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Evaluation of Microcollimated Pars Plana External Beam Radiation in the Porcine Eye

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Abstract

Purpose: To evaluate the clinical and histological side effects of a prototype stereotactic radiotherapy system delivering microcollimated external beam radiation through pars plana in porcine eyes.

Methods: Five Yucatan mini-swine (10 eyes) were randomized to five treatment groups. Eight eyes were dosed with X-ray radiation on Day 1, and two eyes served as untreated controls. Treated eyes received doses up to 60 Gy to the retina and up to 130 Gy to the sclera using single or overlapping beams. The treatment beams were highly collimated such that the diameter was approximately 2.5 mm on the sclera and 3 mm on the retinal surface. Fundus photography, fluorescein angiography (FA), and spectral domain optical coherence tomography (SD-OCT) were obtained on days 7, 30, 60, and 110. Images were examined by a masked grader and evaluated for abnormalities. Animals were sacrificed on day 111 and gross and histopathological analysis was conducted.

Results: Histological and gross changes to eye structures including conjunctiva and lens were minimal at all doses. Fundus, FA, and SD-OCT of the targeted region failed to disclose any abnormality in the control or 21 Gy treated animals. In the 42 and 60 Gy animals, hypopigmented spots were noted after treatment on clinical exam, and corresponding hyperfluorescent staining was seen in late frames. No evidence of choroidal hypoperfusion was seen. The histological specimens from the 60 Gy animals showed photoreceptor loss and displacement of cone nuclei.

Conclusion: Transcleral stereotactic radiation dosing in porcine eyes can be accomplished with no significant adverse events as doses less than 42 Gy.

Keywords: Radiation; External beam; Macula; Porcine eyes; Macular degeneration

Introduction

The past decade has brought about a dramatic upgrade in our management of neovascular age related macular degeneration (AMD). The advent of photodynamic and anti-VEGF therapies, in particular, has greatly increased the prognosis for preservation and improvement of vision in this disease population.[1,2] However, all these therapies have some drawbacks. Photodynamic therapy (PDT) reduces moderate visual loss only to a modest degree, and only a few patients experience improved vision. Furthermore, the recurrence rate is high, with over 90% of patients requiring re-treatment after 3 months. [3] Ranibizumab, the most efficacious treatment of neovascular AMD to date, still leaves room for improvement. For example, 30% of patients remain in the 0 to 3 lines of vision loss category, and the treatment requires multiple re-injections as suggested by the Prospective Optical Coherence Tomography Imaging Patients with Neovascular AMD Treated with Intra-Ocular Lucentis Study (PrONTO), with the mean of injections for the first year being 5.6. [4] Intravitreal injections also carry the risk of retinal tears, rhegmatogenous retinal detachment, vitreous hemorrhage, hypotony, endophthalmitis, pseudoendophthalmitis, and possibly thromboembolic events in populations at high risk. [5,6] Furthermore, the social and budgetary burdens of repeated visits, injection fees, and pharmaceutical costs are large, and given the expanding population of AMD patients, alternative therapies with less re-treatment would be highly desirable.

Most chronic diseases are treated via multi-modality therapies because they typically have complex and multiple etiologies. Combining therapies so as to attack the disease from multiple pathways leads to synergies in outcome. In the case of neovascular AMD, one alternative to anti-VEGF therapy and photodynamic therapy is external beam radiation. 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. [7] Theoretically, precise radiation delivery to the macula can selectively inhibit proliferating endothelial cells with limited destruction of retinal tissue and no systemic side effects. Moreover, Takahasi and colleagues found that new capillaries or vessels are more sensitive than larger vessels or fibroblasts. [8] Vascular endothelial cells in particular are more radiosensitive than other mesenchymal cells types such as fibroblasts and smooth muscle cells. [9] As an additional benefit, ionizing radiation can inhibit the inflammatory response, which is thought by many to play a role in the formation of CNV in AMD.

The rationale for radiotherapy in neovascular AMD is multifold. First, it would attenuate the acute and delayed inflammatory response and subsequent CNV reactivation. Second it would inhibit the rapid formation of fibroblasts after treatment, and thus lead to less scar formation (as it does in the treatment of dermal keloids). Finally, it

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would lead to the closure of rapidly dividing endothelial cells which are the main pathological component of the CNV. This has been most recently demonstrated by the use of epiretinal radiation (Epi-Rad90™ Ophthalmic System), with results similar to anti-VEGF therapy alone. [10].

This study utilized a prototype stereotactic radiotherapy system for the delivery of micro collimated external beam radiation. Three sequential beams were delivered transconjunctivally, through pars plana, converging upon the retina. The study evaluated radiation to the eye of Yucatan mini-swine in escalating doses. This study examined the technological proof of concept as well as the dose range for potential adverse events using clinical exam, gross and microscopic histology, fundus photography, fluorescein angiography (FA), and spectral domain optical coherence tomography (SD-OCT).
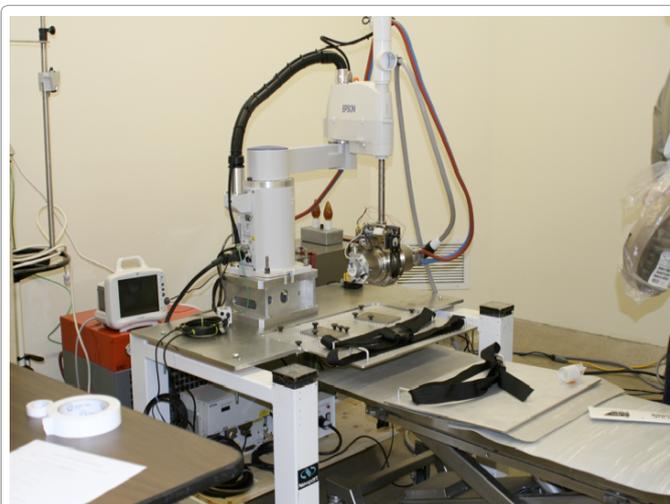

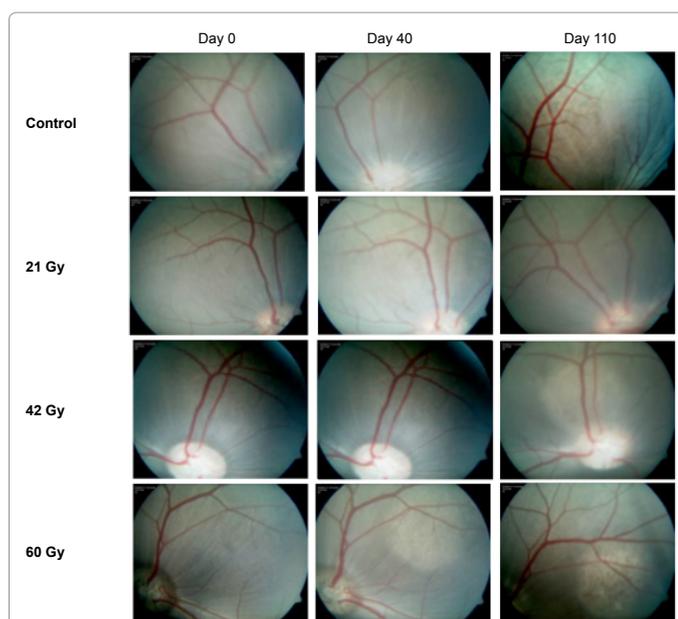**Figure 1:** Oraya Animal Test System (ATS) orthovoltage X-ray robot device used for delivery of radiation.

Figure 2: Fundus photos of control, 21 Gy, 42 Gy, and 60 Gy doses at Days 0, 40, and 110 Days.

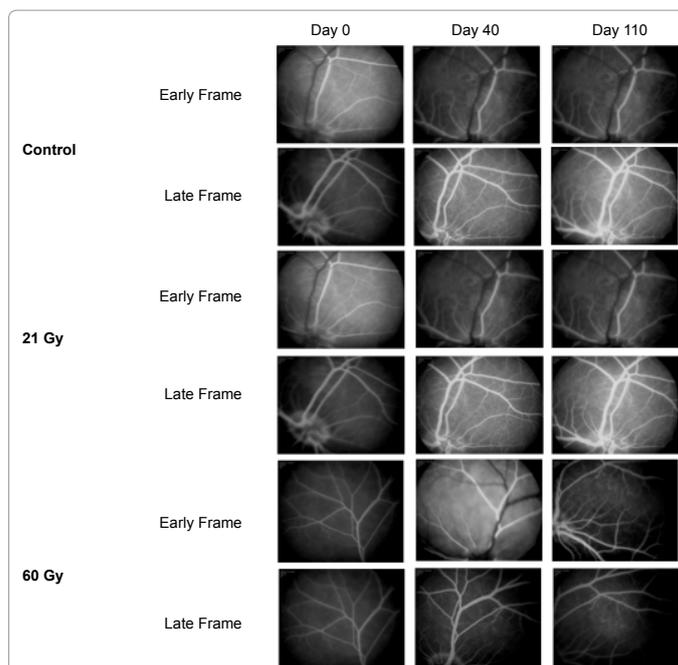**Figure 3:** Fluorescein angiography in control, 21 Gy, and 60 Gy doses at Day 0, 40, and 110 Days.
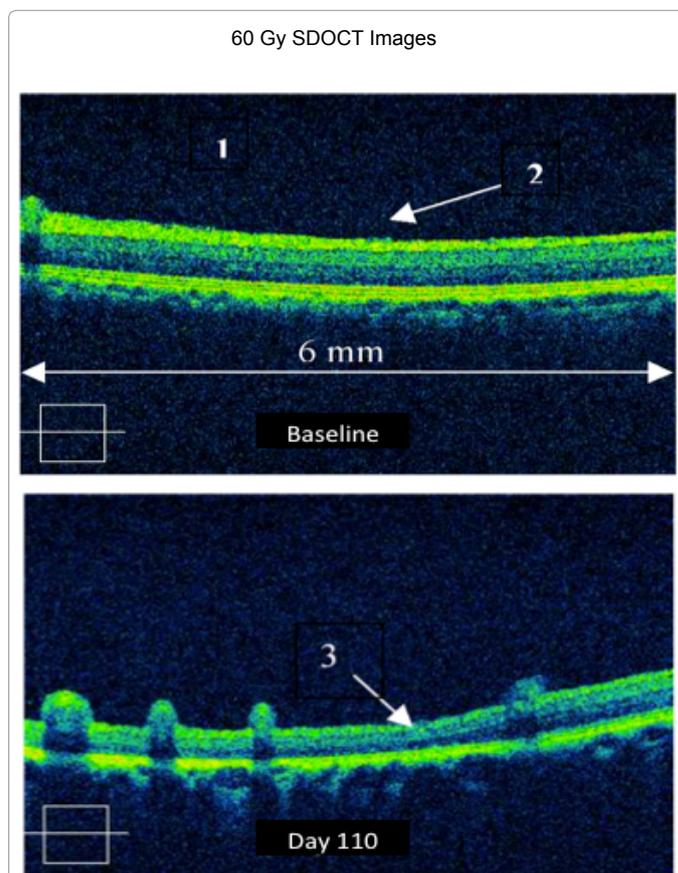

Figure 4: Cirrus spectral domain images of 60 Gy dose at Day 0 and Day 110 demonstrating retinal thinning. Arrow 2 demonstrates the normal inner retinal layers seen at the baseline exam. At day 110, the OCT reveals early signs of the retinal thinning with a clear transition zone marked as arrow 3.

Animal No:	1		2		3		4		5	
Eye	OS	OD	OS	OD	OS	OD	OS	OD	OS	OD
Total Dose to Retina (Gy)	60	C	42	21	C	60	21	42	60	42
Beam Energy (KeV)	100	0	100	100	0	100	100	100	100	100
Total Dose to Sclera (Gy)	129.5	0	84.6	41.1	0	124.5	43.5	87.3	123.9	85.1
Number of Beams	1	3	3	3	3	3	3	3	3	1
Dose per Beam (Gy)	60	0	14	7	0	20	7	14	20	42
Irradiation Time per Beam (mins)	42.5	0	9.8	4.83	0	14.3	5	10.03	14.26	29.6
Total Irradiation Time (mins)	42.5	0	29.4	14.49	0	42.9	15	30.09	42.78	29.6

Table 1: Treatment Dosing Schema of Yucatan Mini-Swine with Oraya Animal Test System (ATS).

Figure 5A: 60 Gy treatment demonstrating sharply demarcated, round tissue reaction that was noted measuring approximately 2.5 mm in diameter.
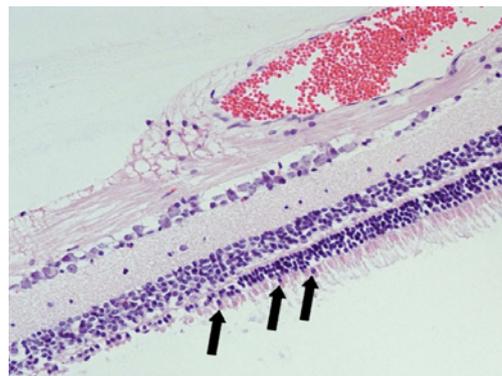

Figure 5C: Histology of single beam 60 Gy specimen 20x magnification, demonstrating photoreceptor loss and transition zone (H&E stain).
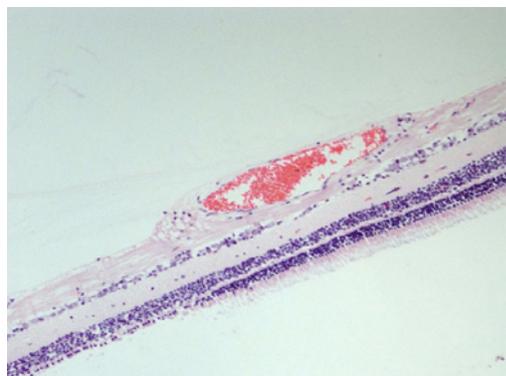

Figure 5B: Histology section of single beam 60 Gy specimen, 10x magnification, demonstrating photoreceptor loss and transition zone (H&E stain).

Figure 5D: Histology of single beam 60 Gy specimen demonstrating flattened RPE cells (H&E stain).

Study design and treatment

Yucatan mini-swine were chosen for this study due to their resemblance in structure, size, holangiotic vascular pattern, and non-tapetal fundus with that of the human eye.[11] Institutional animal care and use committee (IACUC) approval was obtained prior to study commencement and the study adhered to the ARVO animal statement on the treatment of animals. The animals were obtained from S&S Farms (Ramona, CA). Upon arrival, animals were examined to ensure that they were healthy and quarantined for eight days before study enrollment.

Ophthalmic examinations (slit lamp and indirect ophthalmoscopy), fundus photography, and fluorescein angiography (FA) were performed on each eye prior to treatment and at 7, 40, 75, and 110 days. Ophthalmic observations of both eyes were scored and recorded according to the McDonald Shadduck system (as described in *Dermatoxicology*, F.N. Marzulli and H.I. Maibach, 1977 "Eye Irritation", T.O. McDonald and

J.A. Shadduck (pages 579-582). Intraocular pressure (IOP) was measured with a Tono-Pen XL (Medtronic ENT, Jacksonville, FL) on days 7, 42, 75, and 110. Spectral domain optical coherence tomography (OCT) was performed with a Cirrus HD-OCT (Carl Zeiss Meditec, Dublin, CA) and with an RTVue FD-OCT (Optovue, Fremont, CA). Two OCT devices were used to determine whether imaging anesthetized pigs was feasible with both machines. Swine were weighed prior to each anesthesia procedure (radiation dosing or ocular photography), and were euthanized on Day 111 or 112. Globes were collected, fixed in 4% formalin, and submitted for histopathological evaluation.

The radiotherapy was delivered in a single session via the Oraya Animal Test System (ATS). Five animals (10 eyes) were randomized to five treatment groups. Eight eyes were dosed with radiation on Day 0, and two eyes served as untreated controls. A collimated, single-beam device (including a low-energy orthovoltage X-ray source, scleral interface and automated robotic positioning system) was used to deliver

X-ray energy to the retina of each dosed eye (Figure 1). The robotic positioning system allowed for the single beam to be projected to the retina from various angles. Radiation treatments were performed at 100 keV maximum beam energy in 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 2.5 mm and 3 mm on the retinal surface. For six eyes, the radiation dose was delivered in three consecutive beams, through various scleral entry points calculated to place the beams onto a single retinal target. For two eyes, the radiation dose was delivered in one beam. Lateral canthotomies were performed on all eyes prior to dosing for access to the limbal space. Animal received between 0 Gy and 60 Gy and detailed treatment parameters and total radiation delivery are shown in (Table 1).

Results

There was no unscheduled mortality and all swine gained weight during the study. Slit lamp and gross ophthalmic examinations revealed conjunctival irritation (congestion, swelling, and/or discharge) on Day 7 in six of eight 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. Intra-ocular pressure (IOP) was measured on days 7, 42, 75, and 110 and varied throughout the study in all eyes. There was no significant difference noted in intraocular pressure within the same animal over the course of the study and amongst control and treated animals at all timepoints.

Fundus photography, SD-OCT, and FA were performed prior to irradiation and after at days 7, 40, 75, and 110. Each article was examined by a masked grader (Ronald P. Danis, M.D., Professor of Ophthalmology and Visual Sciences, Univ. of Wisconsin) and evaluated for abnormalities. Fundus evaluation revealed no abnormalities of the targeted area except in the 42 and 60 Gy treated animals. At Day 7, a circular retinal lesion with apparent depigmentation was noted in the area targeted with 60 Gy (Figure 2). The lesion persisted on fundus exam until Day 110. Additionally, the 42 Gy single beam eye demonstrated a retinal lesion on day 110, visible as a greenish hue inferior to the optic nerve, and representing a change from pre-treatment fundus photographs. There were no retinal hemorrhages, cotton wool spots, or vascular occlusions/sheathing seen in any specimen. FA demonstrated staining in late frames of the angiogram in the 60 Gy animals, but no evidence of early hypoperfusion changes within the retinal circulation. Additionally, there was no delay within the choroidal fluorescence pattern to suggest ischemia or poor perfusion (Figure 3). The high-resolution OCT of the targeted region failed to disclose any definite abnormality in the control and treated animals up to 42 Gy. In the 60 Gy animals, SDOCT demonstrated subtle retinal thinning with a transition zone. This was first seen at day 30 and didn't not change significantly to day 110 (Figure 4).

Enucleated eyes were prepared for gross and histological analysis at days 111 & 112. Gross examination of the exterior aspects of the globes revealed no evidence of scleral ectasia, scarring or fibrosis, corneal opacity or episcleral tissue reactions. Of note, the sclera in the 60 Gy single-beam group received a dose of 129.5 Gy, without any apparent gross findings seen. Internal examination revealed unremarkable intraocular structures in all eyes, except Group A, the single-beam 60 Gy group. In this eye, in the posterior pole juxta-papillary region (akin to the human macula), a sharply demarcated, round tissue reaction was noted. This ~2.5 mm region appeared to be faintly hypopigmented (Figure 5A).

Histological examination of the anterior segment by a masked pathologist was performed in the radiated regions. The histology of the cornea, limbus, and sclera was normal in all eyes, without signs of necrosis or inflammation. The ciliary body and pars plana regions were free of cellular infiltrates and edema. The sclera, choroid, retinal pigment epithelium (RPE), and neural retina were step-sectioned and examined at multiple levels. There were no abnormalities seen except in the 60 Gy single beam specimen. The gross finding in this eye shown in Figure 3, demonstrated a histological correlate, where photoreceptor loss was seen, with a clearly demarcated transition zone (Figure 5B and 5C). The inner retina was largely unaffected, with a continuous layer of ganglion cells showing a rich complement of Nissil staining (polyribosomes), indicative of health. The RPE layer was intact throughout the lesion, with the only pathology evidenced by some flattening of the RPE cells at the center (Figure 5D), which was not felt to be artifactual but representative of apoptosis induced by radiation.

Discussion

Stereotactic radiation dosing of 8 swine retinas with 21, 42, or 60 Gray delivered in 1 or 3 beams was accomplished successfully using the prototype system. There were angiographic, gross, and histological retinal changes seen at the highest tested radiation doses associated with photoreceptor loss and preservation of the inner retinal layers.

Previous randomized clinical studies have evaluated radiation for the treatment of age related macular degeneration. [12-15] The Radiation Therapy for Age-Related Macular Degeneration (RAD) study was a multicenter, randomized double-masked trial of 205 patients with subfoveal CNV (classic or occult lesions less than 6 MPS 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 utilizing external beam exhibited similar results.

Why have these trials failed to show significant efficacy outcomes? Marcus and colleagues hypothesized that the dose of radiation delivered to the macula was limited by technical inability to precisely localize energy on target. External beam radiation utilize linear accelerators which project a wide beam across ipsilateral and contralateral critical structures. [12] Due to this of imprecision, only a small amount of radiation was applied at each setting. Furthermore, no data supports the assertion that radiation fractions are indeed cumulative in biologic effect on choroidal neovascularization. Also, the patients' eyes were not mapped in space relative to the X-ray beam, and were not immobilized throughout treatment. Thus, confirmation and stability of beam targeting during the therapeutic fraction were not achieved, and fixed-beam high-energy devices directed radiation across critical structures, such as the lens, the ciliary bodies, and the optic nerve. The Oraya ATS system is highly precise in delivery because of the X-ray beam collimation, size, and delivery through the pars plana avoiding significant ocular structures.

The study performed failed to show significant abnormalities associated with excess radiation to the targeted tissues except in the 42 and 60 Gy test group. The threshold for radiation in Yucatan mini-swine are not well documented and appears to be between 42 Gy and 60 Gy based on our results. 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. [16] This description correlates well with the 60 Gy hypopigmented lesions which stained in late frames and manifested photoreceptor displacement/loss and RPE flattening. The failure of the SDOCT to show significant change in the treated animals in lower doses than 60 Gy may be due to insensitivity of the device in detecting photoreceptor loss or RPE flattening and should be re-evaluated in future studies with longer time points. In addition, the choice of a different SDOCT that utilizes image averaging (Heidelberg SDOCT) would substantially improve the evaluation of the inner/outer segments.

Interestingly no gross or histological findings were seen in the anterior segment of the animals treated. Radiation has been used for a variety of anterior segment indications such as ptergia. In the study by Beyer and colleagues, 30 Gy of Strontium 90 beta irridiation decreased ptergia recurrence and was not associated with significant ocular side effects. [17] 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. [18] The Oraya ATS system delivered 129.5 Gy in the highest dose category to the sclera without significant gross or histological damage seen. While the tissue may have slow cell turnover time which may account for the radioresistant properties of the anterior segment, the small size of the beams at the scleral surface (2.5 mm) my account for the lack of side effects seen. Even with the multibeamed animals, no significant adverse events were seen despite the close proximity of each treatment beam.

At face value, the dose of radiation administered in one session between 42 and 60 Gy appears to be relatively safe in this short follow up. The question remains what the toxicity or safety would be in a much longer follow up or whether a change in the beam angles of entry will reduce the potential for delivery of radiation to non-macular structures. [19] Additionally, it is unclear what effect this dose of radiation will have in the compromised macular environment of macular degeneration since the biology of such entity could prove to be different. Finally, evaluation of RPE apoptosis would be useful as an additional safety measurement. These will be addressed in future studies with the actual clinical device and by using an artificially induced animal model of macular degeneration.

In summary, transcleral stereotactic radiation dosing to the mini-swine can be achieved with no significant adverse events seen at doses less than 42 Gy. Further studies are being conducted with the clinical grade device to verify safety and precise targeting in the mini-swine model.

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