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# Treatment of Macular Degeneration Using Embryonic Stem Cell-Derived Retinal Pigment Epithelium: Preliminary Results in Asian Patients

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## SUMMARY

Embryonic stem cells hold great promise for various diseases because of their unlimited capacity for self-renewal and ability to differentiate into any cell type in the body. However, despite over 3 decades of research, there have been no reports on the safety and potential efficacy of pluripotent stem cell progeny in Asian patients with any disease. Here, we report the safety and tolerability of subretinal transplantation of human embryonic-stem-cell (hESC)-derived retinal pigment epithelium in four Asian patients: two with dry age-related macular degeneration and two with Stargardt macular dystrophy. They were followed for 1 year. There was no evidence of adverse proliferation, tumorigenicity, ectopic tissue formation, or other serious safety issues related to the transplanted cells. Visual acuity improved 9–19 letters in three patients and remained stable (+1 letter) in one patient. The results confirmed that hESC-derived cells could serve as a potentially safe new source for regenerative medicine.

## INTRODUCTION

Since their discovery and isolation in 1998, human embryonic stem cells (hESCs) have been considered a potentially valuable tool for generating replacement cells for therapeutic purposes (Lanza et al., 2009). However, despite success in numerous animal models, fears over tumorigenicity and immunogenicity, coupled with ethical concerns, and inefficiencies in differentiation methods have all contributed to delays in carrying out human clinical trials. Only one group has reported the results of the safety and possible biological activity of embryonic stem cell progeny in individuals with any disease (Schwartz et al., 2015), but these investigators only enrolled patients who were mostly Caucasian. Here, we confirmed the potential safety and efficacy of hESC-derived cells in Asian patients.

Loss of the retinal pigment epithelium (RPE) is an important part of the disease process in several retinal disorders, including age-related macular degeneration (AMD) and Stargardt disease. AMD is a degenerative disease that is the leading cause of visual impairment in developed countries, with the dry (nonexudative) form of AMD accounting for 85% to 90% of cases (Age-Related Eye Disease Study Research Group, 2001). Concurrent RPE and choriocapillaris atrophy are present in severe, atrophic dry AMD, with RPE atrophy preceding choriocapillaris atrophy (Schatz and McDonald, 1989; Korte et al., 1984; Leonard et al., 1997). Stargardt macular dystrophy (SMD) is the most common form of juvenile macular degeneration that is due to the production of defective rim proteins encoded by the *ABCA4* gene, leading to the accumulation of di-retinoid-pyridinium ethanolamine (A2E) in the RPE, RPE cell loss, and photoreceptor death (Glazer and Dryja, 2002). There are no known effective treatments to prevent or reverse visual loss for either disease. Since RPE loss is implicated in the pathophysiology of both disorders, RPE replacement has been suggested as a therapeutic intervention for these conditions.

Proper functioning of the RPE is important for maintaining the health and integrity of the outer retina, photoreceptors, and choriocapillaris. Healthy RPE cells play many crucial roles in the retina, including transportation of nutrients such as glucose or vitamin A from blood to the photoreceptors, secretion of growth factors, phagocytosis of the outer segments of the photoreceptors, formation of the blood-retina barrier by tight junctions, and establishment of immune privilege of the eye (Strauss, 2005; Wimmers et al., 2007). Based on the central role of RPE in the pathophysiology of AMD, researchers have attempted allogeneic and autologous RPE cell transplantations for cases of wet AMD (Binder et al., 2002; van Meurs et al., 2004; Algvere et al., 1994) and dry AMD (Algvere et al., 1997, 1999;

Joussen et al., 2007). However, most of these clinical trials have failed to show functional improvements in macular degeneration patients, possibly because of immune rejection and graft failure.

Animal studies have shown that hESC-derived RPE cell transplantation can rescue photoreceptors, resulting in the improvement of visual functions in RPE-oriented retinal degeneration models (Lund et al., 2006; Lu et al., 2009). Clinical trials of hESC-derived RPE cell transplantation have begun recently in the United States and Europe, and Schwartz et al. have reported preliminary safety data on one dry AMD patient and one SMD patient (Schwartz et al., 2012), as well as follow-up data with nine dry AMD and nine SMD patients (Schwartz et al., 2015). The patient population studied in this paper was all Caucasian, except for one African American patient with SMD. Our report provides interim results of the first pluripotent stem cell trials performed in Asian patients, who may carry different risk alleles for the development of some retinal disorders such as AMD. For example, the *Y402H* and *R80G* (in the *C3* gene) variants have been associated with AMD in Caucasians but not in Asians (Chen et al., 2006; Mori et al., 2007; Kim et al., 2008; Ng et al., 2008; Lee et al., 2008; Kondo et al., 2009; Goto et al., 2009; Pei et al., 2009). Herein, we report on four Asian patients with macular degeneration (two with AMD and two with SMD) who underwent subretinal transplantation of hESC-derived RPE and were followed for 1 year to assess safety and tolerability.

## RESULTS

### Derivation of RPE Cells from hESCs

The hESC-derived RPE displayed typical RPE behavior, such as pigmentation during differentiation and maturation, and also exhibited a cuboidal epithelial morphology in tissue culture. During culture, we observed clusters of pigmented RPE cell monolayers that exhibited their unique cobblestone morphology at the edges of clusters (Figures 1A and 1B). Karyotype results using g-banding showed 46XX, a normal female karyotype (Figure 1C). Thawed cells were cultured for 2–3 weeks until fully differentiated to human RPE (hRPE) cells with medium pigmentation (Figure 1D) and were stained for hRPE markers, including ZO1, PAX-6, MITF, and Bestrophin (Figures 1E–1I). We observed that >99% of cells expressed hRPE markers. For cell function analysis, we used phagocytosis assay kits using fluorescence-labeled bioparticles. Visual imaging of the differentiated hESC-derived RPE cells with fluorescence microscopy showed that most hRPE cells could phagocytize the fluorescently labeled beads (Figures 1J–1L). As for the quantification of the potency assay, fluorescence-activated cell sorting (FACS) analysis was conducted with hESC-derived RPE cells immediately post-thawing, which is more relevant to the phenotype of the cells that are actually transplanted (Figure S1). The percentage of cells phagocytized with fluorescence-labeled bioparticles was measured compared to a negative isotype control and an untreated negative control (test group at 37°C: 98.47% ± 0.32%, n = 3; isotype contol at 4°C: 34.47% ± 3.67%, n = 3; untreated negative control at 37°C: 5.52% ± 0.72%, n = 3) (Figure S1). 16-STR (short tandem repeat) genetic analysis using amplified genomic DNA (gDNA) proved that RPE cells originated from MA09. Immunostaining of OCT-4 and NANOG was conducted for impurity testing to confirm that no hESCs were present (Figure 1M). We counted DAPI-stained cells in three different fields and calculated the total cell number, and we did not see any cells that stained positive for $OCT\text{-}4^+$ or $NANOG^+$ within 21-mm dishes (Figure 1M). Additionally, we performed FACS using fluorescent labels for OCT-4 and TRA-1-60 and demonstrated no contamination by hESCs in the final product (PRE-0008) when 10,000 cells were analyzed for each marker: OCT-4, 0.28%; TRA-1-60, 0.02% (positive control: OCT-4, 53.26%; TRA-1-60, 40.96% [hES-MA09 cells were maintained on mouse embryonic fibroblast feeder cells]; negative control [NPC, neural precursor cells]: OCT-4, 0.47%; TRA-1-60, 0.35%) (Figure 1N). On further safety analysis through quality control testing, we confirmed the pathogen- and virus-free status of clinical samples by sterility, mycoplasma, and endotoxin detection following the Korean Pharmacopoeia and the Ministry of Food and Drug Safety (MFDS) guidelines for pathogen and virus testing. For the clinical studies, we transplanted >90% of viable cells after their final formulation in BSS Plus solution.

### Clinical Trial Results

The first advanced dry-AMD patient was a 79-year-old male with an initial best-corrected visual acuity (BCVA) of the study eye of one letter read and of the fellow eye of 20/25 (80 letters) on a Bailey-Lovie chart. During surgery, retinal detachment was difficult to initiate at the first retinotomy site, and subretinal cells were injected at a second site. A small subretinal hemorrhage was noted at this second site (Figure 2B). We estimated that $4 \times 10^4$ cells were injected subretinally. The hemorrhage absorbed spontaneously at postoperative 26 weeks (Figure 2C). Immunosuppression was stopped 4 weeks postoperatively because of repeated elevation of serum creatinine levels, blood urea nitrogen (BUN) levels, and potassium levels, as well as bone marrow suppression and diarrhea; these adverse events returned to preoperative levels after the cessation of immunosuppression. An epiretinal membrane developed at 2 weeks, with dark brown pre-retinal pigmentation from 3 weeks. The epiretinal membrane enlarged until 8 weeks, causing minimal distortion of the underlying inner retina, and the pre-retinal