Cell Stem Cell
In Translation

ESC-Derived Retinal Pigmented Epithelial Cell Transplants in Patients: So Far, So Good

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Many untreatable blinding diseases involve degeneration of retinal pigmented epithelial (RPE) cells, which has prompted exploration of the therapeutic potential of human-pluripotent-stem-cell-derived RPE. The first safety trials reported in *The Lancet* of embryonic-stem-cell-derived RPE cell transplants indicate no serious adverse outcomes and encourage further investigation.

In a recent issue of *The Lancet*, Schwartz et al. (2014) reported on progress in phase I/II clinical trials transplanting embryonic stem cell (ESC)-derived retinal pigment epithelial (RPE) cells in late-stage retinal degenerative disease. As the first report on the mid- to long-term safety of ESCs in cell therapy for any disease, this study is highly significant for the future development of ESC-based therapies.

In the retina, RPE cells serve multiple roles essential for the function and survival of the light-sensitive photoreceptor cells; they form a polarized monolayer (on Bruch's basement membrane) between the blood supply of the choroid and the overlying photoreceptor cells. Degeneration of RPE cells is involved in many untreatable blinding eye diseases, including atrophic age-related macular degeneration (AMD), which is the most common cause of sight impairment in industrialized countries, and Stargardt disease, which is the most common cause of inherited sight loss. The etiology of AMD is complex and polygenic with variant components of the complement cascade increasing the risk of disease, whereas Stargardt disease is usually caused by autosomal recessive mutations in the *ABCA4* gene, an ATP-binding cassette transporter essential for the removal of potentially toxic retinoid compounds from photoreceptor cells (Quazi and Molday, 2014).

The overarching aim of RPE cell transplantation therapy is to delay or prevent the progressive loss of photoreceptor cells by restoring healthy RPE function. Preclinical studies indicating the efficacy of RPE transplantation are limited, partly due to the lack of animal models for AMD, although there is some evidence that healthy RPE may extend the survival of photoreceptor cells and preserve macular function, for example, following translocation of autologous RPE in patients (van Zeeburg et al., 2012). In recent years remarkable progress has been made in establishing protocols for the generation of retinal cells from human pluripotent stem cells (hPSCs) (Nakano et al., 2012). ESC and induced pluripotent stem cells (iPSC) cultures can generate large numbers of RPE cells that form polarized monolayers and perform many of the functions of primary RPE such as rod photoreceptor outer segment phagocytosis (Buchholz et al., 2009; Ramsden et al., 2013; Kamao et al., 2014). The potential for therapeutic application of these cells is widely recognized, though the rationale for which disease context ESC-RPE cells might provide most benefit is a topic of debate. Several studies have evaluated ESC-RPE and iPSC-RPE using the Royal College of Surgeons (RCS) rat model of retinal degeneration, which harbors a mutation in the *Mertk* gene essential for outer segment phagocytosis, and have showed that subretinal injection of RPE can promote photoreceptor survival (Lu et al., 2009; Ramsden et al., 2013), though the mechanism by which this occurs has not been entirely resolved and may be independent of replacement of RPE function.

A number of clinical trials investigating the safety of ESC-RPE and iPSC-RPE transplantation are now underway or planned (Ramsden et al., 2013). Schwartz et al., funded by Advanced Cell Technology (ACT), reported previously the preliminary findings in two patients of safety and tolerability studies (Schwartz et al., 2012) and now describe the mid- to longer-term follow-up (Schwartz et al., 2014). They report the outcomes in nine patients with AMD and nine patients with Stargardt's macular dystrophy receiving subretinal transplants in one eye of 50,000, 100,000, or 150,000 ESC-RPE cells. All patients had end-stage disease with vision ranging from 20/200 (severe vision loss) to hand motion (near blindness). Because the loss of photoreceptor cells is at an advanced stage, the likelihood of recovery of vision in this population is low. In Stargardt disease, RPE transplantation might theoretically prolong photoreceptor survival, though it cannot be expected to correct the underlying photoreceptor gene defect.

The ESC-derived RPE cell preparation, classified as a xenotransplantation product because it involved animal cell coculture, was previously described (Schwartz et al., 2012). It involved differentiation to produce pigmented RPE patches of cells that were cryopreserved before direct subretinal injection as a cell suspension at a preselected transition zone between the central atrophic region and surrounding relatively healthy retina. Other studies plan to introduce cells supported on substrate that mimics Bruch's membrane, rather than as a suspension, which may enhance the generation of an RPE monolayer in vivo. Schwartz et al. report a number of adverse surgical events, including RPE defects, persistent subretinal injection bleb, acute postoperative endophthalmitis, and significant cataract in four patients. These complications were considered by the authors to be in line with the risks of vitreoretinal

surgery. Several adverse systemic events related to immune suppression were also reported. However, there were no ocular or systemic safety concerns attributable to the transplanted ESC-derived RPE cells themselves, such as significant immune reactions, uncontrolled differentiation, hyperproliferation, or tumor formation.

The safety of subretinal transplantation of ESC-RPE cells is also supported by the visual acuity findings. The acuity of the treated eye was maintained in the majority of participants with both AMD and Stargardt disease who did not develop cataract. In fact, the median improvement from baseline of visual acuity measured in those patients with AMD who did not develop cataract was greater in the treated than untreated eyes. It is widely acknowledged that the reliability of visual acuity assessment at low levels of vision is relatively poor and the authors point to the possible influence of placebo effect, examiner bias, and other non-cell-specific effects of surgery. Nevertheless, these initial results provide valuable reassurance that transplantation of ESC-RPE cells appears well tolerated, encourage further investigation, and will help accelerate progress in other planned trials of hPSCs for tissue repair.

The ideal outcome would be for the transplanted RPE cells to attach to Bruch's membrane, align correctly to form a monolayer extending into the atrophic regions between the neural retina and the vascular choriocapillaris, and function properly (e.g., perform efficient phagocytosis, secretion, and transport functions) so that further photoreceptor damage may be limited. It is however very difficult to evaluate transplanted cell behavior without cell labeling techniques using existing in vivo imaging technology because it is not possible to firmly distinguish the transplanted ESC-derived cells from the endogenous cells of the recipient. Schwartz et al. report the development in the majority of eyes of patches of subretinal hyperpigmentation, consistent with successful transplantation and suggestive of a layer of cells lining Bruch's membrane. They rightly caution, however, that the hyperpigmentation does not correlate with changes in visual acuity and may not correspond to transplanted RPE cells that cannot be distinguished noninvasively from surviving recipient RPE cells. Optical coherence tomography (OCT) imaging demonstrates areas of increased signal but cannot determine whether the transplanted cells survive, and the variability on fundus autofluorescence imaging is open to interpretation. In future studies, higher-resolution cellular imaging, for example through the use of adaptive optics techniques, may shed more light on the impact of ESC-RPE cell transplantation on retinal structure. These latest results indicate treatment of patients at earlier stages of disease who are likely to still have photoreceptors to preserve. Additional preclinical studies in animal models using genetic cell labeling techniques to compare the potential of cells delivered as a dissociated suspension, or on support matrices, to form functional RPE monolayers and preserve photoreceptors will be important to guide the development of future trials. Because systemic immunosuppression is undesirable, the use of autologous or HLA-matched hPSCs offers a promising future option (Wright et al., 2014). Ultimately, what will matter most is the impact of intervention on retinal function. In this early study the authors report no clear impact of transplantation on reading speed or conventional static perimetry. In future studies more sophisticated measures of retinal sensitivity, such as microperimetry, may help determine the effect of intervention on retinal function across the transplanted area and identify the relevance of the hyperpigmentation observed.

In summary, this study represents an important and promising first step on the pathway toward stem cell therapies being developed for the treatment of incurable blindness. Lastly, we should applaud the patients for their participation in this pioneering work.

REFERENCES

Buchholz, D.E., Hikita, S.T., Rowland, T.J., Friedrich, A.M., Hinman, C.R., Johnson, L.V., and Clegg, D.O. (2009). Stem Cells *27*, 2427–2434.

Kamao, H., Mandai, M., Okamoto, S., Sakai, N., Suga, A., Sugita, S., Kiryu, J., and Takahashi, M. (2014). Stem Cell Rep. *2*, 205–218.

Lu, B., Malcuit, C., Wang, S., Girman, S., Francis, P., Lemieux, L., Lanza, R., and Lund, R. (2009). Stem Cells *27*, 2126–2135.

Nakano, T., Ando, S., Takata, N., Kawada, M., Muguruma, K., Sekiguchi, K., Saito, K., Yonemura, S., Eiraku, M., and Sasai, Y. (2012). Cell Stem Cell *10*, 771–785.

Quazi, F., and Molday, R.S. (2014). Proc. Natl. Acad. Sci. USA *111*, 5024–5029.

Ramsden, C.M., Powner, M.B., Carr, A.J., Smart, M.J., da Cruz, L., and Coffey, P.J. (2013). Development *140*, 2576–2585.

Schwartz, S.D., Hubschman, J.P., Heilwell, G., Franco-Cardenas, V., Pan, C.K., Ostrick, R.M., Mickunas, E., Gay, R., Klimanskaya, I., and Lanza, R. (2012). Lancet *379*, 713–720.

Schwartz, S.D., Regillo, C.D., Lam, B.L., Eliott, D., Rosenfeld, P.J., Gregori, N.Z., Hubschman, J.-P., Davis, J.L., Heilwell, G., Spirn, M., et al. (2014). The Lancet, in press. Published online October 15, 2014. http://dx.doi.org/10.1016/S0140-6736(14)61376-3.

van Zeeburg, E.J., Maaijwee, K.J., Missotten, T.O., Heimann, H., and van Meurs, J.C. (2012). Am. J. Ophthalmol. *153*, 120–127.e2.

Wright, L.S., Phillips, M.J., Pinilla, I., Hei, D., and Gamm, D.M. (2014). Exp. Eye Res. *123*, 161–172.