2008; 122:375–382.

21 Lofqvist C, Chen J, Connor K, *et al.* IGFBP3 suppresses retinopathy through suppression of oxygen-induced vessel loss and promotion of vascular regrowth. PNAS 2007; 104:10589–10594.

22 Pieh C, Agostini H, Buschbeck C, *et al.* VEGF-A, VEGFR-1, VEGFR-2 and Tie2 levels in plasma of premature infants: relationship to retinopathy of prematurity. Br J Ophthalmol 2008; 92:689–693.

23 McLoone E, O'Keefe M, Lanigan B. Effect of diode laser retinal ablative therapy for threshold retinopathy of prematurity on the visual field: Results of Goldmann Perimetry at a mean age of 11 years. J Pediatr Ophthalmol Strabismus 2007; 144:170–173.

24 Quinn G, Dobson V, Davitt B, *et al.* Progression of myopia and high myopia in the early treatment for retinopathy of prematurity study: findings to 3 years of age. Ophthalmology 2008; 115:1058–1064.

25 • Chung E, Kim J, Ahn H, Koh H. Combination of laser photocoagulation and intravitreal bevacizumab (Avastin) for aggressive zone I retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmology 2007; 245:1727–1730.
This case report utilized Avastin combined with diode LASER to induce regression for AP–ROP.

26 • Travassos A, Teixeira S, Ferreira P, *et al.* Intravitreal bevacizumab in aggressive posterior retinopathy of prematurity. Ophthalmic Surg Lasers Imaging 2007; 38:233–237.
This article reports three cases, of which two cases were treated with Avastin injections in one eye because LASER treatment could not be utilized in these eyes due to anterior segment involvement and one case was treated with Avastin injection in one eye that had failed LASER therapy twice.

27 • Lalwani G, Berrocal A, Murray T, *et al.* Off-Label use of intravitreal bevacizumab (Avastin) for salvage treatment in progressive threshold retinopathy of prematurity. Retina 2008; 28:S13–S18.
This paper reports three cases, which had progressive ROP despite LASER therapy, with Avastin injected in five of the six eyes. This salvage therapy was not uniformly successful and suggests that anti-VEGF agents used earlier could avoid the need for LASER therapy entirely.

28 • Trese M. BLOCK-ROP study. http://www.ClinicalTrials.gov.
This ongoing clinical trial is evaluating the use of Avastin for progressive ROP stages 4 and 5 following LASER therapy. In this clinical trial, one eye of each infant is receiving Avastin with the fellow eye undergoing vitrectomy as a control. Sponsored by Vision Research Foundation. NCT 00702819; 2009.

29 • Kusake S, Shima C, Wada K, *et al.* Efficacy of intravitreal injection of bevacizumab for severe retinopathy of prematurity: a pilot study. Br J Ophthalmol 2008; 92:1450–1455.
This paper reports the use of Avastin for stages 3, 4a, and 4b that developed progressive ROP despite LASER therapy. The authors utilized Avastin following vitrectomy surgery and found a reduction in neovascularization without apparent adverse ocular or systemic effects.

30 Sonmez K, Drenser K, Capone A, Trese M. Vitreous levels of stromal cell-derived factor 1 and vascular endothelial growth factor in patients with retinopathy of prematurity. Ophthalmology 2008; 115:1065–1070.

31 •• Honda S, Hirabayashi H, Tsukahara Y, Negi A. Acute contraction of the proliferative membrane after intravitreal injection of bevacizumab for advanced retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 2008; 246:1061–1063.
This case report emphasizes that injection of Avastin for ROP stages 4 and 5 in the presence of dense fibrous membranes can accelerate retinal detachment. This contraction of fibrous membranes leading to retinal detachment in ROP is similar to that reported in diabetic retinopathy and should not be confused with injection of Avastin for ROP stage 3 with no or minimal fibrous membranes.

32 • Mintz-Hittner H. BEAT-ROP study. http://www.ClinicalTrials.gov.
This ongoing clinical trial is evaluating the use of Avastin in both eyes as a primary therapy without LASER for ROP stage 3. The control infants are receiving standard LASER therapy in both eyes. Sponsored by the University of Texas Health Science Center, Houston. NCT 00622726; 2009.

33 Kwinta P, Bik-Multanowski M, Mitkowska Z, *et al.* The clinical role of vascular endothelial growth factor (VEGF) system in the pathogenesis of retinopathy of prematurity. Graefes Arch Clin Exp Ophthalmol 2008; 246:1467–1475.

34 Bakri S, Snyder M, Reid J, *et al.* Pharmocokinetics of intravitreal bevacizumab (Avastin). Ophthalmology 2007; 114:855–859.

35 Krohne T, Eter N, Holz F, Meyer C. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. Am J Ophthalmol 2008; 146:508–512.

36 •• Mintz-Hittner H, Kuffel R. Intravitreal injection of bevacizumab (Avastin) for treatment of stage 3 retinopathy of prematurity in zone I or posterior zone II. Retina 2008; 28:831–838.
This is the only case series using an intravitreal injection of Avastin as a primary therapy without LASER therapy. It demonstrates the effectiveness of a single low dose of intravitreal Avastin for zone I or posterior zone II ROP stage 3. Normal retinal vessels continue to grow beyond the former vascular–avascular interface to the peripheral retina.

37 • Quiroz-Mercado H, Martinez-Castellanos M, Hernandez-Rojas M, *et al.* Antiangiogenic therapy with intravitreal bevacizumab for retinopathy of prematurity. Retina 2008; 28:S19–S25.
This report of 18 eyes utilized Avastin for ROP stage 4 following LASER, stage 3 as a primary therapy, and when the media prevented LASER. Neovascular regression was found in all but one eye.

38 Shaw P, Narendran V, Tawansy K, *et al.* Intravitreal bevacizumab (Avastin) for post laser anterior segment ischemia in aggressive posterior retinopathy of prematurity. Indian J Ophthalmol 2007; 55:75–76.

39 Kim MJ, Kim S-J, Yu YS. The risk for retinal detachment associated with hemorrhages pre and postlaser treatment in retinopathy of prematurity. Retina 2008; 28:1451–1457.

40 •• Kong L, Mintz-Hittner H, Penland R, *et al.* Intravitreal bevacizumab as antivascular endothelial growth factor therapy for retinopathy of prematurity: a morphologic study. Arch Ophthalmol 2008; 126:1161–1163.
This is the only immunohistopathology on neonatal ocular tissue following intravitreal Avastin injection. It demonstrates that all layers of the retina are intact and inner retinal vessels have proceeded beyond the former vascular–avascular interface.