Purpose: Retinal degeneration often results in the loss of photoreceptors, which leads to permanent vision loss. Generating transplantable photoreceptors using human induced pluripotent stem cells (iPSCs) to replace lost or dysfunctional photoreceptors holds a promise to treat a variety of retinal degenerative diseases. Developing effective methods to produce retinal cells including photoreceptors using available cGMP-manufactured human iPSC lines is a critical step for advancing cell replacement therapy to clinical application. This study aims to make transplantable photoreceptors using a cGMP-manufactured iPSC line. The generated retinal cells were tested for their differentiation capability and integration in a host mouse retina.

Methods: An iPSC line derived in cGMP-compliant conditions was obtained and used to generate retinal cells via a modified version of our previous directed differentiation protocol (Lamba et al 2006). The cells were characterized via qPCR and immunocytochemistry (ICC) at 6 weeks and 3 months after differentiation. GFP labeled retinal cells were injected into the subretinal space of 4-6 week old recipient mice. The eyes were collected at 2 months post transplantation for analysis by ICC.

Results: iPSCs, generated from CD34+ cord blood cells in a cGMP facility at Lonza Bioscience, were obtained from Dr. Zeng’s lab. They were induced to optic field stage for 1 week using small molecules to inhibit Wnt, BMP and TGFβ signaling pathways along with IGF-1 in culture medium. The cells were then expanded to generate neuro-retina and RPE. Neuro-retinal progenitor cells were manually isolated from RPE and expanded further to a relatively pure population of retinal neurons including differentiated photoreceptors by 3 months of culture based on expression of PAX6, OTX2, TUJ-1, CRX and AIPL1. The cells were then analyzed for their ability to integrate into the ONL layer of host retina in IL2rg null humanized mice. We found that a fully-functional RPE-BrM assembly is able to meet the manifold demands of the outer retina, with potential to rapidly benefit patients at risk of advancing blindness.

Results: We showed that a fully-functional RPE-BrM assembly can be made that is suitable for transplantation. This approach has the potential to bring rapid long-lasting benefits to patients with retinal diseases such as AMD or Retinitis Pigmentosa, and could also be adapted for other regenerative treatments.

Conclusions: We show that transplantable photoreceptors can be generated from a cGMP-manufactured human iPSC cell line which could then be fast-tracked to the clinic.

Commercial Relationships: Jie Zhu; Helen Cifuentes, None; Joseph Reynolds, None; Deepak A. Lamba, None

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Program Number: 3616
Presentation Time: 4:00 PM–4:15 PM
A novel biosynthetic RPE-BrM (Retinal Pigment Epithelium-Bruch’s Membrane) assembly suitable for retinal transplantation therapy
Andrew J. Lotery, None; Gareth Ward, None; Philip Alexander, None; David Johnston, None; Anton Page, Angela J. Cree, None; Atul Bhaskar, None; Sumeet Mahajan, None; Martin Grossel, None; J Arjuna Ratnayaka, None

Support: Brian Mercer Charitable Trust, Gift of Sight
Program Number: 3768
Presentation Time: 4:15 PM–4:30 PM
Phase I/II clinical trial of human embryonic stem cell (hESC)-derived retinal pigmented epithelium (RPE) transplantation in Stargardt disease (STGD): One-year results

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Purpose: Stargardt disease is the most prevalent juvenile-onset inherited maculopathy. Defects in the gene ABCA4 lead to accumulation of toxic vitamin A derivatives in cells of the retinal pigment epithelium (RPE), causing RPE dysfunction and degeneration. Retinal function may be protected or promoted by provision of a replenished population of RPE cells. Human embryonic stem cells (hESCs) provide one source of RPE cells for transplantation. Our aim was to investigate the safety and tolerability of subretinal transplantation of a suspension of hESC-derived RPE cells in advanced Stargardt disease.

Methods: We included 12 participants (aged 34 - 45 years) with clinical and electroretinographic features of advanced Group 3 Stargardt disease. Following pars plana vitrectomy we injected subretinally a suspension of hESC-derived RPE cells at doses of 50 K, 100 K, 150 K and 200 K cells. Participants were administered systemic immunosuppression until 3 months after the transplant procedure. We assessed systemic and ocular safety, indicators of cell survival and retinal function for 12 months.

Results: We observed the development of areas of subretinal hyperpigmentation in all participants, suggesting survival and engraftment of hESC-derived RPE cells. The extent of hyperpigmentation correlated positively with the dose of cells administered (R²=0.58, p<0.05). Hyperpigmentation was associated with areas of both hypo- and hyper-autofluorescence. We identified no evidence of tumorigenicity, immune adverse events or other serious safety concerns related to the transplanted cells. Assessment of ETDRS visual acuity, microperimetry, static full field perimetry, colour vision testing and electoretinography demonstrated no significant loss of visual function in the study eye of any participant.

Conclusions: Subretinal transplantation of hESC-derived RPE cells in Stargardt disease appears safe and well tolerated up to 12 months. The favourable safety profile supports the prospect of further studies to investigate the potential for benefit in less advanced disease.

Commercial Relationships: Yasuo Kurimoto, None; Yasuhiko Hirami, None; Masashi Fujihara, None; Chikako Morinaga, None; Midori Yamamoto, None; Kanako Fujita, None; Sunao Sugita, None; Michiko Mandai, None; Masayo Takahashi, None

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Program Number: 3769
Presentation Time: 4:30 PM–4:45 PM

Transplantation of Autologous induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelium Cell Sheets for Exudative Age Related Macular Degeneration : A Pilot Clinical Study

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Purpose: The purpose of this study is to evaluate the structure and function of our orthotopic whole eye transplant model by assessing intraocular pressure (IOP), aqueous humor dynamics using gadolinium (Gd)-enhanced MRI, optic nerve structural integrity with diffusion tensor MRI (DTI) and functional return via electoretinography (ERG).

Methods: Syngeneic transplants were performed in 5 Lewis (RT11) rats. IOP measurements were made using a TonoLab rebound tonometer. MRI: 0.3mmol/kg Gd-DTPA (Magnevist) was injected intraperitoneally after one T1- weighted image at baseline was acquired. Four animals were scanned at 3 weeks after transplantation, and 1 animal was scanned at 10 weeks after transplantation. Scans were performed using a 9.4-Tesla/31-cm Varian/Agilent scanner. ERG: Rats were placed in a black box overnight. An ERG system was integrated with a Dell PC equipped with a fast DSP microprocessor. Recording electrodes gently contacted the corneal

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surface of eyes precoated with a 2.5% hydroxypropyl-methylcellulose solution. A subdermal needle electrode served as common reference while another subdermal needle electrode was inserted at the base of the left leg. A Ganzfield delivered light stimuli with various stimulus strengths.

**Results:** IOPs of the naive and transplanted eye were 15.9±3.1 mmHg and 16.5±3.2 mmHg, respectively. At 3 weeks after transplantation, the right anterior chamber (AC) had a similar time to peak but a significantly lower peak intensity and lower initial increase rate than the left AC. At 10 weeks, the right AC had comparable peak intensity to the left AC. Limited Gd enhancement was observed in the vitreous with no significant difference between left and right eyes (two- tailed paired t-tests, p>0.05). T2-weighted images showed the donor optic nerve had comparable morphology with the uninjured intraorbital optic nerve at 3 weeks. DTI quantitation of the right injured optic nerve showed significantly lower FA and axial diffusivity by 54±6.1% and 24.9±5.7%, respectively, and a significant increase radial diffusivity by 83±29.5% compared to the left uninjured optic nerve (two-tailed paired t-tests, p<0.05). ERG revealed the lack of electrical responses to light stimuli in the transplanted eye.

**Conclusions:** A whole eye transplant model was established revealing the maintenance of aqueous humor dynamics, IOP and preserved integrity of the blood-ocular barriers of the transplanted eye.

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**Fig. 1:** WET graft (A and B). ON: optic nerve; TB: temporal bone; TN: trigeminal nerve; ELG: external lacrimal gland; CCA: common carotid artery; EJV: external jugular vein. Recipient immediately after WET (C). Post WET. Inset shows apposition of optic nerve.

**Fig. 2:** T1-weighted images at 0-10min (left) and 60-70 min (right) after systemic Gd administration, showing rat ocular anatomy of anterior chamber (AC), vitreous and lens 3 weeks after WET.

**Fig. 3:** Average time course of % Gd-enhancement in anterior chamber of both eyes at 3 weeks and 10 weeks after WET.

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