Pilot Study of the Delivery of Microcollimated Pars Plana External Beam Radiation in Porcine Eyes

Mark R. Barakat, MD; Mark Shusterman, MD; Darius Moshfeghi, MD; Ronald Danis, MD; Michael Gertner, MD; Rishi P. Singh, MD

Objective: To investigate the effects of a novel stereotactic radiosurgical system for pars plana delivery of microcollimated x-rays to the retina and determine the retinal radiological dose response and toxicity threshold in a pig model.

Methods: The x-rays were delivered through the pars plana to the maculae of Yucatan miniswine to verify the targeting and safety of a cornea-scleral, stabilized, office-based delivery system. Twelve eyes were randomized to receive 0, 16, 24, 42, 60, or 90 Gy in a single dose to the retina. Eye examinations, fundus photography, fluorescein angiography, and spectral-domain optical coherence tomography were obtained at days 7, 30, 60, and 90. Indocyanine green angiography was done at day 90.

Results: Through day 90 interim analysis, no abnormalities of external structures were noted. A small cortical lens opacity was noted in the 60-Gy group. Fundus evaluation revealed no abnormalities at 16 or 24 Gy. Beginning at day 30, circular pale retinal lesions with sharp margins were noted in the maculae of the eyes that received 42, 60, and 90 Gy. Higher-dose lesions showed late staining on fluorescein angiography, choroidal hypoperfusion on indocyanine green angiography, and defined photoreceptor loss and retinal thinning on spectral-domain optical coherence tomography.

Conclusion: Transscleral stereotactic radiation dosing of porcine eyes demonstrates no apparent clinical abnormalities in doses less than 24 Gy. Doses of 42 Gy or higher led to focal choroidal and retinal damage within the target area.

Clinical Relevance: Radiation can induce small–blood vessel closure and thereby has therapeutic potential in neovascular diseases such as age-related macular degeneration.

Arch Ophthalmol. 2011;129(5):628-632

Author Affiliations: Cole Eye Institute, Cleveland Clinic Foundation, Cleveland, Ohio (Drs Barakat and Singh); Oraya Therapeutics, Newark, California (Drs Shusterman and Gertner); Stanford University, Palo Alto, California (Dr Moshfeghi); and University of Wisconsin, Madison (Dr Danis).

While the past decade has brought about a dramatic improvement in the outcomes of neovascular age-related macular degeneration (AMD) therapy, the current standard-of-care treatment with anti–vascular endothelial growth factor intravitreal agents still leaves room for improvement. With ranibizumab monotherapy, the most efficacious treatment of neovascular AMD reported to date, 30% of patients still have 0 to 3 lines of vision loss with monthly intravitreal injection. Intravitreal injections also carry the risk of retinal tears, rhegmatogenous retinal detachment, vitreous hemorrhage, hypotony, endophthalmitis, pseudoendophthalmitis, and possible thromboembolic events in populations at high risk.[1,2] Furthermore, the social and budgetary burdens of repeated visits, injection fees, and pharmaceutical costs are large and, given the expanding population of patients with AMD, alternative therapies with less retreatment would be highly desirable.

Most chronic diseases are treated via multimodal therapies because they typically have complex and multiple etiologies. The use of low-dose ionizing radiation for control of neovascular growth is based on experimental and clinical evidence and has a sound scientific basis. Ionizing radiation possesses the ability to destroy vascular tissue, and low-dose radiation has been shown to inhibit new blood vessel growth.[3] Theoretically, precise radiation delivery to the macula can selectively inhibit proliferating endothelial cells with limited destruction of retinal tissue and no systemic adverse effects. Moreover, Takahasi and colleagues[4] found that new capillaries or vessels are more sensitive than larger vessels or fibroblasts. Vascular endothelial cells in particular are more radiosensitive than other mesenchymal cell types such as fibroblasts and smooth-muscle cells.[5] As an additional benefit, ionizing radiation can inhibit the inflammatory response, which is thought by many to play a role in the formation of choroidal neovascularization (CNV) in AMD.

This study used a prototype stereotactic radiosurgical system for the delivery of microcollimated external beam radiation. Three sequential beams were delivered transconjunctivally, through the pars plana,

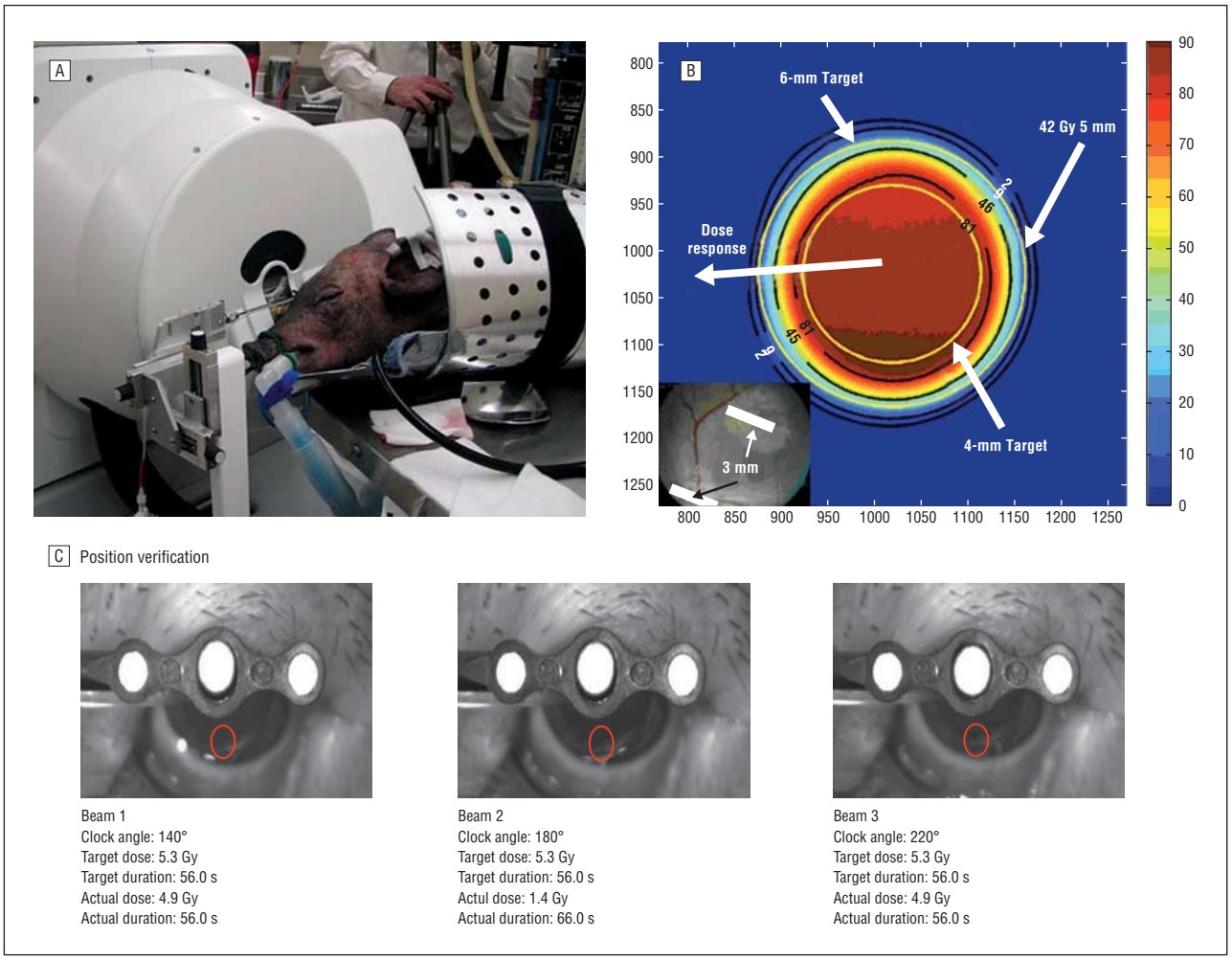

Figure 1. IRay System (Oraya Therapeutics, Newark, California). A, A miniswine is depicted in the clinical system at baseline size. The animal is anesthetized but not paralyzed. B, The dose distribution delivered to the retina is based on bench-top and Monte Carlo simulation and validated in this animal study. This picture was obtained using average motion data obtained during irradiation in a feasibility subject. It is a best fit for the dose on the retina inclusive of movement of the subject or animal. The 4-mm target ring is that which is obtained during a static bench-top experiment. In a static experiment, the penumbra, or distance to the 10% isodose, would be less than 1 mm. C, Treatment planning depicts 3 beams applied to the inferior pars plana region that converge on the macula of the minipig. The I-guide lens holds the eye within the same area on the x, y, z, and rotational axes. (To convert gray to rads, multiply by 100.)

converging on the retina. The study evaluated escalating doses of radiation to the eyes of Yucatan miniswine. This study examined the technological proof of concept as well as the dose range for potential adverse events using clinical examination, gross and microscopic histology, fundus photography, fluorescein angiography, indocyanine green, and spectral-domain optical coherence tomography (OCT).

METHODS

In a previously conducted study, an early prototype of the device (IRay; Oraya Therapeutics, Newark, California) was evaluated in miniswine. A premanufactured robot with an x-ray tube and collimator assembly retrofitted onto it delivered collimated x-ray beams to the retina through the pars plana in porcine eyes. The histological and imaging results revealed a sharp transition zone in the retina indicative of where the highest dose of radiation was delivered. Subsequently, a custom robotic system was developed (IRay) that was used in the current study.

The IRay system was designed for human eyes and modeled taking into account the axial length of the eye and key anterior segment structures for delivery of radiation through the pars plana region. The eyes of Yucatan miniswine were chosen for this study owing to their resemblance in structure, size, holangiotic vascular pattern, and nontapetal fundus to that of the human eye.[6] Although the axial length can be up to 1 cm smaller in the miniswine compared with the human eye, the cornea and anterior curvature are closer to human size. Institutional animal care and use committee approval was obtained prior to study commencement, and the study adhered to the Association for Research in Vision and Ophthalmology animal statement on the treatment of animals. The animals were obtained from S&S Farms (Ramona, California). On arrival, animals were examined to ensure that they were healthy and quarantined for 8 days before study enrollment. Globe axial lengths of all eyes were determined using the PalmScan A2000 (Micro Medical Devices, Calabasas, California) A-scan immersion ultrasonography.

The radiotherapy was delivered in a single session using a low-energy microcollimated x-ray source, scleral interface, and automated robotic positioning system (**Figure 1**). Six animals (12 eyes) were randomized to 6 different dose treatment groups, and the study end points were determined at day 270. The stereotactic robotic positioning system allowed for multiple beams to be projected at the retina from various angles. Radiation treatments were performed at 100 keV maximum beam energy in

Table. Treatment Dosing Schema of Yucatan Miniswine With IRay System

Animal No.	Right Eye				Left Eye			
	Total Dose, Gy	Axial Length, mm	Single-Beam Retinal Dose, Gy	Single-Beam Scleral Dose, Gy	Total Dose, Gy	Axial Length, mm	Single-Beam Retinal Dose, Gy	Single-Beam Scleral Dose, Gy
1	60	17.39	20.00	43.8	24	17.54	8.00	17.7
2	90	17.55	30.00	66.2	42	17.40	14.00	30.7
3	24	17.16	8.00	17.3	0	17.91	0.00	0.0
4	16	17.00	5.33	11.5	60	17.08	20.00	43.2
5	0	17.70	0.00	0.0	90	17.72	30.00	66.7
6	42	17.93	14.00	31.5	16	17.71	5.33	11.9

SI conversion factor: to convert gray to rads, multiply by 100.

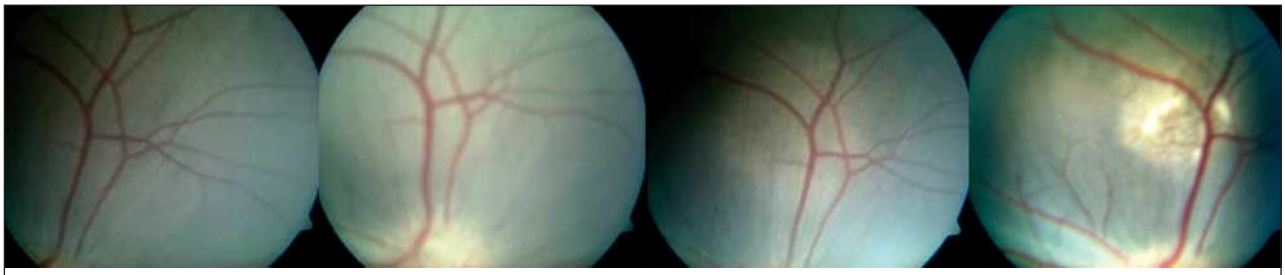

Figure 2. Time-dependent response to 90 Gy (to convert gray to rads, multiply by 100) during the treatment period. Note the evolution of the treatment spot.

which the ratio of surface to target dose was approximately 2.5:1. The treatment beams were highly collimated such that the diameter at the sclera was approximately 2.5 mm and 3 mm on the retinal surface. The radiation dose was delivered in 3 consecutive beams through various scleral entry points calculated to place the beams onto a single retinal target.[7] The low-voltage x-ray source creates minimal spread and scattering of the 3 beams for accurate targeting. In addition, synchronous real-time ocular tracking and robotic aim adjustment prevents the inadvertent targeting of the optic nerve. Lateral canthotomies were performed on all eyes prior to dosing for access to the limbal space. Treatment parameters and radiation delivery to both retina and sclera are shown in the **Table**.

Ophthalmic examinations (slitlamp and indirect ophthalmoscopy), fundus photography, and fluorescein angiography were performed on each eye prior to treatment and at 7, 30, 60, and 90 days. Ophthalmic observations of both eyes were scored and recorded according to the McDonald-Shadduck system.[8] Intraocular pressure was measured at each follow-up period (Tono-Pen XL; Medtronic ENT, Jacksonville, Florida). Spectral-domain OCT was performed at days 30, 60, and 90 with a Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, California).

RESULTS

There were no mortalities, and all swine gained weight during the study. Slitlamp and gross ophthalmic examinations revealed conjunctival irritation (congestion, swelling, and/or discharge) on day 7 in 6 of 12 treated eyes; the irritation was generally mild and related to the canthotomies. There were no other significant external findings seen during the remainder of the study. Intraocular pressure was measured on days 7, 30, 60, and 90 and varied throughout the study in all eyes. There was no significant difference noted in intraocular pressure within the same animal during the course of the study and among control and treated animals at all time points.

At day 90, a small focal cataract was detected on slitlamp examination in one eye that received 60 Gy (to convert gray to rads, multiply by 100). The cataract was verified by 2 masked ophthalmologists and was described as cortical and peripheral. However, it was unclear whether the cataract formation occurred on the radiation beam path.

Fundus photography and fluorescein angiography were performed prior to irradiation and after at days 7, 30, 60, and 90. Each image was examined by a masked grader (R.P.D.) and evaluated for abnormalities. The appearance of the treatment spots evolved in a dose- and time-dependent manner (**Figure 2**, **Figure 3**, and **Figure 4**). No abnormalities were noted on any images for the control group or the 16- and 24-Gy groups. In 2 of 2 eyes in the 42-Gy group, a small area of retinal pigment epithelium damage without retinal capillary nonperfusion was noted on fluorescein angiography at day 90. Pigment mottling was noted at day 90 in both eyes in the 60-Gy group, with antecedent retinal whitening at day 30 in one. The 90-Gy group uniformly developed retinal whitening by day 30, with cotton-wool infarcts, microaneurysms, retinal capillary dropout, and choroidal ischemia following by day 90. Indocyanine green angiograms were performed at day 90. No abnormalities were noted in the control, 16-, 24-, 42-, or 60-Gy groups. Choroidal ischemia was found in the 90-Gy group.

The treatment lesions were identical to the treatment spots that received 60 Gy seen in the prior study, which evaluated an early prototype of the IRay system. The region outside the treatment spot appeared normal on fun-

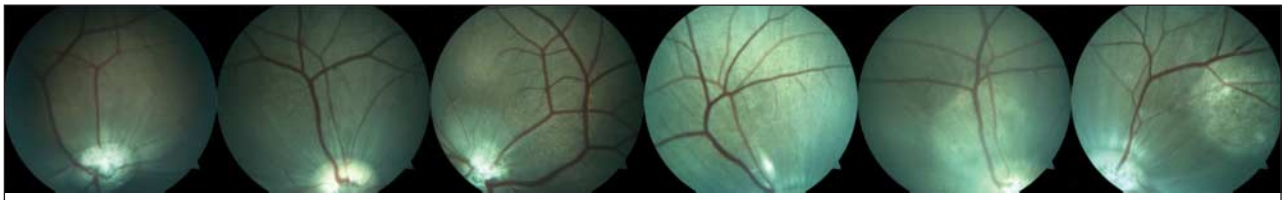

Figure 3. Dose-dependent response by day 90. The treatment spot is easily visible in the 60 Gy and 90 Gy groups, more difficult to locate in intermediate groups, and absent in the 16 Gy and control group (to convert gray to rads, multiply by 100).

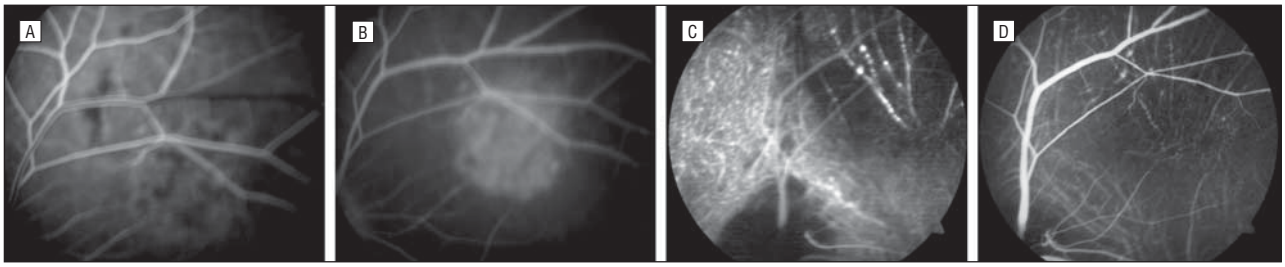

Figure 4. Fluorescein (FA) and indocyanine green (ICG) angiographic images at day 90 of an eye that received 90 Gy (to convert to gray to rads, multiply by 100). An early (A) and a late (B) frame of the FA shows retinal pigment epithelium changes. Early (C) and late (D) frames of the ICG, respectively, demonstrate choroidal ischemia.

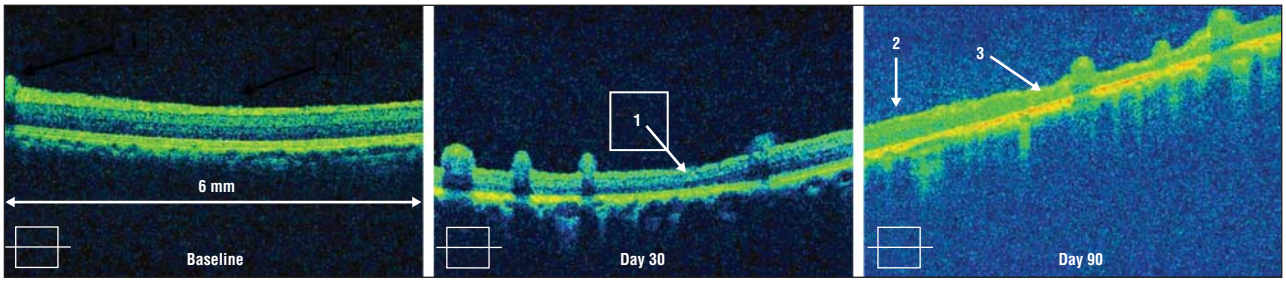

Figure 5. Spectral domain optical coherence tomographic (OCT) raster images demonstrating the progression of retinal thinning seen with the 90- Gy (to convert gray to rads, multiply by 100) group. The penumbra is well defined and is represented by severe retinal thinning. At day 30, OCT revealed early signs of degradation of the retina depicted by arrow 1. By day 90, the region of retina between arrows 2 and 3 is a transition zone between normal-appearing retinal morphology on OCT and clearly abnormal retina.

dus examination, suggesting that a rapid fall-off in dose is occurring as per the performance specification of the IRay. None of the animals who received doses below 42 Gy showed any evidence of effect on the retina.

Spectral domain OCT was performed at days 7, 30, 60, and 90. In the 60- and 90-Gy groups, retinal thinning was noted as early as day 7, and thinning progressed until 90 days after radiation treatment (**Figure 5**). The 42-Gy group showed questionable thinning by day 90, and no spectral-domain OCT abnormalities were found in the lower-dose treatment groups.

COMMENT

Stereotactic radiation dosing of 12 miniswine retinas with doses up to 90 Gy delivered in 3 sequential beams was accomplished successfully using a portable collimated external beam x-ray delivery system. Treatment was well tolerated by all animals and was not associated with any significant external ocular or corneal anomalies or intraocular pressure changes up to postdose day 90. A cortical cataract noted in the 60-Gy group may have been radiation related. The delivery device was developed with the anatomical parameters of a human eye with the assumption of average anatomical length of 24 mm and normal cornea diameters of 12 mm. The pig eyes used in the study averaged 18 mm in length, with similar corneal diameters. Given this, the beam paths could potentially pass anterior to the pars plana and through the peripheral lens in this animal model.

Fundus photography, fluorescein angiography, and spectral-domain OCT demonstrated dose-dependent changes that included a circumscribed lesion spot with significant retinal thinning and retinal depigmentation, cotton-wool spots, microaneurysm formation, retinal capillary nonperfusion, delay choroidal filling, and late angiographic staining—all hallmarks of radiation vasculopathy. While the findings of acute photoreceptor loss and retinal thinning suggest a higher effective dose, a working model failed to show such an increase.[7] No significant findings were made at the lower treatment doses, particularly in the 16-Gy group.

Previous randomized clinical studies have evaluated radiation for the treatment of AMD.[9-12] The Radiation Therapy for Age-Related Macular Degeneration study was a multicenter, randomized, double-masked trial of 205 patients with subfoveal CNV (classic or occult lesions of less than

6 macular photocoagulation study disc areas). Patients were randomized to either 8 fractions of 2-Gy external beam radiation or to control sham therapy. At 1 year, no significant benefit to radiation therapy was noted, as moderate vision loss (≥3 lines) occurred in 51.1% of the treatment group and 52.6% of the control group. Other studies using an external beam exhibited similar results.

However, a recent study of strontium-90 radiation in conjunction with 2 bevacizumab injections in 27 patients with CNV yielded impressive results. Twelve months after targeted delivery of 24 Gy, 96% of patients had stable or improved vision and 48% had a gain of 3 or more lines.[13] This demonstrated benefit with 24 Gy of radiation raises the plausibility of a therapeutic effect in that dose range.

This study failed to show significant abnormalities associated with excess radiation to the targeted tissues except in the 42-Gy and higher test groups. Based on our results, the threshold for radiation retinal toxicity in Yucatan miniswine appears to be above 16 Gy, a potentially clinically relevant dose. Radiation has been known to cause an occlusive microangiopathy secondary to endothelial cell loss and capillary closure occurring after ionizing radiation treatment. Photoreceptors are more resistant to radiation, and animal studies have shown damage to rods with 20 Gy and to cones with 100 Gy. Although damage to photoreceptors has been seen, the inner retinal layers and retinal vascular cells are most affected, causing vessel closure and ischemic retinopathy similar to that in diabetic retinopathy. This description correlates well with the findings presented here. Interestingly, the changes were noted as early as 1 week by spectral-domain OCT, and the evolution of the insult was followed up until day 90.

No gross findings of the anterior segment, excluding the lens, were seen in the animals treated. Radiation has been used for a variety of anterior segment indications such as pterygia. In the study by Beyer,[14] 30 Gy of strontium-90 β irradiation decreased pterygia recurrence and was not associated with significant ocular adverse effects. However, in the cases of external beam radiation for conjunctival melanoma, doses up to 45 Gy have been associated with lash loss, limbal stem cell deficiency, and cataract formation.[15] The IRay system delivered up to 66.7 Gy to the sclera in the highest dose category without any notable damage. While the tissue may have slow cell turnover time that may account for the radioresistant properties of the anterior segment, the small size of the beams at the scleral surface may be of considerable benefit in reducing the undesirable effects of radiation. Even with the use of multibeam treatment patterns, no significant adverse events were seen despite the close proximity of each treatment beam to its predecessor.

The lens in the animal that received 60 Gy showed a focal cortical cataract. The sclera, cornea, and iris were unchanged at these time points. The fact that the cataract is focal and limited to a higher dose may be consistent with the design parameters of the system. The system uses the I-guide vacuum-coupled lens for ocular fixation, and the treatment algorithm is based on the axial length of the human subject. Additionally, the miniswine eyes differ somewhat structurally from human eyes in that the cornea is much larger than the overall size of the eye. Because of these factors, the limbus may have been treated in some instances instead of the beam passing entirely through the pars plana region. Importantly, there were no skin effects (also as expected, because the eyelid is retracted and the x-ray energy is directed sufficiently far from the eyelid to not result in toxic effects). As far as the sclera and cornea are concerned, there were also no detected abnormalities, consistent with a high threshold for damage described in the literature.

In summary, with short-term follow-up, transscleral stereotactic radiation dosing to the miniswine revealed no evidence of apparent abnormality with doses less than 24 Gy and focal destruction of the choroid and retina with doses greater than 42 Gy. More long-term follow-up will be required to determine late radiation effects.

Submitted for Publication: November 18, 2009; final revision received July 21, 2010; accepted August 1, 2010.
Correspondence: Rishi P. Singh, MD, 9500 Euclid Ave, i-32, Cleveland, OH 44195 (singhr@ccf.org).
Financial Disclosure: Dr Singh has received honoraria from Oraya Therapeutics, Alcon, and Genentech. Drs Gertner and Shusterman are employees of Oraya Therapeutics. Dr Moshfeghi serves as a consultant for Oraya and retains equity for his participation. Dr Danis serves as a consultant for Oraya Therapeutics.
Funding/Support: This study was supported by Oraya Therapeutics, California.

REFERENCES

1. Moshfeghi DM, Kaiser PK, Scott IU, et al. Acute endophthalmitis following intravitreal triamcinolone acetonide injection. *Am J Ophthalmol.* 2003;136(5):791-796.
2. Jager RD, Aiello LP, Patel SC, Cunningham ET Jr. Risks of intravitreous injection: a comprehensive review. *Retina.* 2004;24(5):676-698.
3. Finger PT, Chakravarthy U, Augsburger JJ. Radiotherapy and the treatment of age-related macular degeneration: external beam radiation therapy is effective in the treatment of age-related macular degeneration. *Arch Ophthalmol.* 1998; 116(11):1507-1511.
4. Takahashi Y, Teshima T, Kawaguchi N, et al. Heavy ion irradiation inhibits in vitro angiogenesis even at sublethal dose. *Cancer Res.* 2003;63(14):4253-4257.
5. Johnson LK, Longenecker JP, Fajardo. Differential radiation response of cultured endothelial cells and smooth myocytes. *Anal Quant Cytol.* 1982;4(3):188-198.
6. De Schaepdrijver L, Simoens P, Pollet L, Lauwers H, De Laey JJ. Morphologic and clinical study of the retinal circulation in the miniature pig, B: fluorescein angiography of the retina. *Exp Eye Res.* 1992;54(6):975-985.
7. Lee C, Chell E, Gertner M, et al. Dosimetry characterization of a multibeam radiotherapy treatment for age-related macular degeneration. *Med Phys.* 2008; 35(11):5151-5160.
8. McDonald T, Shadduck JA. Eye irritation. In: Marzulli F, Maibach HI, eds. *Advances in Modern Toxicology: Dermatoxicology.* Washington, DC: Hemisphere Publishing Corp; 1977:579-582.
9. Stalmans P, Leys A, Van Limbergen E. External beam radiotherapy (20 Gy, 2 Gy fractions) fails to control the growth of choroidal neovascularization in age-related macular degeneration: a review of 111 cases. *Retina.* 1997;17(6):481-492.
10. Hart PM, Chakravarthy U, Mackenzie G, et al. Visual outcomes in the subfoveal radiotherapy study: a randomized controlled trial of teletherapy for age-related macular degeneration. *Arch Ophthalmol.* 2002;120(8):1029-1038.
11. Marcus DM, Peskin E, Maguire M, et al; AMDRT Research Group. The age-related macular degeneration radiotherapy trial (AMDRT): one year results from a pilot study. *Am J Ophthalmol.* 2004;138(5):818-828.
12. Valmaggia C, Ries G, Ballinari P. Radiotherapy for subfoveal choroidal neovascularization in age-related macular degeneration: a randomized clinical trial. *Am J Ophthalmol.* 2002;133(4):521-529.
13. Heier JS. Concomitant therapy of epiretinal radiation and bevacizumab for neovascular AMD. Paper presented at: Annual Meeting of the American Academy of Ophthalmology; October 2007; New Orleans, LA.
14. Beyer DC. Pterygia: single-fraction postoperative beta irradiation. *Radiology.* 1991; 178(2):569-571.
15. Wuestemeyer H, Sauerwein W, Meller D, et al. Proton radiotherapy as an alternative to exenteration in the management of extended conjunctival melanoma. *Graefes Arch Clin Exp Ophthalmol.* 2006;244(4):438-446.