PI/IIa Study of Subretinally Transplanted Human Embryonic Stem Cell-Derived RPE Cells (OpRegen) in Advanced Dry-Form AMD Patients

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INTRODUCTION

- Age related macular degeneration (AMD) is the leading cause of blindness in people >50y in the developed world. Approximately 90% of these patients suffer from the dry form and currently there are no FDA-approved therapies beyond nutritional supplements.
- In dry-AMD, there is dysfunction and loss of retinal pigment epithelial (RPE) cells in the macular region. In the advanced stage, widespread loss of RPE and photoreceptors in the macular area evolves into geographic atrophy (GA), leading to severe vision loss.
- Attempts to transplant human embryonic stem cells (hESC)-derived RPE cells in patients with AMD, in suspension or on scaffolds, are being conducted by a number of groups.[1-6]
- Our directed differentiation protocol allows derivation of RPE cells from hESCs.[7] These NIH-approved cells, grown under cGMP conditions, underwent rigorous characterization and extensive safety and efficacy testing (**Figures 1-5**).
- In the Royal College of Surgeons (RCS) rat model of retinal degeneration, our hESC-derived RPE cells (OpRegen) settled into monolayers, polarized, and begin functioning (**Figure 5**), improving both structure and function compared with untreated controls.[8]

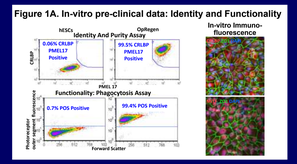

Figure 1A. Flow cytometric analyses and immunohistochemical staining. Differentiated hESC-RPE are >99.5% pure and positive for markers associated with normal human RPE.[8]

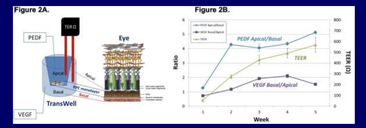

Figure 2. Barrier Formation and Polarized Secretion. A multifunctional potency assay assessing RPE barrier function and ability to secrete PEDF and VEGF in a polarized manner was developed to allow assessment of OpRegen. **[A]** The cells generate a polarized monolayer with barrier function (Trans Epithelial Electric Resistance, TEER). **[B]** Polarized PEDF and VEGF secretion (PEDF to the apical side and VEGF to the basal side) are observed over time. These functions are those associated with function, and correct polarity, of native RPE.

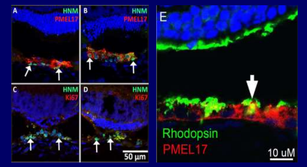

Figure 3. Integration and function of transplanted hESC-RPE in rats. High magnification confocal images of immunohistochemically stained OpRegen at P100 integrated into the host RPE layer. OpRegen stained positively for human nuclear marker (HNM) and human melanasomal protein marker (PMEL17). In addition, transplanted OpRegen did not stain positively for the proliferation marker Ki67 (absence of red in **[C]** and **[D]**). Panel **[E]** demonstrates an immunohistochemical (IHC) analysis of integration of transplanted OpRegen cells into the host rat RPE monolayer. Transplanted eye sections were double stained with rat rhodopsin (green), human specific PMEL17 (red), and DAPI (blue). The panel illustrates rat rhodopsin positive subcellular structures (outer segments) within the transplanted human cells (arrows).[8]

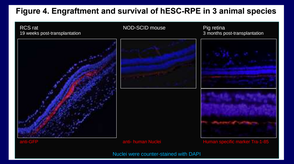

Figure 4. Engraftment and survival of hESC-RPE in 3 animal species. Immunohistochemical staining demonstrating presence and long-term survival in rats, mice, and minipigs.

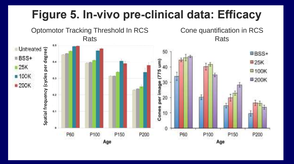

Figure 5. [A] Optokinetic tracking acuity thresholds measured at indicated timepoints. Cell-treated groups outperformed all controls with the mid (100K) and high (200K) dose achieving the best rescue. **[B]** Cone photoreceptor quantification in vehicle control (BSS), low dose (25K cells), middle dose (100K cells), and high dose (200K cells)-treated eyes. Cell-treated eyes had significantly higher numbers of cones compared with controls at all ages.[8]

OBJECTIVES

- The safety and tolerability of OpRegen is being evaluated in a dose escalating Phase I/IIa clinical study in patients with advanced dry AMD accompanied by GA (NCT02286089).
- Safety & imaging data from the first 15 subjects, who received a subretinal transplant of 50k-200k cells in suspension, with >3 years follow up in some, are reported in this poster.

METHODS

- Trial is planned for 24 patients, ≥50 years, with advanced dry AMD and GA
  - **Cohorts 1-2**: Three patients each; **Cohort 3**: six patients (all 3 Cohorts with BCVA ≤ 20/200)
  - **Cohort 4**: Twelve (12) patients, BCVA ≤ 20/64 and ≥ 20/250
  - Doses have ranged from $50x10^3$-$200x10^3$ in 50-100µl of balanced salt solution (BSS)
  - Staggering intervals within and between cohorts initially with periodic independent Data and Safety Monitoring Board (DSMB) review and approval before proceeding to next cohort.
- Transplantation was performed by subretinal injection following conventional 23 or 25G vitrectomy (N=15).
- Systemic immunosuppression is administered 1 wk. prior to transplantation and up to 6 wks. post
- Systemic and ocular safety is closely monitored. Retinal function & structure are assessed using various techniques including BCVA, and color, OCT and fundus autofluorescence (FAF) imaging.

RESULTS

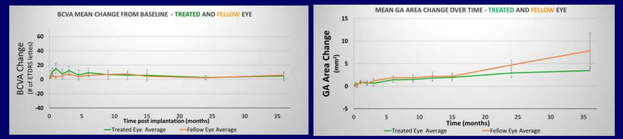

Figure 6. Best corrected visual acuity (BCVA) in Cohort 1-3 subjects did not decrease in treated eyes and has remained largely stable in fellow eyes. There appears to be a trend towards slower GA progression in treated eyes (NS). Of interest, one of three Cohort 4 subjects has shown clinically significant improvement (>3 lines), which has been maintained over 1 year (data not shown).

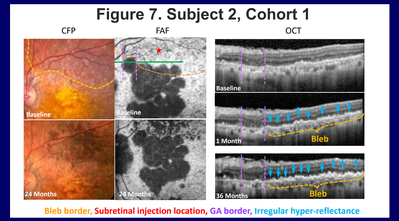

Figure 7. FAF, CFP, and OCT images demonstrate potential signs of long-term engraftment and survival of transplanted cells. Note subretinal pigmentation on color fundus photos and hypo/hyperfluorescent spots on FAF imaging in area of transplant persisting to >3 years. On OCT, irregular subretinal hyper-reflectance is visible as soon as Month 1 and throughout follow-up. Of note, systemic immunosuppressive therapy was discontinued shortly after implantation, which may suggest that potential immune rejection is limited or not present.

SYSTEMIC AND OCULAR SAFETY AND TOLERABILITY OBSERVATIONS IN SUBJECTS TREATED (N=15):

- BCVA remained largely stable over time in treated eyes
  - One cohort 4 subject has experienced and maintained a marked, clinically relevant, improvement (>3 lines)
  - One cohort 4 subject experienced a sharp decline due to ERM, BCVA returned to a few letters above baseline after ERM peeling and continues to improve
  - One cohort 1 subject experienced a sharp improvement followed by a decline but remains above baseline
- GA remained largely stable over time in treated eyes
- No IOP elevations occurred
- All subjects reported at least one AE (N=246)
- Most AEs were of mild intensity (88.6%)
- Most frequently reported systemic disorders
  - Asthenia and malaise reported by 4 subjects
  - All others AEs reported were single events with no trends
- The most reported AEs were related to Eye Disorders System; most frequent were:
  - Conjunctival hemorrhage after surgery – reported by 8/15, recovered rapidly
  - Eye irritation – reported by one subject
  - Subretinal fluid persisting over 24 hours post-injection reported by 4 subjects, all absorbed within 72 hours
  - Subretinal pigmentation – developed in 8 subjects (as reported by site)
  - One subject developed choroidal neovascularization (CNV) over two years after implantation
  - Epiretinal membrane (new/worsening) – reported by 13 subjects, most mild to moderate, one required interventional surgical peel, which was successful
  - Lamellar hole reported by 2 subjects (associated with ERM)
  - Retinoschisis (related to ERM) – reported by one subject

Two Ocular SAE

- Retinal detachment was reported in one subject 2 weeks after surgery. Unknown whether a result of surgical procedure, implanted cells, or a combination of events
- In one subject a severe ERM developed rapidly after surgery – intervention required – ERM peel was performed successfully – BCVA improved to a few letters above baseline, retinal structure restored

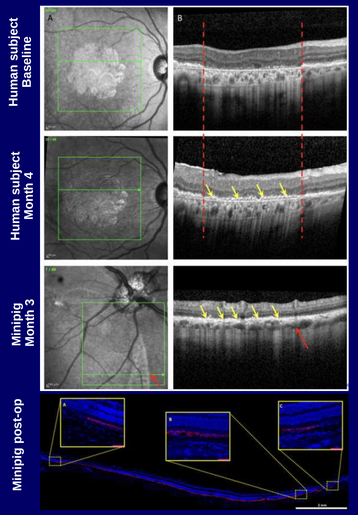

Figure 8. Comparison of OpRegen transplantation in human subject and minipig eye. In-vivo OCT images in a human and in a minipig following transplantation demonstrated similar findings, namely irregular subretinal hyper-reflectance (column B, yellow arrows) which is especially prominent in the human subject in an area of GA (delineated by dashed red lines). In the pig eye, this irregular hyper-reflectance abruptly stops at the border of the surgical bleb (red arrow). It was later possible to show by IHC in the pig eye that the transplanted cells survived and formed a subretinal layer with an intact layer of photoreceptors above them (lowest panel, paraffin-embedded pig eye sections stained with a human-specific marker, anti-Tra-1-85 (red); DAPI nuclear counterstain (blue). The results in the pig eye support the possibility that hyper-reflectance on OCT imaging correlates with presence of the transplanted cells. Orange arrow indicates limit of the surgical bleb in the minipig (below).

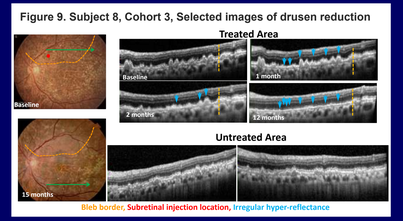

Figure 9. Changes in appearance, and reduction of drusen in area of transplant. Note reduction and change in the appearance of soft and confluent drusen over the span of a few months post-transplantation in a treated area (first two rows) from superior macula inside area of bleb. The outer nuclear (photoreceptor) layer and ellipsoid zone assume a more regular appearance. In an untreated area (lower row), from the inferior macula, the drusen persist.

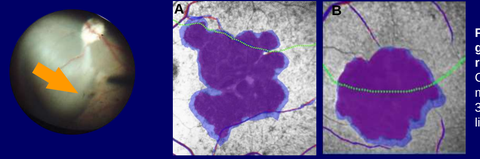

Figure 10. Asymmetrical, reduced growth of GA in the treated areas receiving OpRegen. [A] Subject 2, Cohort 1. **[B]** Subject 9, Cohort 3 at 12 months. GA at baseline (red-violet) vs. 36 months (light blue). The dashed green line is the surgical bleb border.

CONCLUSIONS

- Following subretinal transplantation of OpRegen (hESC-RPE) in suspension there is rapid healing of the injection sites and visual acuity has remained largely stable throughout the study with follow-up of up to >3 years.
- Subretinal pigmentation in the treated area is observed in 10/15 subjects, which has remained stable for >3 years in some subjects.
- There are additional signs suggesting potential RPE engraftment in the area of implantation, particularly subretinal hyper-reflective areas seen on OCT both in human subjects and in pigs; in the pigs this correlated with presence of transplanted cells on histology.
- Within the area of transplant, signs of a reduction and change in drusen material, as well as improvements or possible restorations of the ellipsoid zone and RPE layers, have persisted. The photoreceptor layer and ellipsoid zone assumed a more regular structural appearance in areas of the transition zone where OpRegen was administered.
- Asymmetrical, reduced growth of GA in the treated areas has been observed in some subjects.
- These findings require additional follow-up and observation
- New or worsening ERMs were observed in 13/15 subjects in cohorts 1-4, most were mild to moderate in severity. One required intervention and the ERM was peeled 10-weeks post-transplant with full recovery.
- There was one case of retinal detachment 2 weeks post-op, unknown whether a result of surgical procedure, implanted cells, or a combination of events.
- Overall, OpRegen appears well-tolerated with preliminary evidence of improved structural changes and potential improvement in visual acuity following treatment observed in some patients.
- Cohort 4 is ongoing, treating subjects with better vision, smaller areas of GA, and a known history of recent progression. Exploration of the suprachoroidal route of administration for subretinal injection using the FDA 510k cleared Orbit Subretinal Delivery System (SDS) is being utilized in new subjects.

REFERENCES: 1) L. Cruz et al. Nature Biotech. 2018;36: p328–37. **2)** A. Kashani et al. Science Transl Med. 2018;10(435) **3)** W. Song et al. Stem Cell Reports. 2015;4(5): p860–72. **4)** S. Schwartz et al. Lancet. 2015;385(9957):p509–16. **5)** Mandai, M., et al., NEJM 2017, 376: p1038-46. 6) Schwartz, SD, et al. Invest Ophthalmol, 2016, Vol. 57, pp. ORSFc1–9. **7)** M Idelson et al. Cell Stem Cell. 2009;5(4): p396-408. 8) T. McGill et al. Transl Vis Sci Technol. 2017;6(3):17.

DISCLOSURES: Ghesal Razag: Lineage Cell Therapeutics (BioTime Inc.), Employment; Diana Angelini: Lineage Cell Therapeutics (BioTime Inc.), Employment; Susan Meckler: Cell Cure Neurosciences, Employment; Judy Chen: Investigator; Katie Miani: Investigative site; Erin Nickerman: Investigative site; Sofia Gomez-Gudiel: Investigative site; Janet Kurokouchi: Investigative site; Diana V. Do: Consultant, Investigator; Rita Ehrlich: Investigator

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