accurate delivery of 10 microliters than with a standard 1 cc syringe. Short-term success is defined as improvement by no later than 5 days after treatment, and no recurrence of type 1 ROP or severe neovascularization requiring additional treatment within 4 weeks. Late recurrences are recorded, and any additional treatment is at investigator discretion. A study examination at 12 months of age will assess visual attentiveness, ocular alignment, retinal structure and cycloplegic refraction. Plasma levels of bevacizumab and VEGF are collected pre-injection and at 2 and 4 weeks after treatment. Results should be available in early 2017, and will hopefully provide guidance for dose selection of future larger studies.

In summary, we have a long way to go to have an evidence-based paradigm for anti-VEGF treatment. There are many unanswered questions about which drug, what dose, relative benefits and possible side effects. Consequently, there are many opportunities for high-quality comparative studies that will shape our future treatment of premature infants and aid in reducing the burden of blindness from ROP.

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Intravitreal injections of bevacizumab: timing, technique, and outcomes

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In March 2008 a prospective, randomized, controlled (intravitreal bevacizumab monotherapy vs laser therapy), multicentered clinical trial for retinopathy of prematurity (ROP) began—Bevacizumab Eliminates the Angiogenic Threat of Retinopathy of Prematurity, or BEAT-ROP. This clinical trial was based on animal models of ROP, and on bevacizumab clinical trials in human adults with other neovascular retinal disorders.

The BEAT-ROP clinical trial was a prospective, randomized, controlled, multicenter clinical trial comparing intravitreal bevacizumab versus conventional laser therapy. It reported improved efficacy in the bevacizumab group for zone I ROP in 2011[1] and decreased high myopia in the bevacizumab group for zone I and posterior zone II ROP in 2014.[2] However, unanticipated and troublesome delayed recurrences had become a serious problem in follow-up of infants treated by intravitreal bevacizumab monotherapy due to lack of any guidelines. Thus, delayed recurrences

were comprehensively reviewed in cases from the BEAT-ROP clinical trial and a subsequent consecutive case series. These recurrences occurred especially in infants with aggressive posterior ROP, extended hospital stays associated with multiple nonocular complications, and very low birth weight.[3] Many articles reporting the advantages of bevacizumab over laser therapy in terms of efficacy and myopia have also reported multiple cases of delayed recurrence, mostly with disastrous outcomes. From the initial publication,[1] the need for proper case selection (timing), careful injection (technique), and appropriate long-term follow-up for parameters (outcomes) both advantageous and adverse have been stressed.

Timing

It cannot be overemphasized that intravitreal bevacizumab monotherapy is only appropriate for stage 3+ or APROP. It has been emphasized that bevacizumab is *not* a prophylactic drug and that injections administered too early[4] (before Type 1 ROP has developed) could cause interruption of normal vascularization and accompanying retinal development (especially of the vulnerable macula). This corresponds to the period before vascular endothelial growth factor (VEGF) is sufficiently elevated to allow increased anti-VEGF therapy without damaging underlying retinal differentiation. Similarly, it has been cautioned that bevacizumab is not a salvage drug and that injections for cases identified too late[5] (stages 4 and 5) but nonetheless given bevacizumab without the rapid availability of vitrectomy could produce accelerated contraction of thick membranes and lead to rapid retinal detachment. This corresponds to the period after VEGF has decreased and transforming growth factor-beta (TGF-β) has increased, thus anti-VEGF therapy only initiates the process of contracting fibroblastic elements rather than the process of closing neovascular elements.

Technique

It is essential that an appropriate injection technique is utilized—local analgesia to reduce pain, thorough sterility to prevent endophthalmitis, and adequate stabilization of the eye at the time of the injection (with a short, small-gauge needle aimed toward the posterior vitreous) to avoid hitting the lens (causing dislocation or cataract) or the retina (causing retinal hemorrhage, tear or detachment).[1]

Outcomes

Normal inner retinal vascularization is not complete until approximately 42 weeks. Prior to the completion of inner retinal vascularization, the interface between the vascularized and the avascular retina depends on gestational age (GA) at birth and the hospital course that occurs in the early neonatal period. The very smallest infants (22-26 weeks' GA) will never vascularize completely to the ora serrata (whether the hospital course is uneventful or extremely devastating), because precursor vascular cells have not migrated to the farthest extent of the peripheral retina. These vascular precursors are required for completion of vascularization and subsequent differentiation of the underlying retina. The presence of this avascular, undifferentiated retina is not pathologic, but developmental.[6]

Advantageous Outcomes

The enrollment of infants into the BEAT-ROP clinical trial from 2008 to 2010 currently allows comparison of outcomes in infants 6-8 years of age. Follow-up examinations of infants treated by intravitreal bevacizumab monotherapy versus laser therapy provide evidence of both structurally and functionally improved outcomes in the infants treated by bevacizumab compared to laser. All benefits are greater in zone I patients.

First, bevacizumab allowed an increased number of successfully treated retinas. This translated to *improved efficacy*, especially in zone I cases, with recurrences of 2 of 31 bevacizumab-treated eyes versus 23 of 31 laser-treated eyes ($P = 0.002$).[1] Efficacy was originally reported at 54 weeks in the BEAT-ROP clinical trial but has not decreased with continued observation over the years.

Second, bevacizumab preserved peripheral retinal growth factors and allowed the anterior segment growth to proceed with flatter corneas, deeper anterior segments, and thinner lenses. This translated to *less measurable myopia by refraction*, especially in zone I cases, with mean spherical equivalent of −1.51 D in 52 bevacizumab-treated eyes versus −8.44 D in 35 laser-treated eyes ($P < 0.001$).[2] This study was continued to age 30 months, which was selected because development of high myopia generally levels off at this age but not before.

Third, bevacizumab was not associated with substantial inflammation in the macula, especially in zone I cases, that would require an increased number of laser applications. Thus, the macula is permitted to form a distinct fovea rather than an indistinct macula, usually without a definitive fovea.[7] This translates to *measurable foveal formation by OCT* and to *documented better visual acuity* (unpublished data).

Fourth, bevacizumab allows preservation of the peripheral retina leaving an increased area of retina to differentiate as vascularization proceeds. This translates to *measurable increase in visual field by perimetry* (unpublished data).

Adverse Outcomes

A. Ocular: The serious problem of recurrence was presented (including 6 eyes of 4 infants demonstrating pathology from severe macular dragging to total retinal detachment) before the completion of enrollment into the BEAT-ROP clinical trial (Poster, Second World ROP Congress, New Delhi, India, November 27, 2009). However, this small number of cases observed were

inadequate to characterize 1. incidence, 2. risk period, 3. risk factors, and 4. characteristics of recurrence.

1. Incidence of recurrence reported at 65 weeks adjusted age following 0.625 mg of bevacizumab was 8% (20/241) for infants and 7% (34/471) for eyes.

2. The increased recurrence risk period (90%) between 45 and 55 weeks' postnatal age was not published until 2016.[3] It should be noted that this incidence and risk period is likely dependent on dose and drug. Lower doses may cause an increased recurrence incidence with a risk period that is shorter, whereas higher doses may cause a decreased recurrence incidence with a risk period that is longer (data unpublished). The selection of larger anti-VEGF molecules (eg, bevacizumab with a half-life of 20 days [149 kD]) may decrease the number of recurrences, because these larger molecules persist longer and allow vascularization to be completed. In contrast, the selection of smaller anti-VEGF molecules (eg, ranibizumab with a half-life of 2 hours [48 kD]) may increase the number of recurrences, because these smaller molecules exit the eye before vascularization is completed.[8]

3. The risk factors that were identified were APROP, increased duration of hospital stay (due to multiple systemic complications), and decreased birth weight.[3] Undoubtedly, other risk factors will be associated with ROP recurrence, just as risk factors associated with the development of ROP itself continues to expand.

4. The characteristics of recurrence always includes the return of plus disease (that does not resolve after retreatment) and the return of intravitreal neovascularization (that does resolve after retreatment). Neovascularization can occur at the advancing edge in cases that presented as either as ROP stage 3+ or as APROP; however, neovascularization can also occur at the former ridge and extraretinal fibrovascular proliferative complex in cases that presented as ROP stage 3+. It was further observed that in cases that did recur following the initial injection of bevacizumab, the advancing edge proceeds for a *shorter distance* (length in disk diameters [DD]) and at a *slower pace* (rate in DD per week) compared to the length and rate in cases that did not recur. Thus, in recurrent cases the measurement of vascular growth was a mean of 1.76 DD in length at a rate of 0.11 DD/week. This was significantly different compared to nonrecurrent cases, whose measurement of vascular growth was a mean of 4.48 DD in length at a rate of 0.23 DD/week.[3]

B. Systemic: Virtually all publications have warned of possible systemic complications; however, to date, no complication has been established and there are no suggested systemic screening protocols to establish any correlation between bevacizumab and adverse organ development or malfunction. This of course does not establish safety regarding the use of intravitreal bevacizumab monotherapy for ROP.

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