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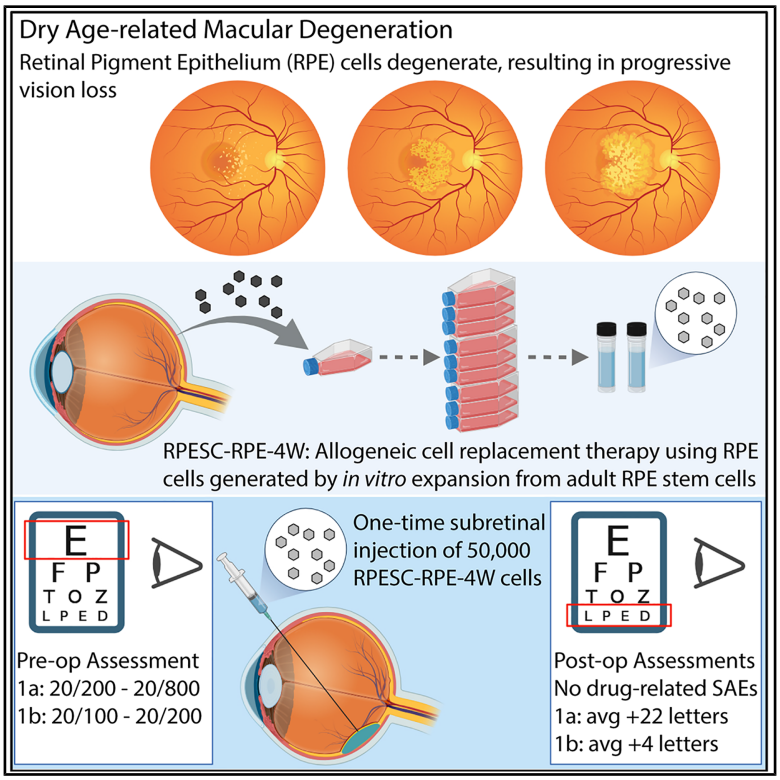
Clinical and Translational Report

Safety and tolerability of RPESC-RPE transplantation in patients with dry age-related macular degeneration: Low-dose clinical outcomes

Graphical abstract



Highlights

- Adult RPE stem cells generated RPE progenitor cells at 4 weeks of differentiation
- Progenitor-stage RPE cells were implanted to treat dry AMD
- Clinical outcomes from low-dose cohort 1 met primary safety endpoints
- Visual acuity improvements were greater in worse-seeing subjects

Authors

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In brief

Retinal pigment epithelium (RPE) cell loss in dry age-related macular degeneration leads to progressive vision decline. Stern and others implanted adult RPE stem cell-derived RPE progeny at a 4-week progenitor stage of differentiation (RPESC-RPE-4W). First-in-human cohort 1 met primary safety endpoints, with greater visual improvement observed in worse-seeing eyes.

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Clinical and Translational Report

Safety and tolerability of RPESC-RPE transplantation in patients with dry age-related macular degeneration: Low-dose clinical outcomes

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SUMMARY

Retinal pigment epithelium (RPE) cell atrophy in dry age-related macular degeneration (AMD) compromises photoreceptor cell function, leading to vision loss. Stem cell-based RPE replacement therapy aims to reverse disease progression and restore vision. RPESC-RPE-4W, a post-mitotic adult RPE stem cell-derived RPE (RPESC-RPE) progenitor cell product, exhibits consistent safety and efficacy in preclinical studies. The first-in-human clinical trial of RPESC-RPE-4W completed low-dose cohort 1 interventions (NCT04627428). Six subjects received a subretinal suspension of 50,000 RPESC-RPE-4W cells. No significant inflammation, tumor, or product-related serious adverse events were observed. Best-corrected visual acuity in the three worse-seeing group A subjects improved by an average of +21.67 letters from baseline at 12 months. Three better-seeing group B subjects improved by an average of +3.0 letters at 6 months. The positive safety and tolerability outcomes for low-dose cohort 1 enabled dose escalation to mid-dose RPESC-RPE-4W therapy for dry AMD.

INTRODUCTION

Dry age-related macular degeneration (AMD) is a frequent cause of visual loss that affects millions of patients in our aging population.[1,2] Retinal pigment epithelium (RPE) cell atrophy occurs early in advanced dry AMD pathogenesis.[3,4] RPE cell loss compromises overlying retinal photoreceptor (PR) cell function, resulting in vision loss.[5,6] As dry AMD progresses, localized areas of geographic atrophy (GA) appear as pale lesions that lack RPE cells. Although treatment is available to slow GA lesion growth,[7–9] no approved treatment is available to reverse cell loss and improve vision. Stem cell-based cell replacement therapies are under development to implant RPE cells into areas of RPE atrophy to improve vision for dry AMD patient benefit.[10,11] Stem cells provide a ready source of RPE cells to replace those lost in dry AMD.[12] Clinical trials of pluripotent stem cell (PSC)-derived RPE (PSC-RPE) transplantation have shown a good safety profile and modest vision improvement.[13] A variety of PSC-RPE cell types delivered as a suspension or on a scaffold are currently under investigation to improve stem cell-based RPE replacement therapy for dry AMD.[14–20]

Our approach to RPE replacement utilizes a unique stem cell source: the adult RPE stem cell (RPESC) that is derived from the native RPE layer of eyes donated to qualified eye banks.[21,22] RPESCs are committed to the RPE lineage and self-renew in culture to produce large quantities of RPE progeny.[21,22] The RPESC isolated from a donor proliferate and then differentiate into cells that closely resemble native RPE cells.[23] Preclinical *in vitro* and *in vivo* studies show a good safety profile with preservation of RPE identity, lack of tumor formation and lack of toxicity. Post-mitotic progenitor-stage RPESC-derived RPE (RPESC-RPE) obtained at passage 2 after 4 weeks of differentiation (RPESC-RPE-4W) were found to increase engraftment and vision rescue compared with other stages of development.[24,25] We report here early clinical outcomes of RPESC-RPE-4W progenitor cell therapy for dry AMD patients.

The phase 1/2a dose-escalation safety and tolerability clinical trial implants RPESC-RPE-4W cell suspensions in three dose cohorts: cohort 1 (50,000 cells), cohort 2 (150,000 cells), and cohort 3 (250,000 cells). Cohort 1a is composed of 3 worse-seeing subjects with best-corrected visual acuity (BCVA) of 20/200 – 20/800, assessed by reading the standardized Early

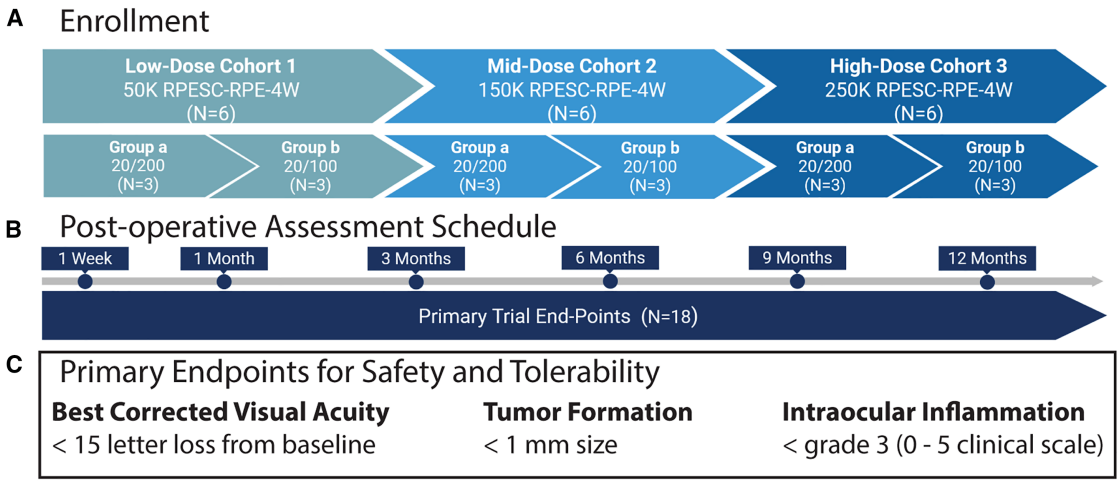

Figure 1. Trial design

(A) Dose escalation occurs over three dose cohorts, each composed of two groups based on best-corrected visual acuity (BCVA). Dose cohorts enroll sequentially, with subjects receiving either 50,000, 150,000, or 250,000 RPESC-RPE-4W cells. Cohort 1a subjects enroll with 20/200–20/800 ("20/200") BCVA followed by three cohort 1b subjects with 20/70 to < 20/200 ("20/100") acuity.

(B) Primary outcomes are assessed over 12 months of follow-up.

(C) Primary endpoint thresholds indicate allowable limits for safety and tolerability.

Treatment Diabetic Retinopathy Study (ETDRS) eye chart. Cohort 1b is composed of 3 better-seeing subjects with BCVA of 20/70 to <20/200. Cohorts 1a and 1b enroll sequentially. The trial primary endpoints assessed over 12 months comprise absence of BCVA loss > 15 letters, intraocular inflammation > moderate severity, tumor formation > 1 mm size, and lack of ocular or non-ocular severe adverse events (SAEs) related to the investigational product (IP) or trial intervention. Secondary or exploratory endpoints comprise macular sensitivity loss < 10 decibels (dB), change in BCVA from baseline to 12 months, change in GA lesion size, change in optical coherence tomography (OCT) macular thickness from baseline, and structural evidence of engraftment. A schematic of the trial design is shown in Figure 1. The first-in-human trial, safety and tolerability of RPESC-RPE transplantation in patients with dry AMD (NCT04627428), has completed low-dose cohort 1 interventions. Outcomes were obtained over 12 months for cohort 1a and over 6 months for cohort 1b. No SAEs related to the IP occurred. Key efficacy findings of BCVA improvement averaged +21.67 ETDRS letters gained from baseline for cohort 1a at 12 months and +4.0 letters for cohort 1b at 6 months. Cohort 1 subjects receiving a low dose of 50,000 RPESC-RPE-4W cells showed no cell-product-related SAEs and a promising efficacy signal. Progress to the next dose cohort was reviewed and approved by the independent data and safety monitoring committee (DSMC) to allow enrollment in cohort 2, implanting 150,000 RPESC-RPE-4W cells as therapy for dry AMD.

RESULTS

Trial conduct

The first-in-human open-label phase 1/2a interventional dose-escalation trial treated 6 cohort 1 subjects. Each subject received 50,000 RPESC-RPE-4W cells under the macula in the eye that exhibited more advanced vision loss. Implantations occurred in the superior temporal macula. Three subjects in the worse-seeing cohort 1a were treated, followed by 3 subjects in the better-seeing cohort 1b. Primary endpoints and retinal thickness were assessed at screening after 1 day, 1 week, and 1, 3, 6, 9, and 12 months. Secondary exploratory endpoints of macular sensitivity and fundus autofluorescence (FAF) were assessed at baseline and at visits at 6 and 12 months. Assessment method details are provided in the STAR Methods section.

Characterization of clinically implanted RPESC-RPE-4W

The trial utilized a single master cell bank (MCB) derived from a single donor that met Food and Drug Admininistration (FDA)-approved criteria for clinical use (Table S1). A schematic of the manufacturing flow is shown in Figure 2A. Clinical doses were prepared by culturing an MCB vial over 3.7 weeks for 5 subjects and for 4.7 weeks for 1 subject (−017). Clinical release criteria values for the six clinical doses implanted in cohort 1 are shown in Figure 2B. Manufacturing details for the RPESC-RPE-4W cell product are provided in the STAR Methods section.

Subject demographics

A total of 18 subjects were screened. 6 subjects met enrollment criteria of vision loss due to dry AMD without confounding retinal disease. The average age of treated subjects was 76 years, with a range of 71–86 years. Three male and 3 female subjects were treated. The average baseline BCVA in the 3 cohort 1a subjects was 32 ETDRS letters in the eyes that would receive treatment and 69 letters in the eyes that would remain untreated. The 3 better-seeing cohort 1b subjects had an average baseline BCVA of 52 letters in the eyes that would be treated and 60 letters in the eyes that would remain untreated. The level of participant vision loss overlapped with the range in which retina patients are most willing to undertake the risk of stem cell treatment.[26] Average baseline values for secondary measures in treated eyes were macular thickness = 249 mm (range 222–291 mm), GA

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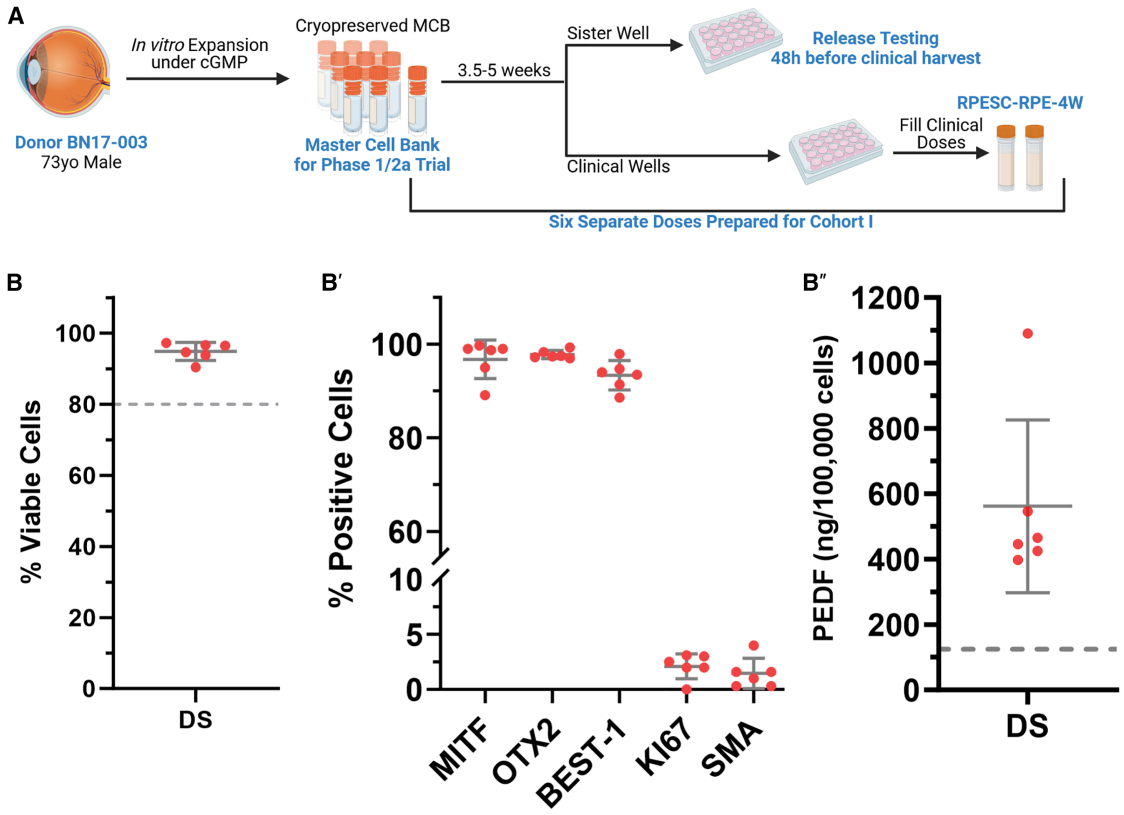

Figure 2. Release criteria values for clinical doses

(A) Schematic of RPESC-RPE-4W manufacture.

(B–B″) Quality control results for drug substance (DS) batches used in cohort 1 ($n = 6$). Gray bars represent mean ± standard deviation (SD). (B) Viability measured by automated cell count with propidium iodide. (B′) Identity and purity markers assessed by immunocytochemistry. (B″) Concentration of secreted PEDF measured by ELISA normalized to media volume and cell number. Note that the elevated PEDF level for one dose was obtained after 4.5 weeks of culture (subject −017), whereas the others were obtained after circa 3.5 weeks of culture. Dashed lines in (B) and (B″) indicate acceptable release levels. Bars show average ± SD. The values for SMA+ = 1.467% and KI67+ = 2.1% of the 50,000 cells implanted were below the release criteria of <5% SMA and <10% KI67.

area = 8 mm^2 (range 0.4–15 mm^2), and macular sensitivity = 15 dB (range 8.4–17.9 dB). Sensitivity was not measured in 2 subjects due to breakdown of the microperimetry instrument. Baseline participant characteristics are shown in Table 1.

RPESC-RPE-4W implantation

One vitreoretinal surgeon (RR) performed all implantation procedures. Pars plana vitrectomy (PPV) with removal of the posterior hyaloid membrane was followed by subretinal injection of approximately 100 microliters (μL) of buffered saline solution (BSS plus, Alcon) through a 38G subretinal cannula (Med-One PolyTip) to create a subretinal bleb. BSS injections occurred in the superotemporal macula approximately 5 mm from the foveal center. The extent of separation of the neural retina from the RPE layer within GA lesions during bleb formation was variable, with >50% of GA lesion area separated in subjects −002 and −018 and <50% separation in the remaining subjects. After bleb formation, a vial of RPESC-RPE-4W suspension was drawn through a 20G cannula into a 1 cc syringe. The 38G cannula was placed on the syringe and 100 μL of RPESC-RPE-4W suspension containing 50,000 RPESC-RPE-4W cells was injected into the bleb. After cell implantation, the vitreous cavity was washed with BSS. One subject (−007) received intraoperative laser treatment for a peripheral retina tear. Subjects left the operating room in a supine position that was maintained for 2 h.

Clinical outcomes

Primary endpoints were assessed at screening and at 1-day, 1-week, and 1, 3, 6, 9, and 12 months after implantation (Figures 1B and 1C). Limited basic ophthalmic exams at 18 and 24 months were carried out to capture late SAEs should they occur. The worse-seeing cohort 1a subjects completed 12-month study visits and the better-seeing cohort 1b subjects completed 6-month study visits. No tumor, significant inflammation (greater than 3 on a 0–5 clinical scale), or cell-product-related SAEs occurred in any subject. The overall BCVA at 6 months for combined cohort 1a and 1b subjects was a gain of +11 letters from baseline. The average difference between treated and untreated eyes for combined cohorts 1a and 1b at 6 months was +12.5 letters. Although rigorous protocols for measuring visual acuity were employed (see STAR Methods), intrinsic variability known to occur in patients with GA[27,28] is reflected in the individual BCVA results.

Cohort 1a BCVA

The raw BCVA measurement data and average BCVA change for cohort 1a are shown in Table 2. The average BCVA change from

Table 1. Baseline characteristics of treated participants

Group # (Snellen)	Subject number	Age (years)	Sex (M/F)	BCVA (letters read)	FAF area (mm^2)	Mean macularthickness (μm)	Macularsensitivity (dB)
A (20/200–20/800)	101002	71	M	29	9	232	15.3
	101004	73	F	35	15	247	8.4
	101005	86	F	32	10	252	–
B (20/70–20/100)	101007	74	F	50	4	249	17.7
	101017	74	M	52	9	222	–
	101018	80	M	55	0.4	291	17.9

BCVA was measured by ETDRS letters read for cohorts 1a and 1b. FAF area and corrected macular thickness were acquired using a Heidelberg Spectralis device. Baseline macular sensitivity was not obtained for two subjects due to instrument failures. "–" indicates microperimetry instrument failure.

baseline was +21.0 letters at 1 month, +22.0 letters at 3 months, +19.0 letters at 6 months, and +21.67 letters at 12 months. Untreated cohort 1a eyes lost −1.67 letters at 3 months, −2.0 letters at 6 months, and −1.33 letters at 12 months. When comparing treated with untreated eyes in cohort 1a, the average BCVA gains were + 23.67 letters at 3 months, +21.0 letters at 6 months, and +23.0 letters at 12 months.

The time course for vision changes in treated eyes showed a small improvement in BCVA at 1 week (+3.33 letters) that increased at 1 month (+21 letters) and remained stable to 12 months (+21.67 letters). Acuity loss in subject −002 during months 6–9 were due to cataract formation and were reversed after cataract surgery. Untreated cohort 1a eyes showed a BCVA decrease of −1.33 letters over 12 months, consistent with the natural course of dry AMD.[29–32] BCVA measurements for cohort 1a subjects shown in Table 2 are plotted in Figure 3A.

Cohort 1b BCVA

The 3 better-seeing cohort 1b subjects showed an average gain from baseline of +1.67 letters at 1 week that increased to +3.67 letters at 1 month, +3.33 letters at 3 months, and +3.0 letters at 6 months of follow-up (Table 3). Untreated cohort 1b eyes showed a decrease of –1.0 letters over the 6-month period. Comparing treated with untreated cohort 1b eyes, a difference of +4.0 letters occurred at 6 months of follow-up. Increases in visual acuity contrast the gradual decline expected from the natural history of dry AMD.[29–32] The raw data describing BCVA letters read, with Snellen equivalent for cohort 1b subjects, are shown in Table 3, accompanied by calculated average values.

Tumor formation and inflammatory response

Tumor formation was not observed in any subject at any time point. All subjects had mild post-surgical inflammation typical after intraocular surgery that resolved with topical steroid treatment. Significant posterior or delayed inflammation did not occur.

Secondary measures

Secondary measures of macular thickness, sensitivity, and autofluorescence were obtained at each study visit.

Neural retinal thickness, measured using the standard Heidelberg Spectralis optical coherence tomography (OCT) algorithm, underwent manual correction of mis-identified retinal layers

Table 2. BCVA of worse-seeing cohort 1a eyes

BCVA worse-seeing cohort 1a			Screen	1W	1M	3M	6M	9M	12M
–002	**Treated**	**Letters read**	29	40	39	40	30	31	43
		Snellen	20/250	20/160	20/160	20/160	20/250	20/250	20/160
	Untreated	**Letters read**	77	75	73	72	76	71	71
		Snellen	20/32	20/32	20/40	20/40	20/32	20/40	20/40
–004	**Treated**	**Letters read**	**35**	**29**	**50**	**50**	**51**	**42**	**50**
		Snellen	20/200	20/250	20/100	20/100	20/100	20/160	20/100
	Untreated	**Letters read**	53	46	53	52	50	53	55
		Snellen	20/100	20/125	20/100	20/100	20/100	20/100	20/80
–005	**Treated**	**Letters read**	**32**	**37**	**70**	**72**	**72**	**73**	**68**
		Snellen	20/250	20/200	20/40	20/40	20/40	20/40	20/50
	Untreated	**Letters read**	77	74	77	78	75	78	77
		Snellen	20/32	20/32	20/32	20/32	20/32	20/32	20/32
Average change in letters, treated eye			**N/A**	**+3.33**	**+21.0**	**+22.0**	**+19.0**	**+16.67**	**+21.67**
Average change in letters, untreated eye			**N/A**	**–4.0**	**–1.33**	**–1.67**	**–2.0**	**–1.67**	**–1.33**
Difference in treated vs. untreated eye			**N/A**	**+7.33**	**+23.33**	**+23.67**	**+21**	**+18.33**	**+23**

Visual acuities measured following ETDRS protocol at each study visit over 12 months. The number of letters read are shown with Snellen equivalent. Treated eyes (light blue) show marked increases in BCVA, whereas untreated eyes show small decreases in BCVA. Average change from baseline and between treated and control eyes are calculated from the raw acuity data.

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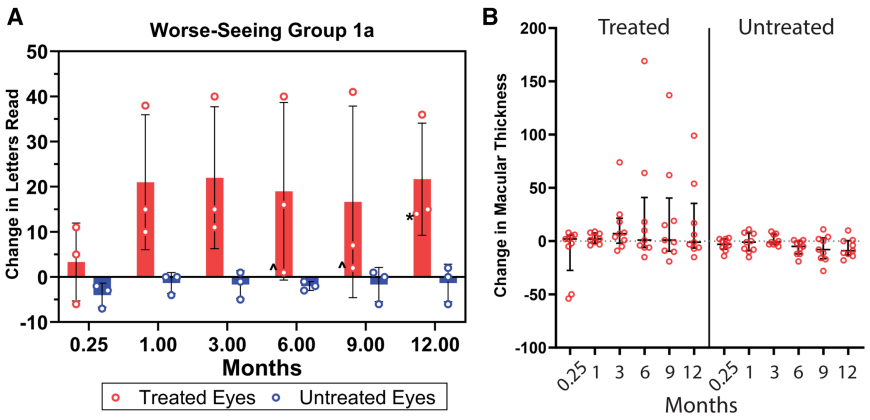

Figure 3. Cohort 1a visual acuity and retinal thickness

(A) BCVA for treated (red) and untreated (blue) cohort 1a eyes. Circles indicate individual eyes and bars represent mean change ± SD. ^ indicates cataract and * indicates cataract removal.

(B) Macular thickness measured from three macular locations (inner, central, and outer) for each subject at each time point (9 measurements per time point). Bars indicate median value and interquartile range.

within GA lesions by an individual masked to treatment conditions (Figures S1 and S2). The thickness measurements for cohort 1a are shown in Figure 3B and Table S3. Cohort 1a average thickness increased +16 μm from baseline in treated eyes at 12 months. Average thickness in untreated cohort 1a eyes decreased –7 μm at 12 months (Figure 3B; Table S3). The average corrected thickness in treated cohort 1b eyes decreased by −1 μm in treated eyes and −11 μm in untreated eyes at 3 months (Table S4). En face heatmaps of retinal thickness in cohort 1a (Figure 4) show that the thickness increase occurred mainly in subject −002, consistent with corrected thickness measurements (Table S4). OCT assessments of retinal thickness did not show significant epiretinal membrane (ERM) formation in any subject.

Macular sensitivity and fixation measurements by microperimetry (CenterVue Macular Integrity Assessment [MAIA]) were incomplete due to instrument breakdown. A minor trend of increased fixation and minimal change in overall sensitivity between treated and untreated eyes were considered unreliable due to the repeated instrument failures. An improved microperimetry technique[33] is needed to describe the effect of RPESC-RPE-4W implantation on fixation and macular sensitivity.

Autofluorescence area (AF) increased over 12 months from 11.3 to 16.5 mm in cohort 1a-treated eyes and increased from 8.6 to 10.5 mm in untreated cohort 1a eyes. At 6 months, there was little change in treated cohort 1a eyes. FAF measurements from cohort 1b showed little change over 6 months of follow-up. Note that RPESC-RPE-4W cells lack intrinsic pigment and autofluorescence to affect FAF. Measurements of FAF for cohort 1 (Tables S5 and S6) indicate a small increase in the area lacking autofluorescence in both treated and untreated eyes. We note that, unlike native RPE cells, RPESC-RPE-4W cells lack pigment and autofluorescence.

Fundus photography of cohort 1a and 1b subjects is shown in Figure 4. Small changes in pigmentation apparent between pre- and post-treatment color images suggest a modest expansion of unpigmented area in some subjects and no detectable change in others. This result is consistent with the lack of pigmentation in the implanted RPESC-RPE-4W cells. Fluorescein angiography at baseline, 6 months, and 12 months did not show choroidal neovascularization in any subject (data not shown).

Immune suppression

Mycophenolate mofetil and tacrolimus treatment begun 2 weeks prior to implantation was continued for 6 months. Immune suppression was monitored by a specialized physician. Subject (−004) was non-compliant after day 173 (~5.8 months). Subject −005 discontinued medications due to a periumbilical rash on day 23, started mycophenolate sodium on day 28, resumed tacrolimus on day 41, and then discontinued both medications on day 142 (~4.7 months). Subject (−007) was non-compliant with tacrolimus from day 80 to 91. Notably, discontinuation of immune suppression was not accompanied by inflammation or BCVA loss.

Adverse events

A total of 28 AEs were reported, including 18 AEs in the study eye, one AE in both eyes, and nine non-ocular AEs. AEs were of severity Grade 1 (22) or Grade 2 (6) using the Common Terminology Criteria for Adverse Events version 6.0 scale. The 18 study eye AEs were related to minor intraretinal hemorrhage at the retinotomy site in 4 subjects and subconjunctival hemorrhage in 9 subjects that resolved spontaneously in each case. A retinal tear in 1 subject was treated intraoperatively with laser and fluid-air exchange. Cataract occurred in 2 phakic subjects (−002 and −007). Subject −002 underwent cataract extraction after month 9. In summary, no IP-related SAEs occurred in any subject. The only procedure-related SAE was a retinal tear found intraoperatively in 1 subject. Implantation-procedure-related AEs occurred at a rate and severity expected from the PPV procedure.[17,34]

DISCUSSION

Preclinical studies show that RPESC-RPE cells at the 4-week intermediate progenitor stage of differentiation are more effective than other stages of development at engraftment and vision rescue.[24,25] Enhanced effectiveness of intermediate stage progenitor cells has been reported in transplantation studies of a variety of other central nervous system cell types, including dopaminergic neurons,[35] spinal cord neurons,[36] and photoreceptor cells.[37] Our clinical trial utilized 4-week progenitor-stage cells for RPE replacement therapy of dry AMD. The first-in-human study completed low-dose cohort 1 interventions with positive results. RPESC-RPE-4W were well tolerated, without significant overgrowth, inflammation, or IP-related SAEs. BCVA improvements indicate safety and an early signal of efficacy. Cohort 1

Table 3. Visual acuity of better-seeing cohort 1b

BCVA better-seeing Cohort 1b			Screen	1W	1M	3M	6M	9M	12M
–007	**Treated**	**Letters read**	50	54	57	57	54	49	44
		Snellen	20/100	20/80	20/80	20/80	20/80	20/100	20/125
	Untreated	**Letters read**	63	54	60	61	62	59	60
		Snellen	20/63	20/80	20/63	20/63	20/63	20/63	20/63
–017	**Treated**	**Letters read**	52	51	52	51	57	50	53
		Snellen	20/100	20/100	20/100	20/100	20/80	10/100	20/100
	Untreated	**Letters read**	59	57	61	58	62	60	57
		Snellen	20/63	20/80	20/63	20/80	20/80	20/63	20/80
–018	**Treated**	**Letters read**	55	57	59	59	55	N/A	N/A
		Snellen	20/80	20/80	20/63	20/63	20/80	N/A	N/A
	Untreated	**Letters read**	57	58	59	57	62	N/A	N/A
		Snellen	20/80	20/80	20/63	20/80	20/63	N/A	N/A
Average change in letters read, untreated eye			**N/A**	**–3.33**	**+0.33**	**–1.0**	**–1.0**	**N/A**	**N/A**
Average change in letters read, treated eye			**N/A**	**+1.67**	**+3.67**	**+3.3**	**+3.0**	**N/A**	**N/A**
Difference in treated vs. untreated eye			**N/A**	**+5.0**	**+3.33**	**+4.3**	**+4.0**	**N/A**	**N/A**

BCVA in ETDRS letters read with Snellen equivalent. Average change from baseline and average change between treated and control eyes were calculated from the raw data at each time point. A small increase in BCVA in treated eyes and a small decrease in BCVA in untreated eye occurred.

outcomes suggest that low-dose RPESC-RPE-4W therapy is safe and well tolerated.

BCVA in treated cohort 1 eyes improved compared with untreated fellow eyes. The BCVA gains found were not expected from the natural history of dry AMD,[29–32] which describes BCVA loss that accelerates when a GA lesion enters the fovea and slows after foveal involvement is complete. Group A subjects had large GA lesions of 5- to 10-mm diameter with complete foveal RPE loss. Group B subjects had smaller GA lesions with partial foveal involvement. Large visual acuity improvements such as those in cohort 1a are rare in untreated dry AMD. Additional data are needed for statistical analysis to definitively determine the significance of the observed vision improvements. The cohort 1a average BCVA gain of +21.67 letters from baseline was greater than reported by other RPE replacement trials for dry AMD.[38] The time course for BCVA improvement occurring between 1 and 4 weeks after RPESC-RPE-4W implantation is consistent with the engraftment mechanism of action described in preclinical studies[24] and a published study of PSC-RPE engraftment.[39] The small BCVA gain during the first week after RPESC-RPE-4W treatment may be attributed to the release of trophic factors such as pigment epithelium-derived factor (PEDF) that support photoreceptor cell function,[40] variability in visual acuity measurements, or an as yet unidentified cause. The distinct 1-week and 1-month time courses for vision improvement suggest that more than one mechanism underlies vision rescue.[41]

The worse-seeing cohort 1a, with baseline BCVA of 20/200–20/800, had greater vision gains than the better-seeing cohort 1b subjects with baseline BCVA of 20/70 to <20/200. Potential mechanisms for the greater improvement in worse-seeing than better-seeing subjects include (1) less densely packed native RPE cells in more advanced disease increased the intercellular space into which RPESC-RPE-4W cells engraft, (2) fewer native RPE cells in advanced dry AMD resulted in a greater ratio of RPE cells replaced, (3) a ceiling for vision improvement may limit improvement in the better-seeing subjects, and (4) mechanisms of early disease, such as thickening of Bruch's membrane, are less responsive to RPE replacement than later RPE cell atrophy that is directly targeted by RPE replacement. Independent of the underlying mechanism, worse-seeing subjects with the greatest need experienced more improvement while better-seeing subjects benefited from a smaller vision gain that reversed disease trajectory.

The finding of more vision gain in the worse-seeing participants contrasts with reports that PSC-RPE transplantation results in no vision gain in the worse-seeing participants and vision gain only in the better-seeing eyes.[38] Differences in the cell type implanted presumably underlie this difference in vision rescue. An important difference between PSC-RPE and RPESC-RPE is that PSC-RPE require extensive differentiation to minimize tumor risk from residual source cells while RPESCs lack tumor-forming potential, enabling implantation of less-differentiated 4-week progenitor-stage cells. Preclinical studies of the RPESC-RPE-4W progenitor stage show enhanced motility and engraftment. Our clinical results describe a relationship between RPESC-RPE-4W and recipient disease state. Dose escalation to better understand the dose-response relationship between RPESC-RPE-4W and BCVA is needed to determine whether increased doses continue to differentially affect worse- and better-seeing subjects.

The extent of vision gained varied from subject to subject. A potential cause is the different extent of GA lesion area treated. An impact of GA lesion coverage on vision outcome has been reported for PSC-RPE transplantation.[38] Lesion coverage by RPESC-RPE-4W may similarly depend on the extent of retinal separation during implantation. Other potential mechanisms for

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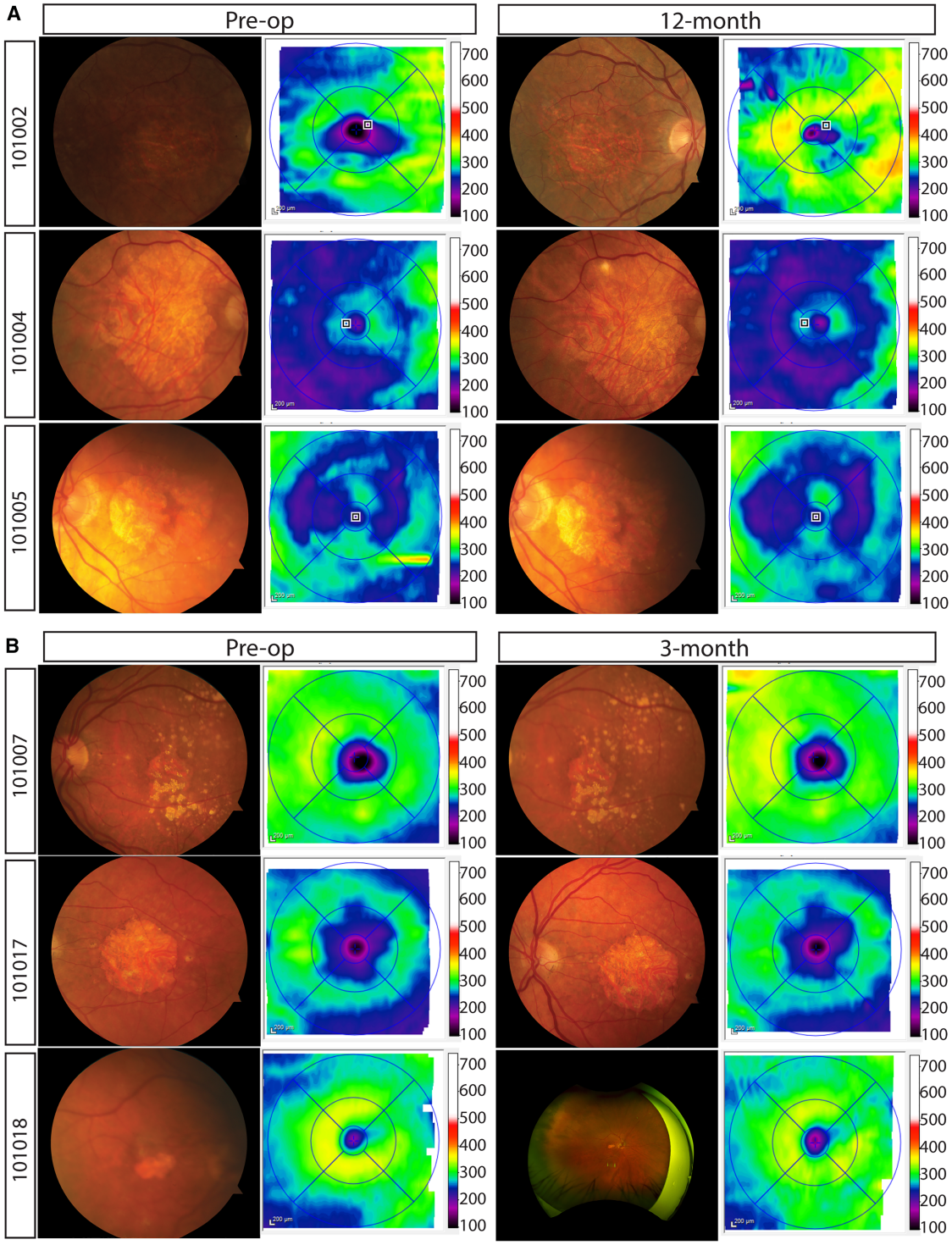

Figure 4. Color fundus and OCT heatmap images

(A) Fundus photos and macular thickness heatmaps at baseline and at 12 months for cohort 1a and (B) at 3 months for cohort 1b. Thickness maps were generated using automated Spectralis software. Little change was apparent after RPESC-RPE-4W treatment.

subject-to-subject variability include the baseline extent of atrophy and subtype of dry AMD/GA.[41,42]

The current study enrolled subjects with a spectrum of dry AMD disease state. Vision gain by RPE replacement is often attributed to rescue of dysfunctional cone photoreceptor cells located in the transition zone at the edge rather than the center of GA lesions. Photoreceptor cell loss in the center of a GA lesion[6,43,44] is often considered too advanced for rescue. Retracted cone photoreceptor cells that have lost outer and inner segments, however, are present within GA lesions.[6,45] Rescue of dormant, retracted cone cells has been reported for peripapillary atrophy,[46] macular telangiectasia,[47] and retinitis pigmentosa.[48,49] It is possible that rescue of residual cone cells within a GA lesion similarly contribute to vision improvement after RPE cell implantation. Our enrollment criterion of baseline macular thickness > 125 μm targets incomplete RPE and outer retinal atrophy when significant residual cone cells are present.[50,51] Further research and subgroup analysis is needed to better understand the mechanisms that may link recipient GA lesion morphology or disease stage[52,53] to RPESC-RPE-4W treatment efficacy.

Independent review of trial progress found "significant potential to address the unmet medical need" of dry AMD patients that resulted in regenerative medicine advanced therapy (RMAT) designation for RPESC-RPE-4W treatment. The trial was designed to assess safety and tolerability, not to determine efficacy. Vision gains met the primary safety endpoint of BCVA loss < 15 letters. The BCVA gains found also provide an early signal of vision rescue useful to guide future studies that enroll a larger number of participants.

RPE cell implantation as a suspension or sheet is a subject of active discussion in the field of RPE replacement.[14] Considerations favoring suspension delivery include (1) fewer surgical complications from the small retinotomy used for cell implantation and (2) direct contact of implanted cells with the native RPE layer to facilitate engraftment. Benefits of a scaffold include improved survival and preserved cell polarity for PSC-RPE.[54] Our preclinical experiments found that a RPESC-RPE suspension engrafts into the native RPE-Bruch's complex to maintain RPE phenotype, polarity, and survival. The clinical findings reported here show that suspension delivery can be a safe and tolerable route of administration for RPE replacement.

Neural retinal thickness measurements were manually corrected[55] to accurately identify retinal layers within a GA lesion that were mis-identified by the automated Spectralis segmentation program (Figure S1). Thickening occurred mainly in two subjects (−002 and −007), with little change in other eyes (Tables S3 and S4). The time-series of the OCT image from subject −002 (Figure S2) shows that minimal retinal edema and mild retinal thinning, consistent with dry AMD progression, was found in untreated eyes (Tables S3 and S4). Thickness measurements do not distinguish between structural change, edema, or subretinal debris. Spectral-domain OCT can resolve retinal layers in healthy eyes, but an improved segmentation technique is needed to resolve the thickness of specific retinal layers in diseased eyes.[50]

RPESC-RPE-4W implantation did not result in the significant ERM formation common in subjects receiving PSC-RPE transplants.[56–58] The lack of ERM may be attributed to the restricted potential of RPESC to form non-RPE progeny[21–23,59] and the reduced tendency of RPESC compared with PSC to undergo epithelial to mesenchymal transition[59] associated with ERM formation. The lack of ERM is a safety finding that distinguishes RPESC- from PSC-based RPE replacement.

RPE cell autofluorescence (AF) increases with age.[60,61] The loss of accumulated autofluorescence that accompanies RPE atrophy (FAF) is a frequently used surrogate measure of RPE cell loss and GA lesion area.[62] However, endogenous restoration of the RPE layer after RPE rips occurs without increased FAF.[63,64] Adult RPESC-RPE-4W cells notably lack autofluorescence and are not expected to increase FAF (Tables S5 and S6). Further, implanted RPESC-RPE-4W cells may displace compromised native RPE cells to reduce FAF lesion area. For these reasons, FAF has limited value to detect RPESC-RPE cells that lack autofluorescence or their effect on GA lesion area.

Immune suppression was provided for 6 months to allow recovery of RPE layer immune privilege that was disturbed by surgery.[65] After immune suppression was stopped, there was no evidence of rejection. This is consistent with reports of multiyear tolerance without immune suppression after allogenic PSC-RPE transplantation.[66–68] Our findings contribute evidence that RPE layer immune privilege[69] can support the survival of implanted allogenic RPE cells over extended intervals.

Limitations of the study

Major limitations of the cohort 1 report are that only six interventions were performed under open-label by one surgeon at a single clinical site. The interim results reported are considered preliminary until additional data are obtained from the 18 phase 1/2a subjects. Intrinsic variability in ETDRS BCVA or placebo effect, which are generally <5 letters in dry AMD studies, limit statistical interpretation of outcomes obtained from the 6 subjects treated. Nevertheless, the BCVA improvements found in cohort 1a are larger than reported for related RPE replacement studies. The lack of reliable retinal sensitivity measurements is a weakness of our trial. The inability to fixate is a recognized challenge in obtaining accurate microperimetry measurements from patients with advanced central vision loss.[70] This was compounded in our trial by instrument failures. A trend toward improved fixation reliability after treatment was found, but the measurements of retinal sensitivity were not considered to be sufficiently reliable for inclusion in this interim report.

In conclusion, interim results from the clinical study of RPESC-RPE-4W replacement therapy for dry AMD met the primary endpoints: lack of significant inflammation, lack of tumor formation, and lack of IP-related SAEs. BCVA showed clinically meaningful gains in the worse-seeing subjects. The positive safety and tolerability found for cohort 1 have enabled progress to mid-dose cohort 2 RPESC-RPE-4W therapy for dry AMD.

RESOURCE AVAILABILITY

Lead contact

Requests for further information and resources should be directed to, and will be fulfilled by, the lead contact, Brigitte L. Arduini (brigittearduini@neuralsci.org).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

Additional information required to reanalyze the data reported in this paper is available from the lead contact upon request. Details of subject medical records will remain confidential to protect patient confidentiality. Only de-identified, processed versions of medical data can be provided. Details of the clinical trial protocol, including enrollment inclusion and exclusion criteria, study plan, and a listing of outcome assessments have been deposited at clinicaltrials.gov (NCT04627428).

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AUTHOR CONTRIBUTIONS

Conceptualization, J.S. and S.T.; methodology, J.S., R.C.R., B.L.A., S.B., D.S., and S.T.; validation, D.S.; formal analysis, J.S., B.L.A., and C.R.; investigation, R.C.R., J.S., S.T., B.L.A., and S.B.; resources, R.C.R., B.L.A., and D.S.; analysis, K.W., S.K., and C.N.; data curation, B.L.A., C.N., K.W., and G.F.; writing—original draft, J.S. and B.L.A.; writing—review & editing, J.S., B.L.A., K.D., R.C.R., S.T., and K.W.; visualization, J.S., B.L.A., and C.R.; supervision, J.S., R.C.R., S.T., D.S., C.S., P.L., and K.W.; project administration, G.F., B.L.A., and K.W.; funding acquisition, J.S., S.P., E.O., K.D., and S.T.

DECLARATION OF INTERESTS

Drs. Stern and Temple co-founded Luxa Biotechnology. They are inventors in RPESC patents licensed to Luxa Biotechnology.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Data analysis
  - Clinical quality control
- ADDITIONAL RESOURCES

SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
anti-KI67	Abcam	ab92742; RRID: AB_10562976
anti-aSMA	Abcam	ab7817; RRID: AB_262054
anti-OTX2	Abcam	ab183951; RRID: AB_3076432
anti-BEST1	Novus Biologicals	NB300-164; RRID: AB_10003019
anti-MITF	Abcam	ab212611; RRID: AB_3677293
AlexaFluor 647 Goat Anti-Rabbit	Thermo Fisher Scientific	A21245; RRID: AB_2535813
AlexaFluor 647 Goat Anti-Mouse	Thermo Fisher Scientific	A21235; RRID: AB_2535804
DAPI	Thermo Fisher Scientific	D1306
Chemicals, peptides, and recombinant proteins		
DMEM/F12 50/50, 1X	Corning	10-0920CV
Minimum Essential Medium, Alpha 1X (aMEM)	Corning	15-012-CV
MEM NEAA (100X)	Gibco	11140-050
GlutaMAX (100X)	Gibco	35030-061
Sodium Pyruvate (100mM)	Gibco	11360-070
N1 Medium Supplement (100X)	Sigma	N6530
CTS N-2 Supplement	Thermo Fisher Scientific	A1370701
HI-FBS	Gibco	10082-147
1M Niacinamide	Spectrum Chemical	N1-105
Earle's Balanced Salt Solution (EBSS)	Gibco	14155-063
Hydrocortisone	Sigma	H6909
Taurine	Sigma	T4571
Triiodothyronine (TLT)	Sigma	T5516
1M NaOH	Spectrum Chemical	SO170
Calcium-, Magnesium-Free Dulbecco's Phosphate-Buffered Saline (CMF-DPBS)	Corning	21-031-CV
Synthemax II-SC Substrate	Corning	CLS3535
Cell Culture Grade Water	Corning	25-055-CV
0.25% Trypsin-EDTA	Life Technologies	25200-072
DNase I	Roche	3724751103
Cryostor CS2	Biolife Solutions	202102
Balanced Salt Solution Combination 2 (BSS)	McKesson	alc0065079550
CAS-Block Histochemical Reagent	Thermo	00-8120
Paraformaldehyde solution 4% in PBS	Santa Cruz Biotechnology	sc-281692
Triton X-100	Sigma	T9284-100ML
Nunc MiniTrays with Nunclon Delta surface (Terasakis)	ThermoFisher Scientific	163118
Critical commercial assays		
PEDF ELISA Kit	Abcam	ab246535
Experimental Models: Cell lines		
Normal Human Dermal Fibroblasts	Lonza	Cat# CC-2511
Software and algorithms		
GraphPad Prism	www.graphpad.com	10.1.2

METHOD DETAILS

Trial conduct

Oversight

Approval for RPESC-RPE-4W implantation therapy for dry AMD was obtained from the FDA and the University of Michigan Institutional Review Board (IRB). An IRB-approved informed consent form was reviewed with each subject and signed prior to screening. Clinical data was de-identified to safeguard subject privacy. Trial outcomes were evaluated monthly by the trial executive committee composed of 4 retinal specialists, a National Eye Institute (NEI/NIH) representative, the trial PI, study coordinator and the Emmes contract research organization. Clinical outcomes were reviewed after the first and last Cohort 1 subject by the independent DSMC composed of 2 retinal specialists, 2 stem cell scientists and a clinical trialist. The trial is listed on clinicaltrials.gov (NCT04627428). The trial adhered to the tenets of the Declaration of Helsinki. Release of clinical outcomes follows a prospective plan that includes executive committee and DSMC review and approval after completion of interventions in each dose cohort. The executive committee and DSMC have approved publication of the Cohort 1 outcomes described in this publication. The DSMC reviewed the Cohort 1 data and approved progress to cohort 2 mid-dose interventions implanting 150,000 RPESC-RPE-4W cells, which are ongoing. The prospective plan for release of clinical outcomes limits data release to Cohort 1.

Enrollment

Subjects referred by their attending ophthalmologist were screened by a qualified study coordinator and investigator based on inclusion and exclusion criteria that specify vision loss attributed to dry AMD, fitness to tolerate immune suppression and fitness to undergo implantation surgery. Worse-seeing group A subjects had complete foveal involvement and better seeing group B subjects had partial foveal involvement. Ocular exclusion criteria included confounding ocular comorbidities of macular edema, active choroidal neovascularization, advanced disciform scar and visual axis opacity. The extent of retinal atrophy was limited by an inclusion criterion that specified central macular thickness >125um. Complete inclusion and exclusion criteria can be accessed on clinicaltrials.gov (NCT04627428).

Immunosuppression

Subjects received 6-months of mycophenolate mofetil (1g bid) and tacrolimus (1mg bid) beginning 2 weeks prior to RPESC-RPE-4W implantation. The immunosuppression regimen was based on related PSC-derived RPE replacement studies.[17,69,71,72] Based on the 3-to-12-month durations of suppression in prior studies, we used a 6-month duration. Liberal criteria for discontinuation reduce systemic risk. Only one subject has discontinued immunosuppression early. Systemic medications were managed by a specialized physician experienced with relevant drug levels and toxicities.

Implantation procedure

Two vials of RPESC-RPE-4W with instructions for use were delivered by qualified carrier to the operating room within 48 hours of manufacture. Only FDA approved instruments and devices were used to complete the 3-port pars plana vitrectomy implantation procedure. A 38-gauge Med-One subretinal cannula was used to inject the RPESC-RPE-4W suspension into the subretinal space. The cannula was placed under the retina twice, initially to create a bleb and minutes later to inject the cell suspension. Cannula placement targeted a location 4-8 millimeters super-temporal to the fovea center. Injection occurred over 5-10 seconds to minimize shear damage of the cell product. Implantation surgery was performed by a board-certified retinal ophthalmologist using a Constellation Vision System (Alcon). Small retinotomies limited reflux and washout of the vitreous cavity removed refluxed cells.

Assessments

Primary endpoints (BCVA (<15 letter loss), tumor formation (<1mm size), intraocular inflammation less than moderate and lack of product- or implantation procedure-related SAEs were assessed for all subjects at screening, 1 day, 1 week, and 1, 3, 6, 9 and 12 month visits. Secondary and exploratory endpoints of macular thickness, FAF area, and macular sensitivity were assessed at screening, 6-, and 12-month study visits except for macular thickness which was assessed at each study visit. (OCT images were corrected through 12 months for Cohort 1a and over 3 months for Cohort 1b.) Basic ophthalmic exams at 18 and 24 months were performed to detect delayed adverse events should any occur. Fluorescein angiography (FA) was performed at screening, 6 and 12 months. Assessments were carried out by a dedicated study coordinator experienced in clinical research data acquisition. A complete list of assessments can be accessed at clinicaltrials.gov (NCT04627428).

Best Corrected Visual Acuity (BCVA). BCVA measurements were recorded by a qualified ophthalmic technician or study coordinator using a certified Series 2000 ETDRS chart following ETDRS protocol that includes refraction at each study visit. The ETDRS chart was placed at a comfortable viewing angle. The right eye was tested first. The subject was instructed to read each letter, left to right, beginning with the top of the chart. Subjects were asked to read slowly to achieve the best identification of each letter. When it became evident that no further meaningful readings can be made, the examiner noted the number of letters missed or read incorrectly. For subjects unable to visualize the eyechart, acuity was recorded as count fingers, hand motions or light perception. All assessments were performed using the same lighting conditions. Selected acuity measurements were repeated by an independent study coordinator who was masked to the study treatment. The ETDRS protocol to measure BCVA has high test-retest reliability with variability generally < 5 letters.[73]

Fundus Photography and Angiography. After dilated exam at screening, and month 3, 6, 9 and 12 visits, color photographs of the central retinal field were obtained by certified study personnel using a mydriatic Zeiss FF450 or equivalent fundus camera. The lowest light intensity to achieve a clear image at a 30° setting was utilized. After fundus photos were obtained during screening, months 6 and 12 visits, fluorescein angiography was performed by injecting 3-5 cc of 10% or 2 cc of 25% sodium fluorescein dye intravenously over

10 seconds. Using filters to detect dye fluorescence, images were obtained at early (30-120 second), mid (3-5 minute) and late (10-12 minute) intervals. Images were securely stored electronically.

Slit Lamp Examination and Ophthalmoscopy. A Haag-Streit BM 900 or equivalent slit lamp adjusted for subject comfort was used by a qualified retinal physician at the lowest light level to obtain clear visualization. Intraocular pressure was measured by Goldmann applanation tonometry. Slit lamp biomicroscopy of the anterior segment was performed at each visit. Inflammation was recorded using a clinical scale of mild, moderate, severe or very severe, specifying location as anterior chamber, posterior chamber or posterior segment. Indirect ophthalmoscopy was undertaken to examine the peripheral retina.

Optical Coherence Tomography. OCT was obtained using a Heidelberg Spectralis SD-OCT spectral-domain device. Measurements were obtained by a certified technician or study coordinator covering the ETDRS grid. Retinal thickness was recorded at the vertical outermost edge for each of three 2mm zones; center subfield, inner subfield and outer subfield. A dense volume scan (20x20, 49 section, ART 16 high speed) and a 7-line raster scan (30x5, 7 section, ART 25, High Resolution) centered on the fovea were obtained. Spectralis automated thickness algorithm were found to frequently mis-identify Bruch's membrane (BM) in areas of GA (Figure S1). Manual correction traced BM using Heidelberg's manual segmentation tools (Figure S1). Masking during manual correction was undertaken to avoid bias. Macular AF images obtained using the Spectralis device are not corrected.

Microperimetry. Macular sensitivity and fixation reliability were measured using a CenterVue MP-3 MAIA microperimeter over the central 10-degree field in a dim room. A threshold strategy specified a 68-stimuli grid with a 3° diameter red fixation target and 4–2 threshold strategy. A standard 3° Goldmann III stimulus size, with the background luminance at 31.4 asb and maximum luminance set at 10,000 asb produces a stimulus dynamic range of 20 dB. The settings provided a large, bright stimulus to maximize reliability and shorten testing time. Readings for a 68-point grid were averaged to quantitate overall global macular light sensitivity. Frequent failures of the microperimetry device precluded obtaining reliable measurements.

RPESC-RPE-4W Manufacturing

The Safety and Tolerability of RPE Stem Cell-derived RPE Transplantation in Patients with Dry Age-related Macular Degeneration (NCT04627428) trial utilized a single master cell bank (MCB) that was manufactured under Good Manufacturing Practice (GMP) at the University of Rochester Upstate Stem Cell GMP Facility. The MCB was derived from a single donor that met FDA Good Tissue Practice criteria for clinical use (Table S1). Primary RPE cells harvested from the single donor (BN17-003) were expanded over two passages in TAB2 media to produce c. 30M RPESC-RPE cells that were cryopreserved in c. 60 MCB vials. Each MCB vial contained c.500,000 RPESC-RPE cells. Quality control for the MCB included AMD risk loci, adventitial agents, cytogenetic integrity, microbiology, cell count, viability, morphology, RPE identity and purity (Table S1). RPESC-RPE-4W IP were prepared at the Cedars-Sinai Biomanufacturing Center by culturing an MCB vial in TAB2 media over 3.5 to 5 weeks ('4-weeks') in 24 well format (Figure 2A). The final culture generated 4-5 wells each containing c.300,000 RPESC-RPE-4W cells. One well was harvested 48 hours prior to final IP formulation to assess release criteria of: 1) Immunohistochemistry (ICC) identity using OTX2 >90%, BEST-1 >85% and MITF by report, 2) ICC purity using KI67 <10% for proliferation and SMA <5% for epithelial to mesenchymal transition (EMT), 3) cobblestone morphology by phase microscopy, 4) potency by normalized Enzyme Linked Immunosorbent Assay (ELISA) PEDF level >125ng/100,000 cells, 5) sterility by gram stain and culture, 6) mycoplasma and 7) endotoxin level < 0.5EU/ml (Table S2). Normal Human Dermal Fibroblasts (NHDF), used as control cells for manufacturing immunocytochemistry assays, were obtained from Lonza (cat# CC-2511) and cultured according to the manufacturer's instructions. The P2 RPESC-RPE-4W cells implanted had been shown to closely resemble native RPE cell morphology, ionic pumping, phagocytosis, and RNA expression.[23–25,74] Two cryovials each containing 75,000 RPESC-RPE-4W cells in 150ul of injection vehicle were shipped live to the clinical site at 2-8°C by World Courier to arrive at the clinical site within 48 hours of formulation. Post-thaw viability of the formulated cells was stable over > 48 hours at 4°C in injection vehicle. Injection through the 38-gauge cannula did not significantly affect cell count. Temperature monitoring and chain of custody documentation were maintained during shipping.

Data analysis

Data from Cohort 1 subjects contribute to the overall trial statistical analysis plan. In brief, each endpoint is summarized by dose cohort at baseline, by vision sub-group in each cohort and totals, and during each follow-up visit. Summaries include mean, standard deviation, median, IQR, minimum, and the maximum and mean endpoint change from baseline for each visit. Mean change in the endpoint from baseline to the Month 12 visit serves as an estimate of treatment effect. A separate hypothesis test for each endpoint is performed to evaluate the change from baseline to Month 12 for each dose level. Statistical tests are two-sided and performed using a 0.05 significance level. Statistical analysis using SAS version 9.4 or higher and/or R version 3.6.0 or higher will occur after 12-month follow-up data acquisition is completed for the total of 18 Phase 1/2a trial subjects.

Clinical quality control

The Kellogg Eye Center Clinical Research Center investigators oversee study conduct. A dedicated clinical study coordinator is assigned to study assessments and data entry follows Good Clinical Practice. Source data is entered in real time into study forms which are filed in secure subject binder. Data quality control checks are routinely carried out to identify data anomalies including missing data, out-of-range, erroneous or incomplete data across visits. Protocol deviations are reported to the study PI and medical monitor who report to the study sponsor, executive committee and DSMC. Emmes Corporation monitors the clinical site to ensure that operations follow established procedures. Clinical data is password protected.

ADDITIONAL RESOURCES

OCT and FAF image data reported in this paper will be shared by the lead contact upon request. Microperimetry data will be provided upon written request that acknowledges the microperimetry measurements to be provided are considered unreliable.

Cell Stem Cell, Volume *32*

Supplemental Information

Safety and tolerability of RPESC-RPE transplantation in patients with dry age-related macular degeneration: Low-dose clinical outcomes

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SUPPLEMENTARY TABLES AND FIGURES

Table S1. Quality control measures for derivation of Master Cell Bank referring to schematic of the manufacturing flow described in Fig. 2A.

Table S1. Quality control for BN17-003 Master Cell Bank (MCB).

Quality Test	Description	Method	Specification	Result
AMD Risk Loci	ARMS2/HTRA1	Taqman assay (rs10490924) Risk allele: T	Not TT	GG
	CFH	Taqman assay (rs1061170) Risk allele: C	Not CC	CT
Adventitious Agents	CMV	qPCR	Not detected	Not detected
	EBV		Not detected	Not detected
	HBV		Not detected	Not detected
	HCV		Not detected	Not detected
	HHV-6		Not detected	Not detected
	HHV-7		Not detected	Not detected
	HHV-8		Not detected	Not detected
	HIV1		Not detected	Not detected
	HIV2		Not detected	Not detected
	HLTV1		Not detected	Not detected
	HLTV2		Not detected	Not detected
	Parvovirus B19		Not detected	Not detected
	Polyomavirus BK		Not detected	Not detected
	Polyomavirus JC		Not detected	Not detected
	Retroviruses	Fluorescence PCR	Not detected	Not detected
	In Vitro Assay for Adventitious Virus Contaminants	MRC-5, VERO, Hs68 Cells with Hemadsoprtion and Hemagglutination Endpoints	Not detected	Not detected
	In Vivo Assay for Viral Contaminants:	Embryonated hen's egg inoculation	Not detected	Not detected

	European and U.S./FDA Test			
Cytogenetic Integrity	Karyotype	G-banding	Normal 46XX/XY	Normal 46 XY
Microbiology	Bacteriostasis/ Fungistasis	USP / EP	No inhibition	No inhibition
	Endotoxin	USP / LAL	< 0.5 EU/ml	0.1232 EU/mL
	Mycoplasma	MycoAlert + qPCR	Not detected	Not detected
	Sterility A	USP / Immersion	Negative	Negative
	Sterility B	Gram Stain	Negative	Negative
	Sterility C	BacT/ALERT	No growth	No growth
Cell Count	Total Live Cell Number	Hemacytometer	Report	42,350,000
Viability	Percentage of Live Cells	Trypan Blue Exclusion	> 80% viable	99%
Morphology	Cobblestone	Phase Imaging	Positive	Positive
Purity/Identity	BEST-1	Immunocytochemistry (ICC)	≥ 85%	86.8%
	MITF		Report	97.2%
	OTX2		≥ 90%	91.3%
	KI67		≤ 10%	4.5%
	SMA		≤ 5%	0.1%
Potency	PEDF Secretion	ELISA	≥1000 ng/mL	12,151 ng/mL

Table S2. Cell product release criteria referring to Fig. 2A,B.

Table S2. Criteria for release of a clinical RPESC-RPE-4W dose.

Test		Description	Specification
Viability	Cell Count / Viability	Cell Count and Viability assay by trypan blue exclusion	≥ 80%
Purity / Identity	Immunocyto-chemistry Staining	% of OTX2+ Cells	≥ 90%
		% of MITF+ Cells	Report
		% of BEST +1*Cells	≥ 85%
		% of Ki67+ Cells	≤ 10%
		% of SMA+ Cells	≤ 5%
Potency	PEDF level	PEDF supernatant via ELISA	≥125 ng/100,000 cells
Morphology	Microscopy	Phase Image	Positive for Cobblestone
Microbiology	Sterility A.	CFR 610.12 final formulated product	Negative result **after** FCP is transplanted
	Sterility B.	Gram stain final formulated product	Negative result **prior to** FCP release
	Sterility C.	In-process sample 48 hours prior to final harvest	No Growth-result prior to FCP release
	Bacteriostasis/ Fungistasis	Bacteriostatasis/Fungistasis Immersion (USP/EP)	Non-inhibitory
	Mycoplasma	Mycoplasma with Mycoplasmastasis: European and United States Pharmacopeia Guidelines (GMP)	Negative /non-inhibitory
	Endotoxin	BET/LAL Kinetic/Chroml/E Assay- USP(GMP)	< 0.5EU/mL

Figure S1. OCT measurement of macular thickness referring to Table 1.

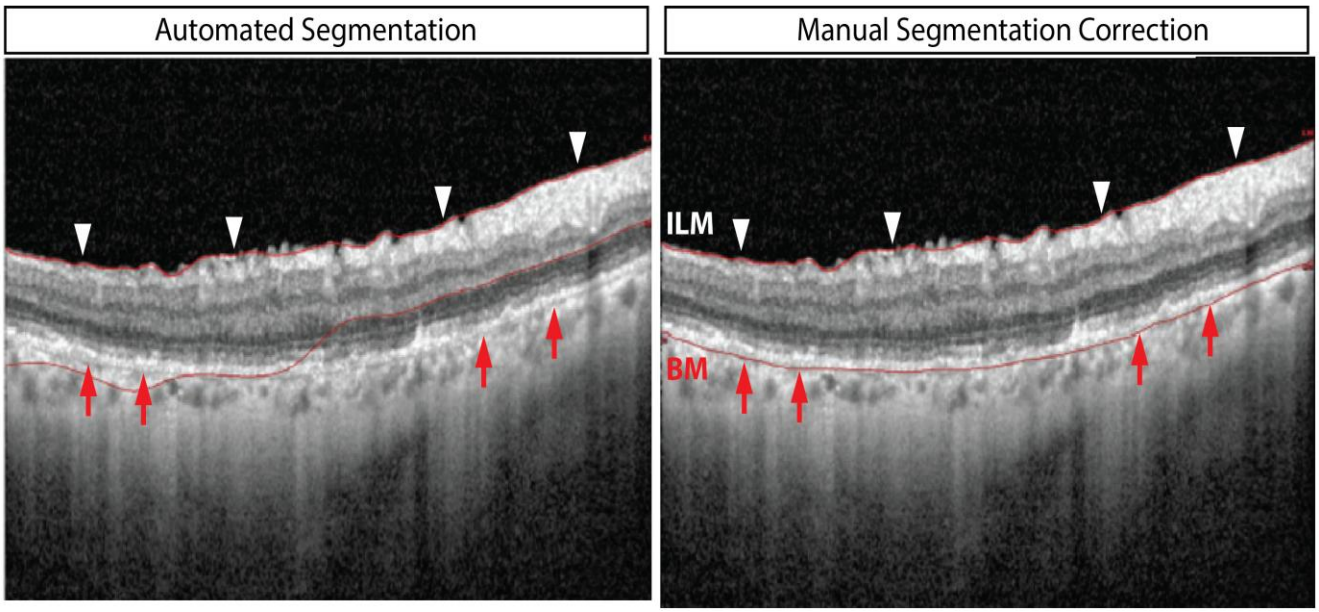

Figure S1. Optical Coherence Tomography (OCT) imaging and segmentation. Automated Heidelberg retinal layer segmentation (left panel, red line) frequently mis-identified Bruchs membrane (BM) in areas of GA. Manual correction was undertaken (right panel, red line) to improve BM identification accuracy. White arrowheads indicate the Inner Limiting Membrane.

Figure S2. Change in corrected thickness referring to Figure 3B.

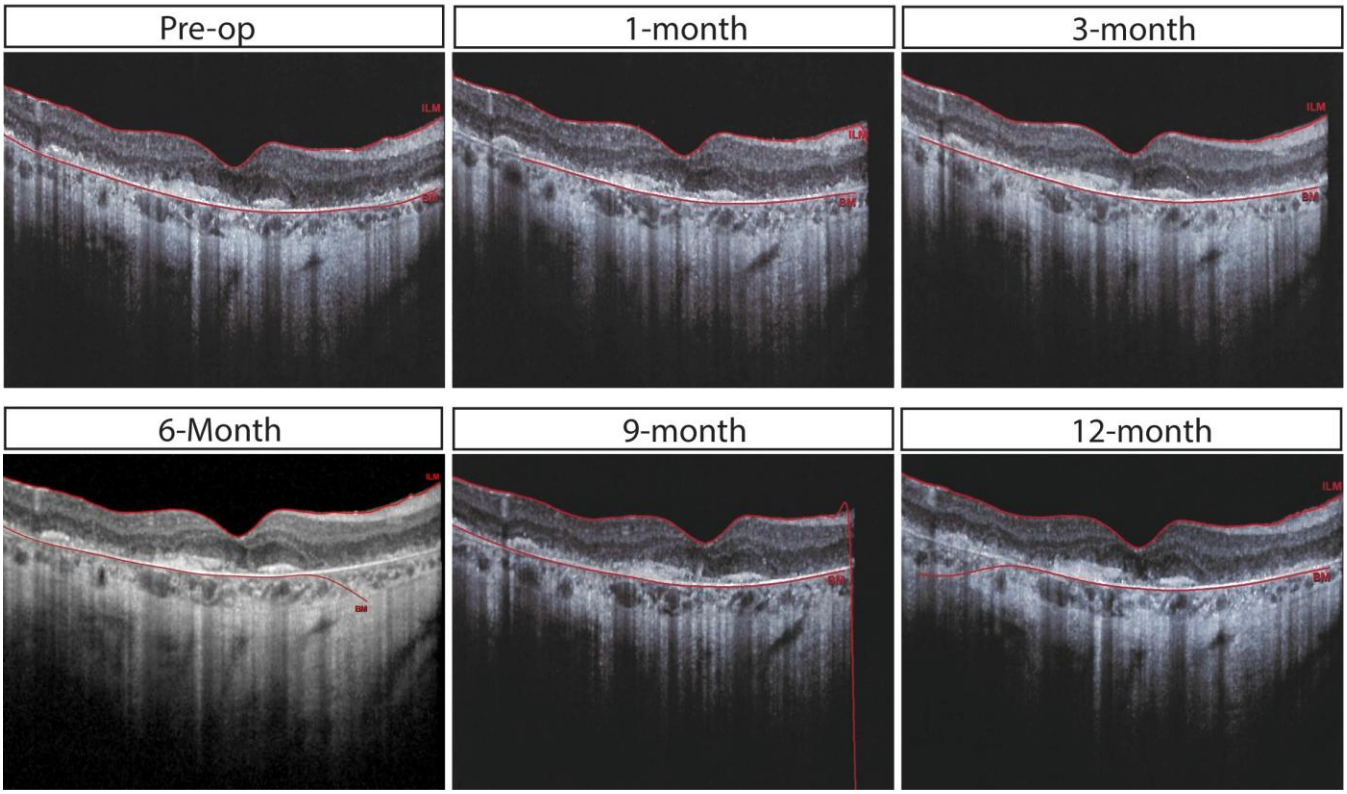

Figure S2. Time series of OCT images for subject -004. Red lines indicate manual correction of segmentation of Bruch's and inner limiting membranes . Retinal layers showed little change over 12 months of follow-up.

Table S3. Macular thickness measurements referring to Figure 3B.

Macular Thickness Cohort 1a (microns)			Screen	1W	1M	3M	6M	9M	12M
-002	Treated	Center Subfield	145	140	152	219	314	282	244
		Inner Subfield	265	211	262	290	329	327	319
		Outer Subfield	286	291	295	294	304	305	303
	Untreated	Center Subfield	265	261	257	263	253	248	251
		Inner Subfield	288	285	287	285	283	280	277
		Outer Subfield	287	287	287	287	286	287	283
-004	Treated	Center Subfield	244	242	243	245	238	235	243
		Inner Subfield	254	204	250	245	239	235	239
		Outer Subfield	244	246	245	239	238	234	237
	Untreated	Center Subfield	299	285	284	294	280	271	281
		Inner Subfield	270	261	260	267	258	254	258
		Outer Subfield	261	255	254	260	254	250	252
-005	Treated	Center Subfield	244	250	252	262	254	259	250
		Inner Subfield	253	261	257	257	249	252	247
		Outer Subfield	261	263	263	268	262	262	258
	Untreated	Center Subfield	264	266	275	270	265	275	274
		Inner Subfield	268	270	275	277	266	270	268
		Outer Subfield	288	291	296	295	289	292	289
Average thickness treated eye			**244**	**234**	**247**	**258**	**270**	**266**	**260**
Average thickness untreated eye			**277**	**273**	**275**	**278**	**270**	**270**	**270**
Treated eye change from baseline			**---**	**-10**	**+3**	**+14**	**+27**	**+23**	**+16**
Untreated Eye change from baseline			**---**	**-4**	**-2**	**+1**	**-7**	**-7**	**-7**

Table S3. Macular thickness for worse-seeing Cohort 1a. Corrected thickness measurements (**Fig. S1**) are shown in micrometers (um) for each study visit. Subfields indicate concentric anatomic regions; center'= 2mm diameter central foveal subfield, 'inner'= 2mm band around the central subfield and 'outer'= 2mm band anterior to the inner subfield. RPESC-RPE-4W implantation occurred in the outer band. Thickness increased in subject -002 with little change in subjects -004 and -005. Mild thinning occurred in untreated eyes.

Table S4. Macular thickness measurements referring to Figure 3B.

Macular Thickness Cohort1b (microns)			Screen	1W	1M	3M
-007	Treated	Center Subfield	163	184	173	165
		Inner Subfield	283	298	283	282
		Outer Subfield	300	310	307	314
	Untreated	Center Subfield	184	156	190	179
		Inner Subfield	279	286	281	281
		Outer Subfield	293	295	293	293
-017	Treated	Center Subfield	166	165	166	165
		Inner Subfield	246	245	246	247
		Outer Subfield	272	268	273	272
	Untreated	Center Subfield	160	158	163	166
		Inner Subfield	239	236	238	238
		Outer Subfield	267	264	264	264
-018	Treated	Center Subfield	254	251	233	225
		Inner Subfield	332	339	324	322
		Outer Subfield	288	300	297	296
	Untreated	Center Subfield	477	397	392	389
		Inner Subfield	419	405	414	409
		Outer Subfield	321	326	321	318
Avg. thickness treated eye			**256**	**262**	**256**	**254**
Avg. thickness untreated eye			**293**	**280**	**284**	**282**
Treated eye change from baseline			**-**	**+6**	**0**	**-1**
Untreated change from baseline			**-**	**-13**	**-9**	**-11**

Table S4. Macular thickness for better-seeing Cohort 1b. Corrected thickness for Cohort 1b are shown over 3 months of follow-up. Subfields indicate concentric anatomic regions; center'= 2mm diameter central foveal subfield, 'inner' refers to the surrounding 2 mm band and 'outer' refers to the 2mm band anterior to the Center and Inner subfields. Thickness increased more in treated than untreated eyes.

Table S5. Change in macular autofluorescence measurements referring to Table 1.

FAF worse-seeing Cohort 1a (mm^2)			Screen	3M	6M	9M	12M
-002	Treated	Area lacking AF	9.2	9.1	9.3	12.2	12.5
	Untreated	Area lacking AF	3.5	4.3	4.7	8.4	5.7
-004	Treated	Area lacking AF	14.5	16.2	17.1	19.8	20.9
	Untreated	Area lacking AF	17.6	19.1	17.9	28.5.	21.0
-005	Treated	Area lacking AF	10.2	12.9	11.4	12.5	16.0
	Untreated	Area lacking AF	6.4	7.8	11.0	10.9	13.8
Treated eye- average area			11.3	12.7	12.6	14.8	16.5
Untreated eye- average area			8.6	10.4	11.2	15.9	13.5
Treated eye- change from baseline			-	1.4	1.3	3.5	5.2
Untreated eye- change from baseline			-	1.8	2.6	7.3	4.9

Table S5. Fundus Autofluorescence area- Cohort 1a. Macular area lacking autofluorescence was assessed at baseline, 3-, 6-, 9- and 12-month intervals in worse-seeing Cohort 1a subjects. Raw data and calculated averages are shown in mm^2.

Table S6. Change in macular autofluorescence measurements referring to Table 1.

FAF better-seeing Cohort 1b (mm^2)			Screen	3M	6M	9M	12M
-007	Treated	Area lacking AF	3.5	4.2	4.1	4.9	5.0
	Untreated	Area lacking AF	4.8	4.6	4.4	5.5	4.7
-017	Treated	Area lacking AF	8.5	8.5	9.2	8.9	9.2
	Untreated	Area lacking AF	8.6	8.6	9.2	9.4	10.1
-018	Treated	Area lacking AF	3.7	1.4	1.9	-	-
	Untreated	Area lacking AF	2.9	0.0	0.2	-	-
Treated eye- average area			5.2	4.7	5.1	-	-
Untreated eye- average area			4.5	4.5	4.6	-	-
Treated eye- change from baseline			-	-0.5	-0.1	-	-
Untreated eye- change from baseline			-	0.0	-0.1	-	-

Table S6. Fundus Autofluorescence area- Cohort 1b. Macular area lacking autofluorescence for Cohort 1b was assessed at baseline, 3 months, 6 months, 9 months and 12 months for subjects -007 and -017 and over 6 months for subject -018. Raw data and calculated averages are shown in mm^2. Minimal change in FAF occurred over 6 months of Cohort 1b follow-up.