

# A Novel Device for Suprachoroidal Drug Delivery to Retina: Evaluation in Nonhuman Primates

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**Received:** December 12, 2022

**Accepted:** April 4, 2023

**Published:** June 8, 2023

**Keywords:** retina; suprachoroidal injection; drug delivery; nonhuman primates

**Citation:** Rotenstreich Y, Sher I, Lawrence M, Mangelus M, Ingerman A, Barak Y. A novel device for suprachoroidal drug delivery to retina: Evaluation in nonhuman primates. *Transl Vis Sci Technol*. 2023;12(6):3. <https://doi.org/10.1167/tvst.12.6.3>

**Purpose:** Evaluation of distribution and tolerance of suprachoroidal injection of indocyanine green (ICG) in nonhuman primates (NHPs) using a novel suprachoroidal (SC) delivery technology.

**Methods:** Three live and three euthanized African green monkeys were injected with 150 or 200  $\mu$ L ICG/eye into the SC space of both eyes, 2.5 mm posterior to the limbus in the inferior quadrant, utilizing a novel SC injector. Eyes were analyzed by imaging of scleral flatmounts. Live animals were observed for 24 hours for general health. Ophthalmic evaluation included slit-lamp biomicroscopy, tonometry, fundus imaging, confocal laser ophthalmoscopy, and spectral-domain optical coherence tomography (SD-OCT) before and at 10 minutes and 1, 3, and 24 hours post-injection.

**Results:** SC dosing was successfully performed in all eyes. Infrared fundus imaging demonstrated ICG distribution throughout the posterior segment, reaching the macula within 24 hours post-injection. No inflammation, intravitreal penetration, SC blebs, retinal detachment, or hemorrhages were detected. No significant changes were observed in retinal thickness by SD-OCT ( $P = 0.267$ , ANOVA). A mild, statistically insignificant elevation in intraocular pressure was observed within 10 minutes post-injection (mean  $\pm$  standard error:  $7.28 \pm 5.09$  mmHg;  $P = 0.061$ ) and was spontaneously resolved within the first hour after dosing.

**Conclusions:** Suprachoroidal injection of 150 to 200  $\mu$ L ICG dye was successfully performed and well tolerated in NHP eyes, with rapid distribution into the macular region and throughout the posterior pole.

**Translational Relevance:** This novel SC drug delivery system may potentially provide safe and effective delivery of therapeutics to the posterior pole region in humans.

## Introduction

Age-related macular degeneration (AMD), diabetic retinopathy, and glaucoma are among the leading causes of blindness and moderate to severe vision impairment worldwide.<sup>1</sup> Developing efficient and safe treatments for these diseases remains a challenge, as these diseases are chronic and refractory and require

delivering the treatment to the posterior segment of the eye.

Repeated intravitreal (IVT) injections of anti-vascular endothelial growth factor (VEGF) and corticosteroids are considered the standard care for neovascular AMD and diabetic macular edema.<sup>2</sup> Although excellent therapeutic effects were obtained in clinical trials, real-world data on patients receiving IVT injections of anti-VEGF therapies suggest worse visual

outcomes compared with clinical trials, most likely due to the high burden of repeated injections and poor patient compliance.<sup>3,4</sup> Some patients do not respond to treatment regardless of the frequency of injections.<sup>5</sup> In addition, several side-effects related to the IVT injection procedure have been reported, including long-term increases in intraocular pressure (IOP)<sup>6</sup> and ocular inflammation.<sup>7</sup> Furthermore, IVT injected therapeutics must cross the inner limiting membrane to reach the target organs — namely, retina, retinal pigment epithelium (RPE), and choroid, which may reduce their therapeutic efficacy.<sup>8,9</sup> Moreover, exposure of the anterior segment and lens to IVT injected corticosteroids is associated with undesirable side effects including cataracts and elevated IOP.<sup>10,11</sup>

With the advancement in regenerative medicine, there is increasing interest in delivering cell and gene therapies for rare inherited retinal degeneration diseases, as well as more common diseases such as AMD.<sup>12–16</sup> Even though some clinical trials reported promising results following IVT delivery of viral vectors and cells, the distance from target tissue, exposure of anterior segment and lens, and recent reported safety concerns<sup>16–18</sup> indicate the need for development of additional safe and efficient posterior segment delivery routes.

Suprachoroidal (SC) delivery is a relatively new promising approach for the delivery of therapeutics in close proximity to the target chorioretina, avoiding direct entry into the inner eye. As the exposure of the vitreous, lens, and anterior chamber is minimal, SC injection potentially reduces the risks for endophthalmitis, retinal detachment, IOP elevation, and cataract formation.<sup>19,20</sup> In the last decade, several preclinical studies and clinical trials assessed the safety and efficacy of SC delivery of small molecules, steroids, and recently also viral vectors for gene therapy using microneedles that are inserted into the sclera at a perpendicular angle.<sup>21–23</sup> The injection procedure is relatively simple and is considered minimally invasive. In general, SC injections of aqueous soluble molecules were found to result in faster clearance compared to conventional intravitreal injections; however, shortly after the SC injection, the concentrations of injected therapeutics in the choroid, RPE, and retina were higher compared to IVT injections.<sup>21–23</sup>

In 2021, the U.S. Food and Drug Administration (FDA) approved the SC delivery of a triamcinolone acetonide injectable suspension (CLS-TA; Clearside Biomedical, Alpharetta, GA) for the treatment of macular edema associated with uveitis, following the successful PEACHTREE and MAGNOLIA clinical studies.<sup>24,25</sup> Although limited therapeutic efficacy was obtained in phase III studies with patients with retinal vein occlusion, no serious adverse events related to

study treatment were observed in any of those clinical trials or in clinical studies with diabetic macular edema.<sup>21,24–27</sup>

Following successful preclinical studies in rats, pigs, and nonhuman primates (NHPs),<sup>28</sup> promising preliminary results were presented for SC delivery of an anti-VEGF fab transgene using adeno-associated virus 8 (AAV8) vector (RGX-314; REGENXBIO Inc., Rockville, MD) in patients with exudative macular degeneration and diabetic retinopathy. The SC injection was well tolerated and patients demonstrated stable best corrected visual acuity and central retinal thickness with a 70% reduction in anti-VEGF treatment burden.<sup>29</sup>

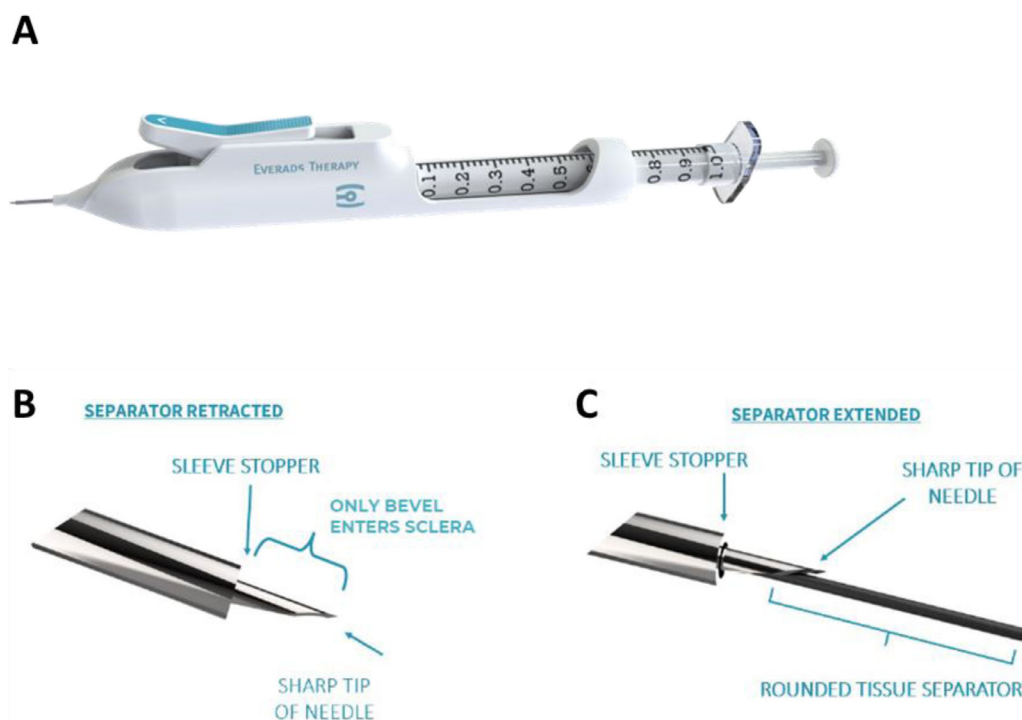
Some of the limitations of SC injection using microneedles include a limited injection volume (0.05–0.1 mL), distribution of injected therapeutics in proximity to the injection site, and backflow of the injected solution.<sup>20,30,31</sup> Another SC drug delivery approach is a microcannulation system that is designed for direct delivery of therapeutics in submacular and peripapillary regions by manual insertion of a catheter through a scleral incision into the SC and advancing toward the macula or optic nerve head. The system contains a curved cannula and a fiberoptic light source for live visualization and maneuvering within the SC space.<sup>32</sup> It has been tested in monkeys and pigs, followed by a clinical trial for the delivery of bevacizumab and triamcinolone into the submacular SC space.<sup>32,33</sup> However, this method is invasive, requiring an operating room environment and experienced surgeons.

In previous studies, we reported the development of a novel approach for SC delivery, based on tangential injection through the sclera, using an injection device that contains a wide needle and a blunt tissue separator. Using this approach, dyes, human cells, nanoparticle suspensions, and antibodies (bevacizumab) were injected into the SC of rats, rabbits, and pigs and resulted in fast distribution of the injected therapeutics across the choroid layers up to the RPE, covering 80% to 90% of the posterior segment with no hemorrhages, retinal detachment, or other apparent side-effects.<sup>9,34–36</sup> Here, we assessed the efficacy and short-term safety of SC injection of indocyanine green (ICG) in NHPs using a novel injector designed for SC delivery in primates.

## Methods

### Animal Care and Handling

Monkeys were housed in the Virscio primate enclosure facility at the St. Kitts Biomedical Research



**Figure 1.** (A) The SC drug delivery device. (B) A close up of the needle and bevel of the device with the tissue separator retracted within the needle. (C) The tissue separator extended.

Foundation (SKBRF) campus, Lower Bourryeau Estate, St. Kitts, West Indies. The SKBRF Institutional Animal Care Committee approved and supervised all procedures and experiments (approval #AC18178). All experiments adhered to the recommendations of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

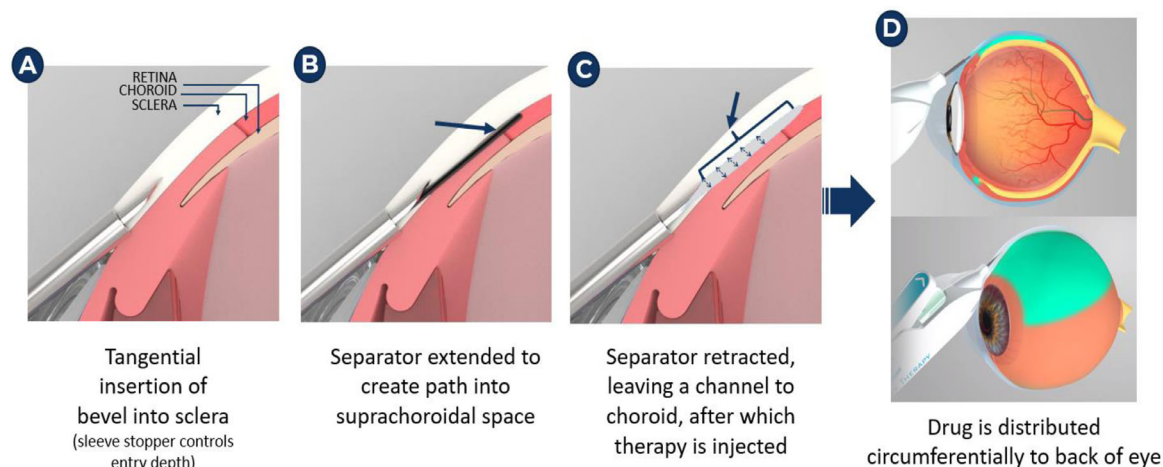
Three monkeys with normal ophthalmic findings that were to be euthanized for other study purposes were assigned to group 1. Three additional monkeys with normal ophthalmic findings were assigned to group 2. All monkeys were adult males with body weight of  $6.2 \pm 0.7$  kg. Monkeys were screened to assess general well-being and ocular health by slit-lamp biomicroscopy and funduscopy, color fundus imaging, infrared fundus imaging, optical coherence tomography (OCT), and tonometry prior to ICG injection (groups 1 and 2).

For baseline assessments in all monkeys and all subsequent procedures in monkeys in group 2, animals were anesthetized with 8.0 mg/kg ketamine (Ketathesia; Henry Schein, Melville, NY) and 1.6 mg/kg xylazine (AnaSed; Akorn, Lake Forest, IL). General wellbeing was assessed before, during, and after sedation. Pupil dilation was achieved with topical 10% phenylephrine and/or 1% cyclopentolate. Animals in group 2 were returned to the colony after confirming

the quality of acquired imaging, and general and ocular health.

## SC Injection

SC injections in group 1 were performed in euthanized animals in globes in situ within the orbit post-euthanasia (for other non-ophthalmic study purposes). Monkeys in group 2 were anesthetized with ketamine/xylazine as indicated above. Eyes of monkeys in group 2 were disinfected with 5% Betadine (Alcon, Geneva, Switzerland) and rinsed with sterile normal saline. The 27-gauge needle of the injector (Fig. 1) was placed 2.5-mm posterior to the limbus in the inferior quadrant at a slight angle, tangential to the sclera, and then flattened and advanced to the tissue stopper, creating a scleral tunnel (Fig. 2A). The tissue separator was then extended beyond the needle tip (Fig. 2A), immediately followed by retraction of the tissue separator (Fig. 2C), thereby creating a channel from the sclera into the suprachoroidal space. This was followed by injection of the ICG (5 mg/mL; Patheon Italia Spa, Monza, Italy) by pressing the syringe plunger (Fig. 2D). After withdrawal of the needle, 0.3% ciprofloxacin ophthalmic solution (Leading Pharma, Fairfield, NJ) was administered topically. Eyes in group 1 ( $n = 6$ ) were injected with 150  $\mu$ L of ICG. Eyes in



**Figure 2.** Schematic representation of suprachoroidal injection steps. First, the injector is inserted tangentially at a slight angle up to the sleeve stopper with the sharp tip of the needle penetrating only the sclera (A). Then, the tissue separator is extended, generating a channel into the choroid (B). Next, the tissue separator is retracted (C), and ICG is injected into the open channel, resulting in quick distribution across the posterior segment (D).

group 2 received an injection volume of either 150  $\mu\text{L}$  ( $n = 2$ ) or 200  $\mu\text{L}$  ( $n = 4$ ) ICG, as indicated in the text.

### IOP Measurement

IOP was measured in anesthetized monkeys using a tonometer (TonoVet, Vantaa, Finland) at baseline in groups 1 and 2. In group 2, additional IOP measurements were taken immediately after the injection and at 1 hour post-injection. Three measurements were taken from each eye to calculate the mean IOP.

### Slit-Lamp Biomicroscopy

Anterior and posterior segments of animal eyes were examined by slit-lamp biomicroscopy (ZEISS 30SL-M; Zeiss, Oberkochen, Germany) at baseline for groups 1 and 2. In group 2, monkeys were also tested within 10 minutes after the injection, and at 1 hour, 3 hours, and 24 hours following injection. Evaluation of the posterior segment and retina were performed using a 90-diopter lens.

### Fundus Imaging

Bilateral color fundus images and fluorescence images were captured at baseline for groups 1 and 2. Monkeys in group 2 underwent additional fundus imaging within 10 minutes after the injection and at 1 hour, 3 hours, and 24 hours post-injection using ICG excitation/emission filters. Images ( $50^\circ$ ) were centered on the fovea using a TRC-50EX retinal camera (Topcon, Tokyo, Japan) with Canon 6D digital

imaging hardware (Canon, Tokyo, Japan) and NewVision Fundus image analysis software.

### OCT Imaging

OCT scans were performed at the intersection of the fovea and optic nerve head (ONH) using a SPECTRALIS OCT Plus (Heidelberg Engineering, Heidelberg, Germany) with eye tracking and HEYEX image capture and analysis software at baseline for groups 1 and 2. For monkeys in group 2, additional OCT testing was performed within 10 minutes following ICG injection and at 1 hour, 4 hours, and 24 hours. At the time of OCT imaging, fundus reflectance images were acquired at 488 nm (blue) and 820 nm (infrared), with ICG images collected in the central and peripheral temporal superior, nasal, and inferior fields of the view using the Composite Image function. Stained ICG area was calculated using ImageJ 1.51a (National Institutes of Health, Bethesda, MD).

### Flatmount Analysis of Eyes in Group 1

Eyes were enucleated within 10 minutes following ICG injection into the SC of eyes of euthanized monkeys in situ in orbit. The anterior segment of the globe was removed. Eye cups were flatmounted followed by successive removal of the retina and choroid to reveal the suprachoroidal space and accumulated ICG overlaying the sclera. Images of the scleral flatmounts were acquired using a Leica camera (Leica Microsystems, Wetzlar, Germany) coupled to a

Wild Heerbrugg M690 microscope (Wild Heerbrugg, Heerbrugg, Switzerland).

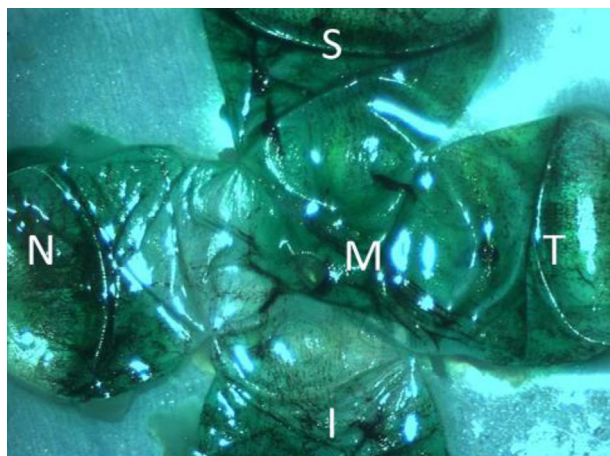
## Statistical Analysis

SPSS Statistics 20.0 (IBM, Chicago, IL) for Windows was used for all statistical analyses. For the pharmacokinetics analysis, due to the small sample size in each group at each time point ( $n = 3$  or 4), nonparametric analyses were used. Repeated-measures ANOVA was used to assess the significance of the differences between baseline and post-injection measurements for outcome measures with normal distribution (IOP and spectral domain optical coherence tomography [SD-OCT] retinal thickness). Kuskal-Wallis analysis was used to assess the significance of the differences in IOP between IVT and suprachoroidal injected eyes. For the SD-OCT thickness analysis, due to the small group size ( $n = 3$ ) nonparametric Friedman followed by Wilcoxon signed-rank tests were used.

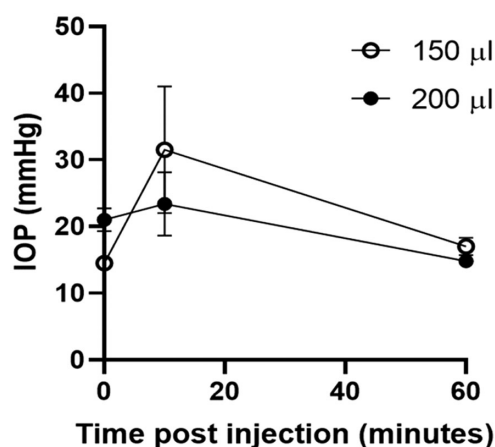
## Results

### SC Injection of ICG in Euthanized NHP Eyes In Situ

Cadaver eyes from three African green monkeys post-euthanasia (cadaver globes in situ within the orbit) were initially studied to assess the feasibility of



**Figure 3.** ICG was distributed throughout the suprachoroid in eyes injected in orbit after euthanasia. Following monkey euthanasia ( $n = 3$ ), six eyes were injected in orbit with 150  $\mu$ L ICG into the SC in the inferior quadrant. Eyes ( $n = 6$ ) were enucleated, and scleral flatmounts were prepared and photographed following removal of the anterior segment, retina, and choroid. S, superior; N, nasal; T, temporal; I, inferior; M, macula.



**Figure 4.** Acute and transient elevation in IOP following SC injection of ICG in live animals. Two eyes received SC injection of 150  $\mu$ L ICG (open circles) and four eyes received SC injection of 200  $\mu$ L ICG. IOP was measured (before,  $t = 0$ ) and 10 minutes and 60 minutes after ICG injection. Data are presented as mean  $\pm$  SE.

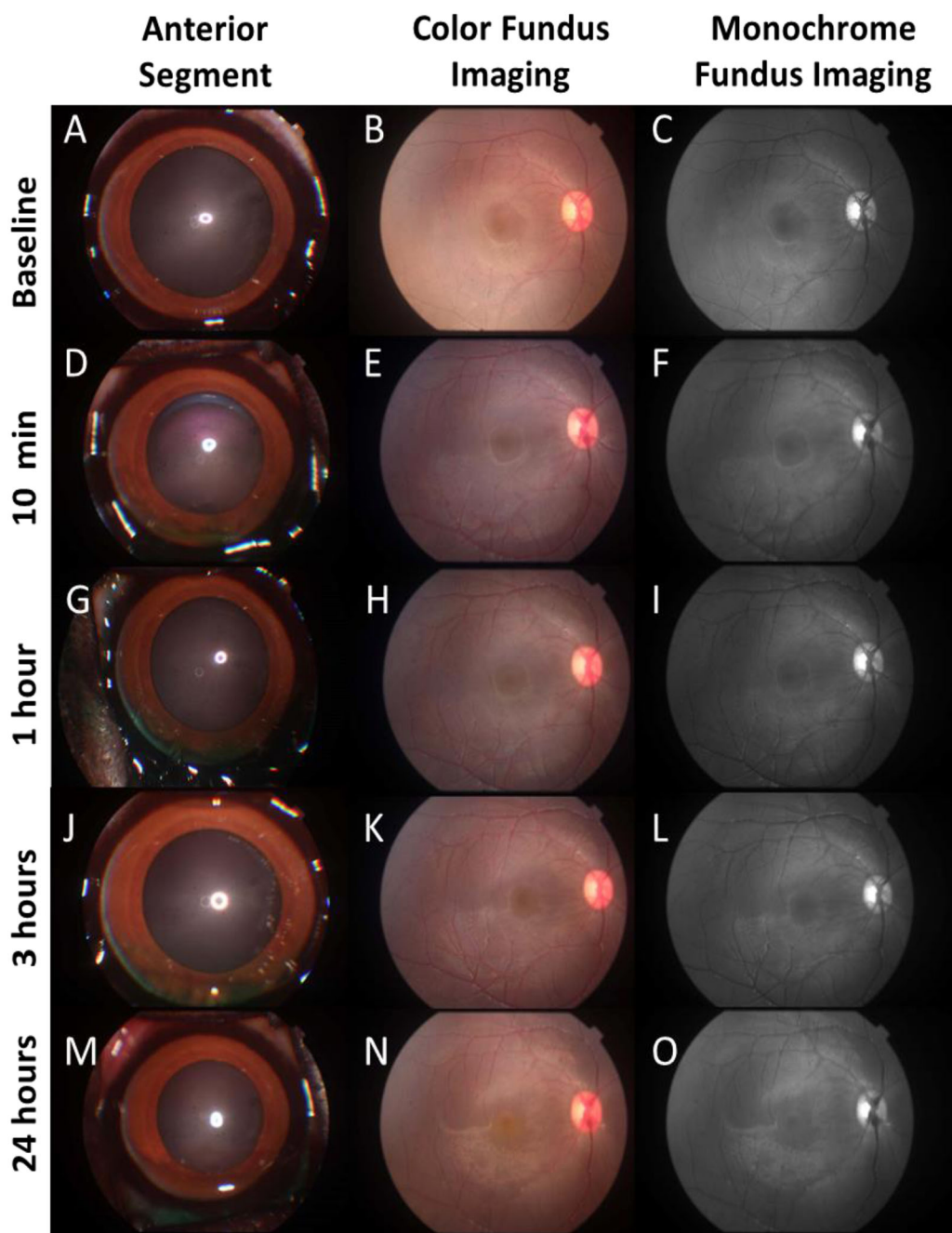
the injection method ( $n = 6$ ). SC injection was successfully administered in all in situ cadaver eyes by insertion of the delivery system needle tangentially into the sclera, followed by extension and retraction of the tissue separator to create a channel into the choroid. The injection was done in the inferior quadrant 2.5-mm posterior to the limbus. Injection volume was 150  $\mu$ L of ICG with minimal to no reflux. No signs of penetration into the vitreous chamber or injection-associated ocular injuries were observed following the suprachoroidal injections. Eyes were enucleated within 10 minutes of the injection and flatmounted. No sign of dye penetration into the vitreous chamber was observed. [Figure 3](#) demonstrates a representative image of a scleral flatmount of an enucleated eye from group 1. Although the injections were done through the inferior quadrant, ICG distribution could be observed throughout all four quadrants, including the macula.

### SC Injection of ICG in Live Monkeys

SC injection was successfully administered in all eyes in the inferior quadrant 2.5 mm posterior to the limbus. Two eyes were injected with 150  $\mu$ L of ICG and four eyes were injected with 200  $\mu$ L of ICG with minimal to no reflux. No sign of penetration into the vitreous chamber or injection-associated ocular injury or infection was observed following suprachoroidal injections in any of the eyes.

### Tolerability of the SC Injection

Slit-lamp biomicroscopy examinations were performed prior to injection, within 10 minutes

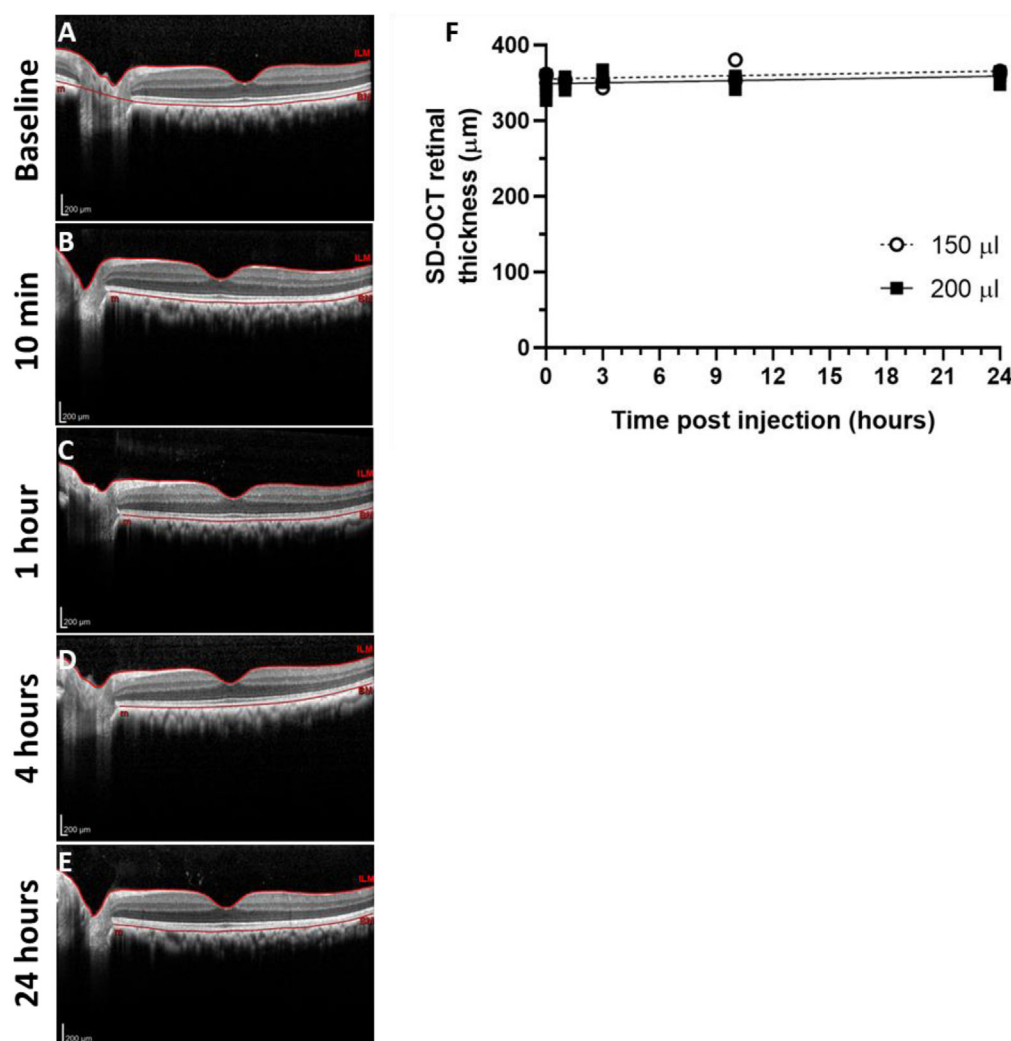


**Figure 5.** Anterior segment and fundus imaging of a representative eye following in vivo ICG SC injection. Representative anterior segment imaging (A, D, G, J) and color (B, E, H, K) and monochrome (C, F, I, L) fundus images of the left eye of a live monkey before (A–C), 10 minutes after (D–F), 1 hour after (G–I), 3 hours after (J–L), and 24 hours after (M–O) SC injection of 200  $\mu$ L ICG.

following SC injection and at 1 hour, 3 hours, and 24 hours post-injection. No inflammatory reaction was observed after SC injection within the time points evaluated. A mild, statistically insignificant elevation in IOP was observed within 10 minutes post-injection (mean difference  $\pm$  standard error [SE],  $7.28 \pm 5.09$  mmHg;  $P = 0.061$ ) and was spontaneously resolved within the first hour after dosing (Fig. 4).

### Ocular Integrity and Retinal Thickness Following SC ICG Injection in Live Animals

Anterior segment, color, and monochrome fundus photography images were captured on all eyes prior to ICG injection, within 10 minutes following injection, and at 1 hour, 3 hours and 24 hours following injection. No ICG was observed in the vitreous. No



**Figure 6.** SD-OCT imaging indicating the integrity of retinal layers maintained up to 24 hours following SC injection. (A–E) Representative SD-OCT imaging of a live animal prior to (A) and within 10 minutes (B), 1 hour (C), 4 hours (D), and 24 hours (E) after SC injection of 200 µL ICG (the same eye as in Figure 5). (F) SD-OCT total retina thickness of group 2 eyes following SC injection of 150 µL ( $n = 2$ ) and 200 µL ( $n = 4$ ) ICG.

choroidal or retinal blebs or hemorrhages or any other disruptions were detected in any time points in all eyes (Fig. 5). SD-OCT scans of the retinas of injected eyes demonstrated no significant differences in total retinal thickness following injection up to 24 hours post-injection ( $P = 0.272$ ) (Fig. 6A). The SD-OCT total retinal thickness did not significantly differ between eyes that received 150 µL or 200 µL ICG ( $P = 0.156$ ) (Fig. 6F).

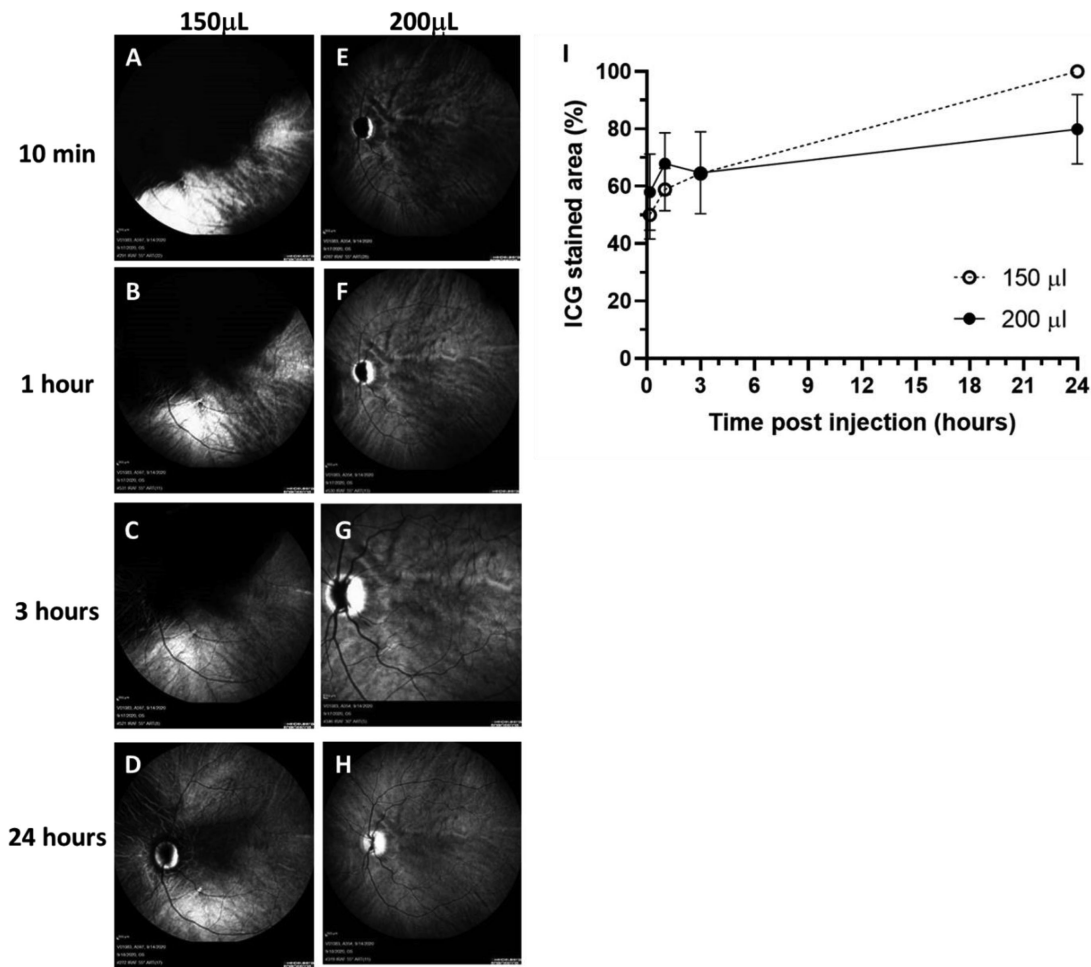
### Assessment of ICG Distribution Across the Posterior Segment of In Vivo Eyes

The dye distribution was assessed by infrared fundus imaging using the Heidelberg SD-OCT device. As shown in Figure 7, ICG migrated within the

suprachoroidal space from the inferior peripheral injection site toward the macula and ONH. Although some variability in ICG stained area was observed between injected eyes, by 24 hours all eyes exhibited ICG signal across the posterior segment reaching the macula.

## Discussion

Recent translational and clinical studies suggest that the SC is a promising target for drug delivery to the posterior segment with high exposure of the choroid, sclera, RPE, and retina. In this study, we demonstrated for the first time, to the best of our knowledge, the safety and efficacy of a new SC injection system in



**Figure 7.** ICG imaging of the posterior segment in live monkeys in vivo. (A–H) Representative ICG imaging of a live animal within 10 minutes (A, E), 1 hour (B, F), 3 hours (C, G), and 24 hours (D, H) of SC injection of 150 µL (A–D) or 200 µL (E–H) ICG. Bright areas indicate ICG signal. (I) Quantification of ICG stained area. Data are presented as mean  $\pm$  SE.

NHP eyes. As proof of concept, ICG dye was used to demonstrate and visualize distribution within the SC. Our results indicate efficient spreading of the dye across the SC with no adverse effects. No subretinal, choroidal, or subchoroidal blebs; bleeding; or retinal or choroidal hemorrhage were observed following SC injection of 150 µL or 200 µL dye. SD-OCT imaging and IOP measurements suggest that SC injection of up to 200 microliter ICG via the novel device was very well tolerated with minimal to no changes in physiological structures.

The SC injection was performed 2.5 mm posterior to the limbus in the inferior quadrant. Infrared fundus imaging demonstrated ICG distribution throughout the posterior segment, reaching the macula within 24 hours post-injection. Similar findings were observed using the same injection method with smaller proof-of-concept devices, in rats, rabbits, and pigs.<sup>35–37</sup> Chen et al.<sup>30</sup> reported the injection of 150 µL ICG into the SC of rabbit eyes using a 30-gauge needle connected to a 250-µL Hamilton syringe. Injection of

150 µL ICG was done more posteriorly compared to our injection (9 mm behind the limbus, in the inferior quadrant) and resulted in more limited spreading of ICG in the SC, covering roughly 65% of the SC.

The imaging and dissection of the eyes for flatmount analyses confirmed no exposure of the anterior segment, vitreous, or lens following the SC injection in live NHPs and cadaver NHP eyes. These findings suggest a major advantage of SC injection with regard to reducing the risk of cataract and glaucoma.<sup>10,11,38,39</sup> The pharmacokinetics of ICG following SC injection is unknown, unlike systemic ICG injection, which has been used for many years and is well studied.<sup>40–42</sup> Our study suggests a gradual spreading of ICG in the SC of NHPs following SC injection (Fig. 7). It is noteworthy that in rabbit eyes, ICG distribution within the SC was found to be similar to the distribution of other injected materials including nanoparticles and stem cells.<sup>35</sup> Further studies are warranted to address the pharmacokinetics of ICG and specific therapeutics injected using the novel SC

injection device in NHP eyes. Additional limitations of our study include the short-term (24 hours) follow-up of live monkeys and the small sample size in each study group. Although no adverse effects were observed, long-term safety monitoring in a larger cohort of NHPs and testing potential therapies are required prior to proceeding to clinical studies with the new SC injection device.

Previous studies have indicated fast distribution of injected materials circumferentially across the choroid layers, with high exposure of the choroid, RPE, and retina, and gradual spreading of injected particles into the sclera within days or weeks, depending on the injected material.<sup>43,44</sup> These studies indicate that clearance rates from the SC depend on the injected molecule or particulate size and tissue distribution properties. We and others have shown fast clearance of bevacizumab in rabbit and porcine eyes following SC injection.<sup>8,43</sup> By contrast, lipophilic small molecules such as steroids and tyrosine kinase inhibitors nanoparticles are retained in the posterior segment for several months after SC injection.<sup>39,44,45</sup> Future studies, injecting various molecules in NHP eyes and including a longitudinal follow-up will enable determination of the biodistribution and pharmacokinetics of injected molecules using the new SC device in NHP eyes.

In conclusion, the new SC injection system enables a safe and well-tolerated injection into NHP eyes of up to 200  $\mu$ L of solution from the periphery, with injected molecule spreading across the posterior segment including the macula within hours of injection with no substantial adverse effects.

## Acknowledgments

Supported by funding from Everads Therapy. Company personnel were not involved in conducting the experiments or data analysis.

Disclosure: **Y. Rotenstreich**, Everads Therapy (C, F); **I. Sher**, Everads Therapy (C, F); **M. Lawrence**, Virscio, Inc. (E); **M. Mangelus**, Everads therapy (E); **A. Ingberman**, Everads therapy (E); **Y. Barak**, Everads Therapy (C)

## References

1. Steinmetz JD, Bourne RRA, Saylan M, et al. Causes of blindness and vision impairment in 2020 and trends over 30 years, and prevalence of avoidable blindness in relation to VISION 2020: the right to sight: an analysis for the Global Burden of Disease Study. *Lancet Glob Health*. 2021;9:e144–e160.
2. Varela-Fernández R, Díaz-Tomé V, Luaces-Rodríguez A, et al. Drug delivery to the posterior segment of the eye: biopharmaceutic and pharmacokinetic considerations. *Pharmaceutics*. 2020;12:269.
3. Ghanchi F, Bourne R, Downes SM, et al. An update on long-acting therapies in chronic sight-threatening eye diseases of the posterior segment: AMD, DMO, RVO, uveitis and glaucoma. *Eye (Lond)*. 2022;36:1154–1167.
4. Ciulla TA, Hussain RM, Pollack JS, Williams DF. Visual acuity outcomes and anti-vascular endothelial growth factor therapy intensity in neovascular age-related macular degeneration patients: a real-world analysis of 49 485 eyes. *Ophthalmol Retina*. 2020;4:19–30.
5. Holz FG, Amoaku W, Donate J, et al. Safety and efficacy of a flexible dosing regimen of ranibizumab in neovascular age-related macular degeneration: the SUSTAIN study. *Ophthalmology*. 2011;118:663–671.
6. Hoguet A, Chen PP, Junk AK, et al. The effect of anti-vascular endothelial growth factor agents on intraocular pressure and glaucoma: a report by the American Academy of Ophthalmology. *Ophthalmology*. 2019;126:611–622.
7. Tolentino M. Systemic and ocular safety of intravitreal anti-VEGF therapies for ocular neovascular disease. *Surv Ophthalmol*. 2011;56:95–113.
8. Olsen TW, Feng X, Wabner K, Csaky K, Pambuccian S, Cameron JD. Pharmacokinetics of pars plana intravitreal injections versus microcannula suprachoroidal injections of bevacizumab in a porcine model. *Invest Ophthalmol Vis Sci*. 2011;52:4749–4756.
9. Sher I, Goldberg Z, Bubis E, Barak Y, Rotenstreich Y. Suprachoroidal delivery of bevacizumab in rabbit in vivo eyes: rapid distribution throughout the posterior segment. *Eur J Pharm Biopharm*. 2021;169:200–210.
10. Kothari S, Foster CS, Pistilli M, et al. The risk of intraocular pressure elevation in pediatric non-infectious uveitis. *Ophthalmology*. 2015;122:1987–2001.
11. José-Vieira R, Ferreira A, Menéres P, et al. Efficacy and safety of intravitreal and periocular injection of corticosteroids in non-infectious uveitis: a systematic review. *Surv Ophthalmol*. 2022;67:991–1013.
12. Campa C, Gallenga CE, Bolletta E, Perri P. The role of gene therapy in the treatment of retinal

- diseases: a review. *Curr Gene Ther.* 2017;17:194–213.
13. Ross M, Ofri R. The future of retinal gene therapy: evolving from subretinal to intravitreal vector delivery. *Neural Regen Res.* 2021;16:1751–1759.
  14. Puertas-Neyra K, Usategui-Martín R, Coco RM, Fernandez-Bueno I. Intravitreal stem cell paracrine properties as a potential neuroprotective therapy for retinal photoreceptor neurodegenerative diseases. *Neural Regen Res.* 2020;15:1631–1638.
  15. Park SS. Intravitreal autologous bone marrow CD34+ cell therapy for retinal vein occlusion: phase I/II clinical trial. *Invest Ophthalmol Vis Sci.* 2018;59:3905.
  16. Heier JS, Kherani S, Desai S, et al. Intravitreal injection of AAV2-sFLT01 in patients with advanced neovascular age-related macular degeneration: a phase 1, open-label trial. *Lancet.* 2017;390:50–61.
  17. Newman NJ, Yu-Wai-Man P, Carelli V, et al. Efficacy and safety of intravitreal gene therapy for Leber hereditary optic neuropathy treated within 6 months of disease onset. *Ophthalmology.* 2021;128:649–660.
  18. Kiss S, Grishanin R, Nguyen A, et al. Analysis of aflibercept expression in NHPs following intravitreal administration of ADVIM-022, a potential gene therapy for nAMD. *Mol Ther Methods Clin Dev.* 2020;18:345–353.
  19. Wan CR, Muya L, Kansara V, Ciulla TA. Suprachoroidal delivery of small molecules, nanoparticles, gene and cell therapies for ocular diseases. *Pharmaceutics.* 2021;13:288.
  20. Hancock SE, Wan CR, Fisher NE, et al. Biomechanics of suprachoroidal drug delivery: from benchtop to clinical investigation in ocular therapies. *Expert Opin Drug Deliv.* 2021;18:777–788.
  21. Naftali Ben Haim L, Moisseiev E. Drug delivery via the suprachoroidal space for the treatment of retinal diseases. *Pharmaceutics.* 2021;13:967.
  22. Chiang B, Jung J, Prausnitz M. The suprachoroidal space as a route of administration to the posterior segment of the eye HHS public access. *Adv Drug Deliv Rev.* 2018;126:58–66.
  23. Kansara VS, Hancock SE, Muya LW, Ciulla TA. Suprachoroidal delivery enables targeting, localization and durability of small molecule suspensions. *J Control Release.* 2022;349:1045–1051.
  24. Yeh S, Khurana RN, Shah M, et al. Efficacy and safety of suprachoroidal CLS-TA for macular edema secondary to noninfectious uveitis: phase 3 randomized trial. *Ophthalmology.* 2020;127:948–955.
  25. Khurana RN, Merrill P, Yeh S, et al. Extension study of the safety and efficacy of CLS-TA for treatment of macular oedema associated with non-infectious uveitis (MAGNOLIA). *Br J Ophthalmol.* 2022;106:1139–1144.
  26. Thomas J, Kim L, Albini T, Yeh S. Triamcinolone acetate injectable suspension for suprachoroidal use in the treatment of macular edema associated with uveitis. *Expert Rev Ophthalmol.* 2022;17:165–173.
  27. Lampen SIR, Khurana RN, Noronha G, Brown DM, Wykoff CC. Suprachoroidal space alterations following delivery of triamcinolone acetate: post-hoc analysis of the phase 1/2 HULK study of patients with diabetic macular edema. *Ophthalmic Surg Lasers Imaging Retina.* 2018;49:692–697.
  28. Ding K, Shen J, Hafiz Z, et al. AAV8-vectored suprachoroidal gene transfer produces widespread ocular transgene expression. *J Clin Invest.* 2019;129:4901–4911.
  29. Khanani AM. Suprachoroidal delivery of RGX-314 gene therapy for neovascular AMD: the phase II AAVIATE study. *Invest Ophthalmol Vis Sci.* 2022;63:1497–1497.
  30. Chen M, Li X, Liu J, Han Y, Cheng L. Safety and pharmacodynamics of suprachoroidal injection of triamcinolone acetate as a controlled ocular drug release model. *J Control Release.* 2015;203:109–117.
  31. Chiang B, Kim YC, Edelhauser HF, Prausnitz MR. Circumferential flow of particles in the suprachoroidal space is impeded by the posterior ciliary arteries. *Exp Eye Res.* 2016;145:424–431.
  32. Olsen TW, Feng X, Wabner K, et al. Cannulation of the suprachoroidal space: a novel drug delivery methodology to the posterior segment. *Am J Ophthalmol.* 2006;142:777–787.
  33. Tetz M, Rizzo S, Augustin AJ. Safety of submacular suprachoroidal drug administration via a microcatheter: retrospective analysis of European treatment results. *Ophthalmologica.* 2012;227:183–189.
  34. Sher I, Bubis E, Ketter-Katz H, Goldberg A, Saeed R, Rotenstreich Y. Efficacy and safety of injecting increasing volumes into the extravascular spaces of the choroid using a blunt adjustable depth injector. *Int Ophthalmol.* 2020;40:2865–2874.
  35. Rotenstreich Y, Tzameret A, Kalish SE, et al. A minimally invasive adjustable-depth blunt injector for delivery of pharmaceuticals into the posterior pole. *Acta Ophthalmol.* 2017;95:e197–e205.
  36. Tzameret A, Sher I, Belkin M, et al. Transplantation of human bone marrow mesenchymal stem

- cells as a thin subretinal layer ameliorates retinal degeneration in a rat model of retinal dystrophy. *Exp Eye Res.* 2014;118:135–144.
37. Tzameret A, Kalish SE, Sher I, et al. Long-term safety of transplanting human bone marrow stromal cells into the extravascular spaces of the choroid of rabbits. *Stem Cells Int.* 2017;2017:4061975.
  38. Kim JH, Chang YS, Kim JW, Lee TG, Lew YJ. Imaging suprachoroidal layer in exudative age-related macular degeneration. *Curr Eye Res.* 2016;41:715–720.
  39. Campochiaro PA, Wykoff CC, Brown DM, et al. Suprachoroidal triamcinolone acetonide for retinal vein occlusion: results of the Tanzanite Study. *Ophthalmol Retina.* 2018;2:320–328.
  40. Chang AA, Morse LS, Handa JT, et al. Histologic localization of indocyanine green dye in aging primate and human ocular tissues with clinical angiographic correlation. *Ophthalmology.* 1998;105:1060–1068.
  41. Menchini U, Virgili G, Lanzetta P, Ferrari E. Indocyanine green angiography in central serous chorioretinopathy ICG angiography in CSC. *Int Ophthalmol.* 1997;21:57–69.
  42. Destro M, Puliafito CA. Indocyanine Green videoangiography of choroidal neovascularization. *Ophthalmology.* 1989;96:846–853.
  43. Sher I, Goldberg Z, Bubis E, Barak Y, Rotenstreich Y. Suprachoroidal delivery of bevacizumab in rabbit in vivo eyes: rapid distribution throughout the posterior segment. *Eur J Pharm Biopharm.* 2021;169:200–210.
  44. Tzameret A, Ketter-Katz H, Edelshtain V, et al. In vivo MRI assessment of bioactive magnetic iron oxide/human serum albumin nanoparticle delivery into the posterior segment of the eye in a rat model of retinal degeneration. *J Nanobiotechnology.* 2019;17:3.
  45. Shen J, Kim J, Tzen SY, et al. Suprachoroidal gene transfer with nonviral nanoparticles. *Sci Adv.* 2020;6:eaba1606.