Regenerative medicine promises tremendous hypothetical benefit for patients with blinding eye disease. The existence and plasticity of human embryonic stem cells (hESCs) was first described more than 15 years ago. Since that time, a number of other potential sources of stem cells have been discovered and proposed as sources for cellular therapies. The transplantation of undifferentiated or differentiated cells into specific anatomical locations for therapeutic effect holds great promise, especially for the treatment of retinal disease. The theoretical advantage of cellular therapy is that it may be utilized in any disease that leads to damage or loss of a particular cell type. Cell-based therapeutics can be derived from several stem cell sources, including adult stem cells, hESCs, and induced pluripotent stem cells (iPSCs).

Adult stem cells are undifferentiated progenitor cells found in a variety of tissues. These cells can differentiate into a limited number of specialized cell types found in that particular tissue, allowing for cellular repair and maintenance. Studies have shown that these cells can be differentiated along mesodermal, endodermal, and ectodermal lineages. However, finding and harvesting these cells can be onerous, and their capacity to divide and differentiate is limited, which poses difficulties for the generation of large quantities of donor cells for transplantation.

Human ESCs are considered a promising source of replacement cells for regenerative medicine to treat a wide range of diseases caused by tissue loss or dysfunction. Lines of hESCs can be generated from a single cell obtained from embryos created for reproductive purposes through in vitro fertilization procedures without sacrificing the embryo. Human ESCs are pluripotent and can differentiate into a multitude of cell types including endothelial cells, neural tissue, blood cells, and hepatocytes. Scalability issues are obviated as these cell lines have a high capacity to divide and differentiate. In addition, hESCs and their differentiated progeny have been shown to be less immunogenic than adult tissues in animal models of xenorejection. This immunogenicity, however, also depends critically on the local immune environment of the transplant site and the presence of antigen presenting cells. Another benefit is that issues related to the surgical delivery of hESC-derived RPE (hESC-RPE), such as cellular survival during transit from the syringe to the subretinal space, can be optimized in vitro and in preclinical studies. However, many hurdles to clinical utilization exist, including the risk of teratoma formation, unwanted differentiation, and immunolog-
ic rejection. Similarly, issues of engraftment, survival, and polarization in a diseased environment pose challenges to acceptable safety and efficacy profiles. Furthermore, despite the fact that hESCs are not abortion-sourced and do not require the loss of an embryo, the social issues have not been completely resolved.

Induced pluripotent stem cells were first derived from mice in 2006, and human iPSCs were developed shortly thereafter. Induced pluripotent stem cells can be dedifferentiated directly from adult cells such as skin fibroblasts. Given that these cells can be derived from and then transplanted into the same patient, there is a theoretically reduced risk of immune rejection. In addition, there is evidence that iPSCs and their differentiated progeny are inherently less immunogenic, but this may depend on the reprogramming technique used.13,19,20

The degree of immunogenicity of autologous iPSC-derived cells also appears to depend on the cell type produced. In an autologous transplantation study using humanized mice, human iPSC-derived smooth muscle cells were highly immunogenic, whereas iPSC-derived RPE cells were minimally immunogenic even when transplanted into skeletal muscle. A major impediment to the use of iPSC-derived cells, of course, is that any genetic defect present in the source cell will also be present in the differentiated iPSC progeny. However, iPSC-derived cells may benefit from the reprogramming and dedifferentiation process such that genetic predisposition to senescence-related disease processes may be obviated, at least in the short term. The development of techniques to repair disease-causing genes may permit the use of gene-corrected or edited, genetically matched donor cells for autologous transplantation. However, human iPSCs may be prone to transcriptional and epigenetic aberrations, and thus may have a propensity to form tumors, undergo premature senescence, or prove difficult to purify and scale on a commercial level. Similarly, methods of dedifferentiation and redifferentiation are still being optimized for safety, efficiency, and scalability. Nonetheless, iPSC technology holds tremendous promise.

Cellular Therapies for Macular Degeneration

The retina is an ideal site for the use of cell-based therapies. First, the transplanted cells can be directly visualized using a variety of imaging modalities assessing both structure and function, without the need for tissue biopsy. Second, the subretinal space in the healthy eye is a relatively immune-privileged site with markedly diminished cellular and humoral responses, which may limit the rejection of foreign cells as long as the blood–retinal barrier remains intact. Third, the size and architecture of the retina allows for a relatively small number of cells to be delivered to a specific location. Further, a successful therapeutic effect can be quantified using a variety of noninvasive functional measures including visual acuity, visual field testing, micropereimetry, color vision, contrast sensitivity, dark adaptation, and autofluorescence imaging. An additional advantage of the retina in comparison to other sites in the nervous system is that it contains a nonsynaptic layer, the RPE, which may be a less challenging target for initial safety and efficacy studies.

Degeneration of the RPE results in photoreceptor cell loss and impaired vision in many retinal diseases including dry AMD and Stargardt disease (STGD). Age-related macular degeneration is the leading cause of severe visual impairment in the developed world. In the dry form, which accounts for 80% of AMD cases, genetic and environmental factors predispose patients to RPE degeneration and geographic atrophy (GA). Stargardt disease is the most common form of juvenile macular degeneration, where defective proteins encoded by the ABCA4 gene result in photoreceptor cell dysfunction followed by RPE and photoreceptor death. There is no current treatment available for either disease, but since RPE loss is central to the pathogenesis of both disorders, RPE replacement has been proposed as a therapeutic intervention. There is evidence that implantation of RPE cells can preserve photoreceptors and prevent vision loss in preclinical models of disease. The use of other cell types to achieve this goal, such as neural or retinal progenitor cells and bone marrow–derived stem cells, will not be reviewed here.

The retinal pigment epithelium consists of a monolayer of hexagonally arranged epithelial, nonsynaptic, polarized cells adherent to Bruch’s membrane. The function of the RPE includes absorption of scattered light, epithelial transport of nutrients from the choriocapillaris to the photoreceptors and elimination of metabolites from retinal tissue to the choriocapillaris, spatial buffering of ions, phagocytosis of photoreceptor outer segment membranes, secretion of growth factors and cytokines, and regeneration of 11-cis retinal. The retinal pigment epithelium exhibits apical-basal polarity necessary for the transport of ions and nutrients, with the apical membrane in contact with the outer segments of the photoreceptors.

Initial studies utilizing autologous and allogeneic primary RPE cells in transplantation or translocation experiments had mixed results in terms of visual outcomes and graft survival. Two major difficulties with transplantation of adult human RPE cells are the high rate of surgical complications from the harvesting procedure and the very small quantities of cells that can be harvested. The use stem cell–derived RPE circumvents many of these pitfalls by permitting the entire process to be highly controlled and scalable such that many recipients could receive the same cellular product. Our initial strategy of using differentiated hESC-RPE in an area of compromised RPE in patients treated with high-dose systemic immunosuppression was designed as a first step into the realm of potential stem cell therapies for human disease.

Preclinical Studies

For the aforementioned reasons, hESC-RPE were utilized for initial preclinical investigation. The line MA09 hESC was used to create a master cell bank using good manufacturing practices. These cells were expanded on mitomycin C–treated mouse embryonic fibroblasts and allowed to differentiate in culture, with most cells expressing a neuronal phenotype. With time, polygonal pigmented RPE cells developed in the differentiating culture of embryoid bodies. These pigmented cells could be isolated, purified, and subjected to extensive testing, including pathogen, karyotyping, phagocytosis assays, differentiation and purity, quantitative PCR, and quantitative immunostaining for RPE and hESC markers (Fig. 1). Quantitative PCR showed downregulation of the hESC markers NANOG, OCT4, and SOX2 and strong upregulation of the transiently expressed neuroectoderm marker Pax6 and RPE markers bestrophin, MITF, and RP665. Expression of Pax6 was lost in mature RPE cell cultures. In addition, the cells demonstrated phagocytic activity and had a normal karyotype.

Preclinical testing in two animal models for efficacy and safety analysis was then performed. We injected hESC-RPE cells subretinally in Royal College of Surgeons rats and Elovl4 mice, two well-established animal models of retinal degeneration. The transplanted cells successfully integrated into the RPE monolayer as demonstrated by immunohistochemistry and persisted for more than 8 months without evidence of tumor formation. These mice demonstrated improved visual function and luminescence threshold response. Importantly, hESC-RPE cells...
transplanted into National Institutes of Health (NIH)-III immune-deficient mice remained localized to the subretinal space with no evidence of tumorigenicity, teratoma formation, or spread to other body parts (Fig. 2).

Although these preclinical models were encouraging, none of them can adequately recapitulate the senescence of Bruch’s membrane that would be seen in patients with dry AMD and which could play a role in hESC-RPE engraftment and survival. Bruch’s membrane has been shown to undergo various alterations with aging including atrophy, thickening, lipid accumulation, and decreased diffusional capacity. Given the stated challenges of engraftment and polarization that hESC-derived RPE face when transplanted in suspension into diseased eyes, it follows that the relative health of Bruch’s membrane may play a pivotal role in the success or failure of this strategy. Bruch’s membrane can be quite senescent in atrophic AMD whereas in STGD it should be theoretically less senescent. In light of this, the first clinical trials in humans...
were planned in part to evaluate two conditions on this spectrum of disease: elderly patients with dry AMD and younger patients with STGD.

**Safety Issues**

Despite the many theoretical advantages of hESC, including scalability, there are also a plethora of safety concerns if the hESC derivatives are to be implanted into humans. The differentiated cell population must be free of pathogens, possess the characteristics of the differentiated cell, and be free of undifferentiated cells. The cells in this study were derived from a female patient, and it is theoretically possible that female hESCs could feature derangements in X-chromosome inactivation and methylation. In addition, the cells need to be tested in various preclinical models, including immunodeficient animals, to demonstrate the absence of teratoma formation, hyperproliferation, or the migration of cells into other organs. Importantly all studies using the hESC-RPE cells in preclinical models showed no evidence of teratoma formation, hyperproliferation, or ectopic tissue formation.

Another major consideration in these studies is the possibility that the transplanted cells could initiate an inflammatory response or trigger immunologic rejection. Although cellular rejection itself would be a disappointing outcome, of greater concern is the possibility that an inflammatory reaction in the subretinal space could incite further damage to areas of retina that remain functional. Despite cross-species transplantation, preclinical models demonstrated little or no inflammation.

The preparation of the cellular material prior to transplantation is critical. The human ESC-RPE in their current formulation must be reconstituted, diluted, cultured, and Gram stained in a good manufacturing practice facility prior to surgery. In our safety trials, two doses were prepared for each patient as a contingency.

Finally, although the subretinal injection procedure involves standard vitreoretinal surgery techniques, care must be undertaken to limit the possibility of adverse outcomes such as infection, retinal detachment, choroidal hemorrhage, wound leaks, elevated intraocular pressure, or damage to the crystalline lens. One important advantage of delivering the cells in suspension via cannula into the subretinal space as opposed to delivering sheets of cells with or without scaffolds is the limited size of the retinotomy. With delivery of cells through a 25/38-gauge soft cannula, the retinotomy is minimized and therefore focuses the safety outcomes on the therapeutic cells rather than the surgical delivery.

Rather than discuss the entire scientific field, this review will more narrowly focus on the rationale and findings from two recent clinical trials studying the safety of hESC-RPE surgically transplanted in a cellular suspension into the subretinal space of patients with advanced dry AMD and STGD. This strategy requires transplanted cells in suspension to safely survive surgical transplantation, engraft onto compromised Bruch’s membrane, polarize, and function in the correct microenvironment such that they enhance native RPE function, and potentially rescue, protect, or regenerate compromised or dormant photoreceptor cells.

**STUDY FRAMEWORK**

Two prospective clinical studies to establish the safety and tolerability of subretinal transplantation of hESC-RPE in patients with STGD and dry AMD were carried out. These studies were phase I/II, open-label, nonrandomized, sequential, multicenter clinical trials. A total of 18 patients (nine with dry AMD and nine with STGD) were selected from four centers in accordance with the inclusion and exclusion criteria, including end-stage disease, genotyping, central vision loss, absence of other significant ophthalmic pathology, no history
of cancer, the absence of contraindications for systemic immunosuppression, the ability to undergo a vitrectomy and surgical procedure under monitored anesthesia care, and psychological suitability to participate in a first-in-human clinical trial involving hESC-derived cells. The protocol was approved by the institutional review boards, embryonic stem cell research oversight, and ethics committees of the respective sites.

**Surgical Considerations**

Transplant engraftment within a completely atrophic central macula is unlikely owing to the loss of choriocapillaris in advanced atrophic disease. In addition, complete macular atrophy does not mimic the state of the macula in earlier stages of degeneration, which likely remains the ultimate therapeutic target for these potential treatments. In both AMD and STGD, RPE loss and dysfunction are thought to precede photoreceptor loss, and therefore the greatest therapeutic benefit may come from early treatment when the overlying photoreceptors are still viable. For these reasons, transplantation sites were chosen carefully at the investigator’s discretion in order to target areas of compromised but still viable RPE and photoreceptors as determined by optical coherence tomography (OCT) and autofluorescence.

**Procedure**

Vials of cryopreserved hESC-RPE were thawed, brought to the appropriate concentration, Gram stained, cultured, and then delivered to the operating room. Pars plana vitrectomy, including the surgical induction of a posterior vitreous separation from the optic nerve anteriorly to the posterior border of the vitreous base, was performed in the eye with worse vision. We injected 150 μL of resuspended hESC-RPE through a 23/38 or 25/38 cannula (MedOne PolyTip cannula; MedOne Surgical, Sarasota, FL, USA), delivering the target dose of cells into the subretinal space near an area of compromised RPE thought to be amenable to rescue.

Three dose cohorts were used for each disorder with each cohort comprising three patients with STGD and three with AMD: cohort 1 received 50,000 cells, cohort 2 received 100,000 cells, and cohort 3 received 150,000 cells.

The immunosuppression regimen included tacrolimus (target blood concentrations 3–7 ng/mL) and mycophenolate mofetil (ranging from 0.25–2.00 g orally per day) a week before the surgical procedure and continued for 6 weeks. At week 6, the regimen called for discontinuation of tacrolimus and a continuation of mycophenolate mofetil for an additional 6 weeks.

**Study Endpoints**

The primary endpoints were the safety and tolerability of hESC-RPE transplantation in patients with dry AMD or STGD. The secondary endpoints measured the efficacy of the cells; study patients were followed up with serial ophthalmic examinations including testing for best-corrected visual acuity, visual fields, slit-lamp biomicroscopy, ophthalmoscopy, OCT, fluorescein angiography, autofluorescence, fundus photography, and electroretinography. Systemic monitoring involved serial physical examinations, vital signs, electrocardiograms, cancer screening, and hematological and serological testing.

Two phase I/II studies were conducted. Nine patients (five female; median age: 50 years, range: 20–71) were enrolled in the STGD trial and nine patients (six female; median age: 77 years, range: 70–88) were enrolled in the AMD trial. The median follow-up period was 22 months—four patients had <12 months follow up, 12 patients had 12–36 months follow up, and two patients had >36 months follow up.

**Safety and Tolerability**

These phase 1 clinical trials met the primary safety endpoint with no adverse events resulting from the cellular therapy. None of the eyes exhibited any sign of acute transplant rejection such as prominent lymphocyte infiltration, acute or chronic uveitis, or cystoid macular edema. There was no sign of hyperproliferation, teratoma formation, or apparent dedifferentiation of the cells. Angiographic analysis revealed no abnormalities in the retinal vascular or choroidal circulations up to 1 year after surgery.

Adverse events were largely limited to the surgical procedure and the systemic immunosuppression. Three eyes had preretinal pigmented foci visible on biomicroscopy and OCT near the injection site. However, no adverse effects such as epiretinal membrane formation or hyperproliferation resulted from these foci. Four eyes developed worsening cataracts requiring cataract surgery. A single case of culture-positive acute postoperative endophthalmitis (Staphylococcus epidermidis) occurred in an STGD patient 4 days after surgery, which was treated successfully with intravitreal antibiotic injections, antibiotic eye drops, and discontinuation of immunosuppression. Importantly, the culture and Gram stain performed on the original hESC-RPE batch were negative, and no subretinal inflammation was observed. Another eye developed vitreous inflammation with an inferior transvitreal band that resolved spontaneously by month 6 without any sequelae.

Other adverse events associated with the treatment were likely related to the immunosuppression. One patient acquired a urinary tract infection that necessitated discontinuation of the immunosuppression. Several other patients reported gastrointestinal symptoms and two reported nonmelanoma skin cancers.

**Visual Outcomes**

As part of the safety analysis visual function was studied. The best-corrected baseline visual acuity in the study eyes ranged from 20/200 to hand motion. In the 9 patients with AMD, visual acuity at 6 months improved by at least 15 letters in 4 eyes, 11 to 14 letters in 2 eyes, and remained stable in 3 eyes (change of ≤10 letters). In the 7 patients with follow-up at 12 months, 3 eyes had an increase of at least 15 letters, 1 eye had an improvement of 13 letters, and 3 were stable (Fig. 3A). Among the 8 STGD patients who had visual acuity assessed at 6 months, 3 eyes improved by at least 15 letters, 4 eyes remained stable, and 1 eye lost 11 letters. At 12 months follow-up, 3 STGD patients improved by at least 15 letters, 3 were stable, and 1 had a decrease of more than 10 letters (Fig. 3B).

Although the focus of these trials was safety of the cellular therapy, we sought structure-function correlations that might explain the improvements seen in some patients. We found 13 of 18 patients (72%) demonstrated increased subretinal pigmentation after transplantation at the border of the atrophic area (Fig. 4). Some of these areas were found to demonstrate reconstitution or thickening of the RPE layer on OCT, possibly suggestive of successful cellular engraftment. However, a major limitation of this study is that these anatomic outcomes cannot be definitively ascribed to the transplanted cells in the absence of higher resolution imaging techniques, microperimetry, or any specific label for the transplanted cells.
SUMMARY AND FUTURE CHALLENGES

Pluripotent stem cells have the capacity for unlimited self-renewal and have been proposed as a potential source of therapeutic cells for regenerative medicine. These studies provided the first description of the short- and long-term safety of hESC progeny transplanted into human patients. Given the excellent safety profiles observed thus far, this work sets the foundation for future trials using cellular therapies for regenerative medicine in humans.

Within the confines of these phase 1 trials, the transplanted hESC-RPE cells appear to be well tolerated. None of the 18 patients had an adverse intraocular or systemic event related to the cells. However, a number of patients had adverse events...
FIGURE 4. Imaging of eyes displaying pigmentation after hESC-RPE transplantation. (A–C) Color fundus photographs and OCT images of a patient with AMD at baseline (black circle shows an outline of the transplanted area), 3 months, and 6 months. Note the presence of a pigmented area (B, C, arrows) that becomes larger and more pigmented by 6 months. Optical coherence tomography images taken along the white dashed lines at each time point show the presence of cells on the inner aspect of Bruch’s membrane at 6 months compared with baseline. (D–F) Color fundus photographs and OCT images from a patient with STGD at baseline, 6 months, and 1 year. Pigmentation is evident at the border of the atrophic area (E) that becomes more prominent at 1 year (F, arrows). Optical coherence tomography images at baseline (D, inset) and 6 months (E, inset) show the formation of a thin hyperreflective line at the level of the RPE (E, arrows) adjacent to bare Bruch’s membrane. (G–I) Color photographs and OCT images of a patient with STGD with a large central area of geographic atrophy (G). Increased pigment is seen in the superior portion of the atrophic zone at 6 months (H) that becomes larger and more pigmented at 15 months (I). The corresponding OCT images illustrate hyperreflective foci at the level of the RPE at 6 months and 15 months post transplantation. Reprinted with permission from Schwartz SD, Regillo CD, Lam BL, et al. Human embryonic stem cell-derived retinal pigment epithelium in patients with age-related macular degeneration and Stargardt’s macular dystrophy: follow-up of two open-label phase 1/2 studies. *Lancet*. 2015;385:7–13. Copyright 2015 Elsevier.
related to the surgery or the immunosuppressive regimen. So while endpoints such as visual acuity improvements and structural changes seem encouraging, enthusiasm must be tempered. Initial safety studies such as this one are typically limited by the lack of a masked control group, the advanced disease present at baseline, and the small number of patients. Visual acuity measurements can be unreliable in patients with advanced geographic atrophy. Furthermore, while anatomic correlates to functional improvement may be addressed through a nuanced evaluation of the transition zone, single-cell resolution imaging may ultimately be required. Thus, challenges remain when trying to correlate functional improvements without clear anatomic changes. The next phase of clinical testing will include microperimetry in order to provide more rigorous structure-function correlations. A second major challenge is the use of solid organ transplant-dose immunosuppressive regimens. Subsequent studies will attempt to reduce the amount of immunosuppression and test whether it is even necessary to any degree. Future study designs will also incorporate a masked sham-surgery group to control for the placebo effect and examiner bias in subjective outcome measures such as visual acuity.

Since our initial report on the interim safety profile observed in two patients, a number of other stem cell treatment strategies have been proposed to address the various challenges and concerns posed by our initial approach. Future methodologies include augmentation of senescent Bruch’s membrane with artificial materials such as polyester or parylene to theoretically improve implantation and polarization of the cells. Other investigators are using sheets of RPE sourced from HLA-matched iPSCs to address rejection concerns.

As always, the burden of proof rests on upcoming randomized, multicenter trials. With more sophisticated multimodal imaging and functional testing such as adaptive optics-based scanning laser ophthalmoscopy and microperimetry, it may be finally possible to determine whether the transplanted cells are having a direct effect on visual function at particular sites in the retina. If this is found to be the case, retinal stem cell-derived RPE transplants could take hold as the first viable treatment for an unmet medical need with large numbers of individuals who might benefit.

Acknowledgments

The authors thank the Ocata Stem Cell Study group. This manuscript was written in collaboration with the Ocata Therapeutics Clinical Study Group. Steven D. Schwartz is the Ahmanson Professor in Ophthalmology, Stein Eye Institute, UCLA Department of Ophthalmology. Aaron Nagiel is the Elsa and Louis Kelton Fellow and the Thelma and William Brand Fellow as well as a recipient of the Heed Ophthalmic Foundation fellowship.

Supported by Ocata Therapeutics and an unrestricted grant from Research to Prevent Blindness, the Price Foundation, UCLA Broad Stem Cell Research Center, and the Stein Eye Institute Clinical Research Center, UCLA Department of Ophthalmology.

Disclosure: **S.D. Schwartz,** Alcon (C, F), Bausch & Lomb (C, F), Allergan (C, F), Genentech (C, F), Regeneron (C, F), Avalanche (C, F); G. Tan, None; H. Hosseini, None; A. Nagiel, None

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