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Concise Review

Recent Progress in Retinal Pigment Epithelium Cell-Based Therapy for Retinal Disease

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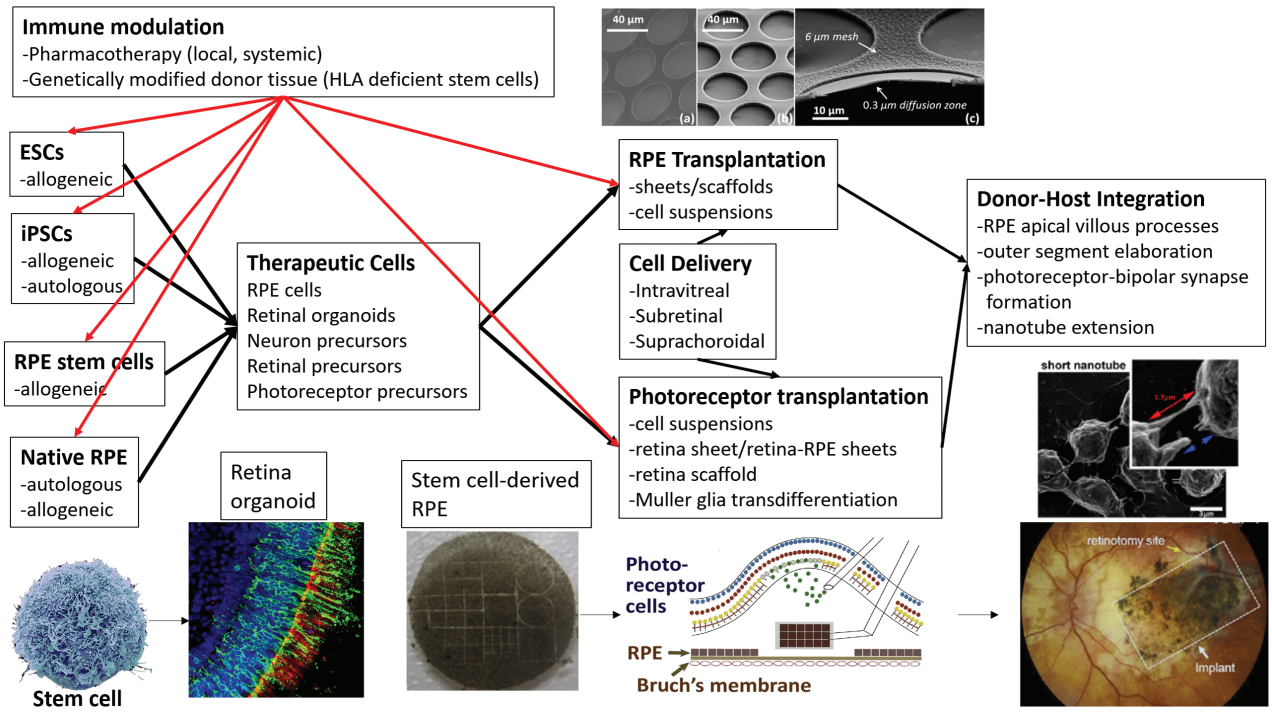
Abstract

Age-related macular degeneration and retinitis pigmentosa are degenerative retinal diseases that cause severe vision loss. Early clinical trials involving transplantation of retinal pigment epithelial cells and/or photoreceptors as a treatment for these conditions are underway. In this review, we summarize recent progress in the field of retinal pigment epithelium transplantation, including some pertinent clinical trial results as well as preclinical studies that address issues of transplant immunology, cell delivery, and cell manufacturing.

Key words: geographic atrophy; macular degeneration; induced pluripotent stem cells; embryonic stem cells; cell transplantation; retina; retinal pigment epithelium; retinitis pigmentosa.

Graphical Abstract



Significance Statement

This review summarizes recent clinical and preclinical studies involving retinal pigment epithelium transplantation as a treatment for age-related macular degeneration and Stargardt disease. Unresolved issues involving transplant immunology, cell delivery, and cell manufacture are explored in detail. We propose areas in which additional research could help accelerate progress in cell-based therapy for blinding retinal disease.

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Introduction

Age-related macular degeneration (AMD) is the leading cause of blindness in the industrialized world among persons aged 55 years and older.[1] By 2040, approximately 288 million persons worldwide will have AMD.[1] In the advanced stages of the disease, patients lose central vision due to the growth of abnormal blood vessels (termed choroidal new vessels) under the central retina (termed the macula), which leads to impairment of reading, driving, recognizing faces, and living independently. This outcome, termed "wet AMD," affects approximately 10%-15% of patients with AMD and can be treated reasonably well with intravitreal injections of agents that interfere with vascular endothelial growth factor (VEFG) signaling.[2] The other form of advanced AMD causing central visual loss involves atrophy of the macular photoreceptors and the subjacent retinal pigment epithelium (RPE) and choriocapillaris and is termed geographic atrophy (GA) or "dry AMD." In the US, approximately 1.2 million patients are projected to have AMD-GA by 2033 with an annual incidence of 160 000.[3] Stargardt disease (STGD) is the most common cause of inherited macular degeneration in children with a prevalence of 1:10 000 worldwide.[4] The most common causative mutation affects the *ABCA4* gene on chromosome 1, typically inherited in an autosomal recessive fashion.[5,6] In most patients, the *ABCA4* mutation affects retinal metabolism in the rod photoreceptors,[7] and the RPE cells degenerate with excessive lipofuscin accumulation associated with retinal outer segment phagocytosis. RPE damage is associated with photoreceptor death and blindness.[8] Central visual loss can occur at a young age or later in life,[9,10] but in most patients visual loss is in childhood.

Recently, medications that block the activation of complement factor 3[11,12] or complement factor 5[13] have been approved by the US Food and Drug Agency (FDA) to treat AMD-GA. These therapies do not seem to restore lost vision but delay the rate at which AMD-GA progresses. Medical as well as gene therapies are being developed for retinal dystrophies,[14] including mutation-agnostic gene therapy.[15] In addition, retinal prostheses are available to treat patients with profound visual loss due to photoreceptor degeneration.[16-18]

An alternative approach to treat the late-stage causes of AMD-associated blindness involves cell-based therapy to replace damaged RPE and/or photoreceptors, work which is in early-phase human trials. This review will focus on recent progress in RPE cell-based therapy for the treatment of AMD-GA. STGD will be mentioned briefly.

During the past several years, some important developments in RPE cell transplantation have occurred:

1. Both embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC)-derived RPE (iPSC-RPE) can be made to good manufacturing practice (GMP) specification.
2. Phase I studies using ESC-derived RPE (ESC-RPE) and iPSC-RPE show no evidence of tumor formation with 1-4 years follow-up.[19-25]
3. Transplants of RPE monolayers on scaffolds can be well tolerated[26,27] but also can be associated with complications such as retinal detachment and proliferative vitreoretinopathy.[28]
4. Transplants of RPE suspensions can be associated with efflux into the vitreous cavity and epiretinal membrane formation, but the establishment of a sustained, extensive monolayer of RPE does not seem to occur with subretinal delivery of RPE suspensions in patients with AMD-GA, AMD-associated choroidal neovascularization, or STGD.[19,22,23,29]
5. Among patients with neovascular AMD, autologous native[30] as well as iPSC-derived RPE transplants[20,25] are well tolerated in the absence of immune suppressive therapy. Modest clinical benefit was demonstrated in visual acuity[30] and in reduced need for anti-VEGF therapy.[20]
6. Allogeneic transplants on scaffolds (and possibly also as suspensions) can survive in the subretinal space of AMD-GA and STGD eyes for at least 2 years with a minimal immune suppressive regimen, although immune surveillance seems to occur, and the absence of rejection has not been unequivocally established.

We will summarize briefly the background and progress in each of these areas and propose areas in which additional research could help accelerate progress in RPE cell-based therapy. Familiarity with the anatomy and physiology of the RPE and retina is presumed in what follows (Fig. 1).

Progress in Human RPE Cell-Based Therapy Studies

A variety of cell sources have been used for human RPE transplant studies (Table 1).

Kashani et al[26] reported 1-year follow-up of a phase I/IIa clinical trial in which monolayers of differentiated human ESC-RPE on parylene scaffolds were transplanted into 16 subjects with AMD-GA. One subject had an adverse ocular event that included retinal hemorrhage and focal retinal detachment. A larger proportion of treated eyes experienced a ≥5-letter gain on the Early Treatment Diabetic Retinopathy Study (ETDRS) eye chart[33] vs their fellow untreated eyes (27% vs 7%, not statistically significant), and a larger proportion of non-implanted eyes demonstrated a >5-letter loss (47% vs 33%, not statistically significant). Each patient received immune suppression using tacrolimus (from day −8 to day 42) to achieve a therapeutic trough range. Tacrolimus dose tapering began on day 42 and was completed by day 60. Patients also received a single intravenous injection of 250 mg methylprednisone on the day immediately before surgery. Silicone oil (5000cS) was used for retinal tamponade. The severity of the baseline GA of the subjects enrolled in this study likely precluded the possibility of significant improvement in their visual acuity. Of note, one subject, who was able to perform microperimetry testing, demonstrated improvement in retinal sensitivity within the retina overlying the transplant. There was no improvement in the retinal sensitivity of co-registered retinal loci in the non-implanted eye. In addition to demonstrating safety, this study demonstrated the stability of the scaffolds once implanted, as none of the scaffolds migrated during the first year of the study, which allowed silicone oil to be removed in 15 subjects by the second year after their surgery. Based on these results, future studies will enroll patients with a shorter disease duration, a smaller region of AMD-GA, and the possibility of greater visual response to the transplant. Some of the technical aspects of the surgery also may be facilitated as the surgeons noted that separation of the retina from the overlying area of GA was easier and faster in less chronic cases. One of the benefits of transplanting a polarized RPE sheet may involve a trophic effect. Ahluwalia et al[34] showed that the polarized RPE secretome preserves photoreceptors in a preclinical model of photoreceptor degeneration, the Royal College of Surgeons (RCS) rat. The

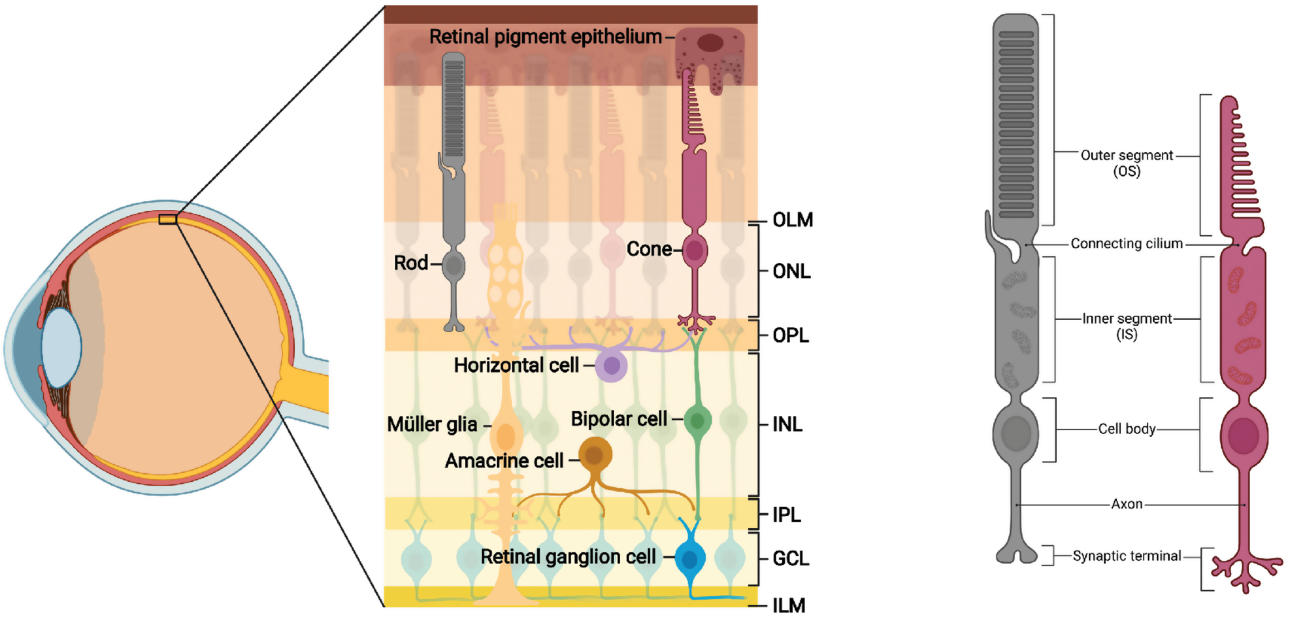

Figure 1. Organization and circuitry of the retina. (**A**) The retina contains 3 layers of cell bodies: the outer nuclear layer (ONL), in which rod and cone cell bodies reside; the inner nuclear layer (INL), containing horizontal cell (HC), bipolar cell (BC), amacrine cell (AC) and Müller glial (MG) cell bodies; and the ganglion-cell layer (GCL) where retinal ganglion-cell (RGC) somata and displaced ACs are found. PRs are supported by close apposition to the retinal pigment epithelium (RPE). The neural retina is bound apically by the outer limiting membrane (OLM) and basally by the inner limiting membrane (ILM), both formed by end-feet of the MG. PRs connect with BCs and HCs via synapses in the outer plexiform layer (OPL). The inner plexiform layer (IPL) contains signal-carrying synapses between BCs, ACs, and RGCs. (**B**) Rod and cone PRs display several distinct morphologic features. The outer segment (OS) contains stacked discs of photosensitive opsins for light detection. The connecting cilium facilitates trafficking between outer and inner segments (IS), the latter of which are rich in mitochondria. Extending from the cell body are axons with synaptic terminals, which interact with inner retinal neurons at triad ribbon synapses. Reproduced with permission from Ludwig and Gamm.[31]

Table 1. Cell sources for RPE transplantation.

Allogenic cell sources	Autologous cell sources
Embryonic stem cells	Induced pluripotent stem cells
Induced pluripotent stem cells –non-HLA matched –HLA matched –HLA genome-edited	Native RPE**
RPE stem cells	
Native RPE	

For references, please see text.
**Although not discussed in this review, autologous native RPE and RPE stem cell transplants have been done in clinical and preclinical studies.[30,32].

secretome reduced retinal cell apoptosis, diminished reactive oxygen species, and reduced oxidative and inflammatory stress in this model. This trophic feature of the transplant allows for the possibility of photoreceptor rescue directly over the transplant as well as the rescue of photoreceptors in adjacent areas to which soluble factors comprising the secretome diffuse. Human histology and in vivo imaging studies demonstrate the presence of viable photoreceptors, primarily cones, at the margins of GA.[35-37] Typically, these dying photoreceptors lack outer segments. Directly or indirectly, if the RPE transplant can reestablish normal physiology in these dysfunctional photoreceptors, then patients may recover some visual acuity as well as improvement in other aspects of visual function.

In contrast to the work cited above involving patients with AMD-GA, Sugita et al[24] reported 1-year follow-up in 5 patients with neovascular AMD who underwent iPSC-RPE transplantation. Salient features of this trial are as follows: (1) iPSC-RPE (established using non-integrating episomal vectors) were transplanted as a suspension (~2.5×10^5 cells); (2) patients displayed choroidal neovascularization incompletely responsive to intravitreal anti-VEGF injections; (3) surgeons did not excise the choroidal neovascular membrane as part of the procedure; (4) patients received intravitreal aflibercept[38] at the end of the procedure; (5) recipients and the donor were matched at 6 human leukocyte antigen (HLA) loci (class I [HLA-A, -B, -C] and class II [HLA-DRB1, -DQB1, -DPB1]); and (6) patients received local steroid injections (which can suppress T-cell activation and production of inflammatory cytokines such as interleukin (IL)-2, interferon-gamma, and tumor necrosis factor (TNF)-alpha) as the sole immunosuppressive treatment. Immune rejection was monitored through clinical surveillance (including routine diagnostic testing such as fluorescein angiography and optical coherence tomography (OCT)) as well as through laboratory testing in which the ability of recipient peripheral blood mononuclear cells to mount an immune response against donor iPSC-RPE was assayed in vitro. (The reaction of CD4 helper T cells, CD8 cytotoxic T cells, CD11b monocytes/macrophages, CD19 B cells, and CD56 natural killer (NK) cells was assessed using fluorescence-activated cell sorting; interferon-gamma levels also were assessed in the fluid phase.) RPE cells constitutively express class I major histocompatibility complex (MHC) antigens and can be induced to express class II MCH antigens as part of an inflammatory response.[39] As expected, the donor iPSC-RPE expressed HLA class I (A, B, C) but not class II (DR, DQ, DP) antigens although they did so when exposed to interferon-gamma. Although all 5 patients initially displayed

resolution of intra- and subretinal fluid, 4 patients developed recurrent fluid during follow-up. Thus, from the standpoint of using the transplant to treat the exudative complications of AMD, the surgery was not successful in 4 of 5 patients. The investigators posited that the number of cells transplanted may not have been sufficient to cover the area of macular pathology. An unusual feature of these transplants is that initially the degree of pigmentation was very slight in the area of the transplant, but pigmentation increased substantially during the course of follow-up in 2 patients, perhaps consistent with the concern regarding inadequate cell delivery. All cases developed epiretinal membranes, which probably indicates the presence of efflux of RPE from the subretinal space into the vitreous cavity during the recovery period following surgery. Although the authors detected clinical evidence of transplant rejection in only 1 patient (ie, increased subretinal fluid over the graft in the absence of dye leakage on fluorescein angiography), in vitro studies demonstrated evidence of an immune response in 3 patients. In one case, there was a proliferative response in CD4 T cells and CD11b monocytes 4 weeks after surgery (in the absence of any clinical signs of inflammation and preceding the development of increased fluid on OCT). The CD4 response was absent by 8 weeks after surgery. CD11b cells continued to proliferate in response to iPSC-RPE exposure until 21 weeks after surgery, and by 36 weeks the response had returned to baseline. (Subretinal fluid resolved following subtenon steroid injection.) In the second case, CD4 cells demonstrated a proliferative response 12 weeks after surgery in the absence of clinical signs of inflammation. In the third case, the patient developed an inflammatory response judged to be due to triamcinolone-related sterile endophthalmitis. By 24 weeks after surgery, this patient developed a positive response to donor iPSC-RPE as judged by CD11b monocyte proliferation, which resolved eventually without treatment. These results indicate that signs of immune surveillance may change over time. If the initial assessment for an immune response to the transplant is at a delayed time point, one may falsely conclude there has been no immune reaction even with laboratory testing. Also, clinical assessment to detect signs of immune rejection may be unreliable. Finally, HLA matching alone may not be adequate to prevent immune surveillance of subretinal transplants of RPE suspensions.

RPE Transplant Immunology: Clinical Results

A number of recent studies have reported no clinical evidence of ESC-RPE[19,21,23,26,27,40-42] and allogeneic iPSC-RPE transplant rejection, and no signs of rejection were noted in a patient who underwent autologous iPSC-RPE transplantation,[25] as expected. (Another phase I/II trial involving scaffold-based autologous iPSC-RPE is in progress [Autologous Transplantation of Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelium for Geographic Atrophy Associated with Age-Related Macular Degeneration, NCT04339764.) However, it is clear from preclinical studies that clinical evidence of inflammation is rarely observed in the setting of immune reaction to subretinal RPE transplants (see below). Kashani et al[43] reported a histopathologic study of a patient with AMD-GA who received a human ESC-RPE transplant on a parylene scaffold (Fig. 2). The implant measured 3.5 × 6.25 × 0.006 mm^3, had a monolayer of approximately 100 000 well-differentiated RPE cells, and was designed to underlie the majority of the macula. The RPE cells were allogeneic and were derived from the H9 human ESC line. The recipient demonstrated mismatch from the RPE cell donor in 14 of the 16 class I and class II HLA alleles that were examined. The recipient also received a short course of immunosuppression consisting of 0.075 mg/kg/day tacrolimus from day -8, to day 42 to achieve a serum trough range of 3-10 ng/mL. At day 42, tacrolimus doses were tapered by half every week until day 60 when immunosuppression was terminated. The recipient demonstrated no improvement in visual acuity at the year-1 and year-2 follow-up visits. Routine histopathology demonstrated a reasonably confluent monolayer of pigmented RPE cells. Occasionally, cells were present on the underside of the parylene scaffold, a phenomenon that had been observed in preclinical studies.[44] RPE cells stained positively for RPE65, a visual cycle protein involved in the conversion of all-*trans*-retinyl ester to 11-*cis*-retinol. The cells also stained positive for Na-K ATPase in a distribution that was largely but not entirely apical. (Normally, this enzyme is apically located in RPE.) The donor origin of the implant-associated RPE cells was confirmed by positive immunostaining for the HLA class I antigen, HLA-A2, expressed by the donor cells but not by those of the recipient. Donor RPE cells also stained positively for bestrophin, a cytosolic calcium-activated ion channel present in RPE cells.[45] RPE cells on the scaffold did not stain for recipient-specific HLA-B7 antigen, although such cells were present in the recipient. There was no evidence of cell proliferation in the implant as demonstrated by the absence of Ki-67 immunoreactivity. Despite the fact that photoreceptor nuclei were not detected over the area of the implant, focal areas of rhodopsin staining associated with photoreceptor-like structures in rosette configurations were noted within the area of GA and immediately above the RPE implant. Some RPE cells exhibited rhodopsin-positive inclusions, which may be phagosomes resulting from phagocytosis of host photoreceptor outer segments. In addition, CD34-positive cells were present subjacent to the implant. CD34 is an endothelial cell marker. The contralateral eye did not demonstrate these subretinal vascular structures. This result may mean that the implant, through expression of VEGF and/or other factors, may have stimulated choriocapillary growth without initiating the development of pathologic choroidal neovascularization. Thus, these data indicate that donor RPE: (1) survived for 2 years, despite a relatively brief immunosuppressive regimen; (2) maintained their differentiation and monolayer configuration; (3) interacted with some host photoreceptors; (4) may have fostered physiologic growth of subjacent vascular structures (eg, choroidal endothelial cells).

Immune cell infiltrates were present in the recipient. CD68 is a marker of macrophages. CD68-positive cells were more abundant and more widely distributed in both the retina and the choroid of the implanted vs the fellow control eye. CD8 is a marker of cytotoxic T lymphocytes. Frequent CD8-positive cytotoxic T cells were present in the choroid and adjacent to the implant and were particularly concentrated in the area adjacent to Bruch's membrane in the untreated eye as well. CD4 is a marker of Th lymphocytes. CD4-positive T cells were present in the retina and choroid surrounding the implant and were prevalent in the choroid of the non-implanted eye. Some of the CD4-positive cells in the retina of the implanted eye were also FOXP3-positive, which may indicate the presence of a regulatory or immune suppression effect of these cells. The investigators assessed the presence of autoantibodies to

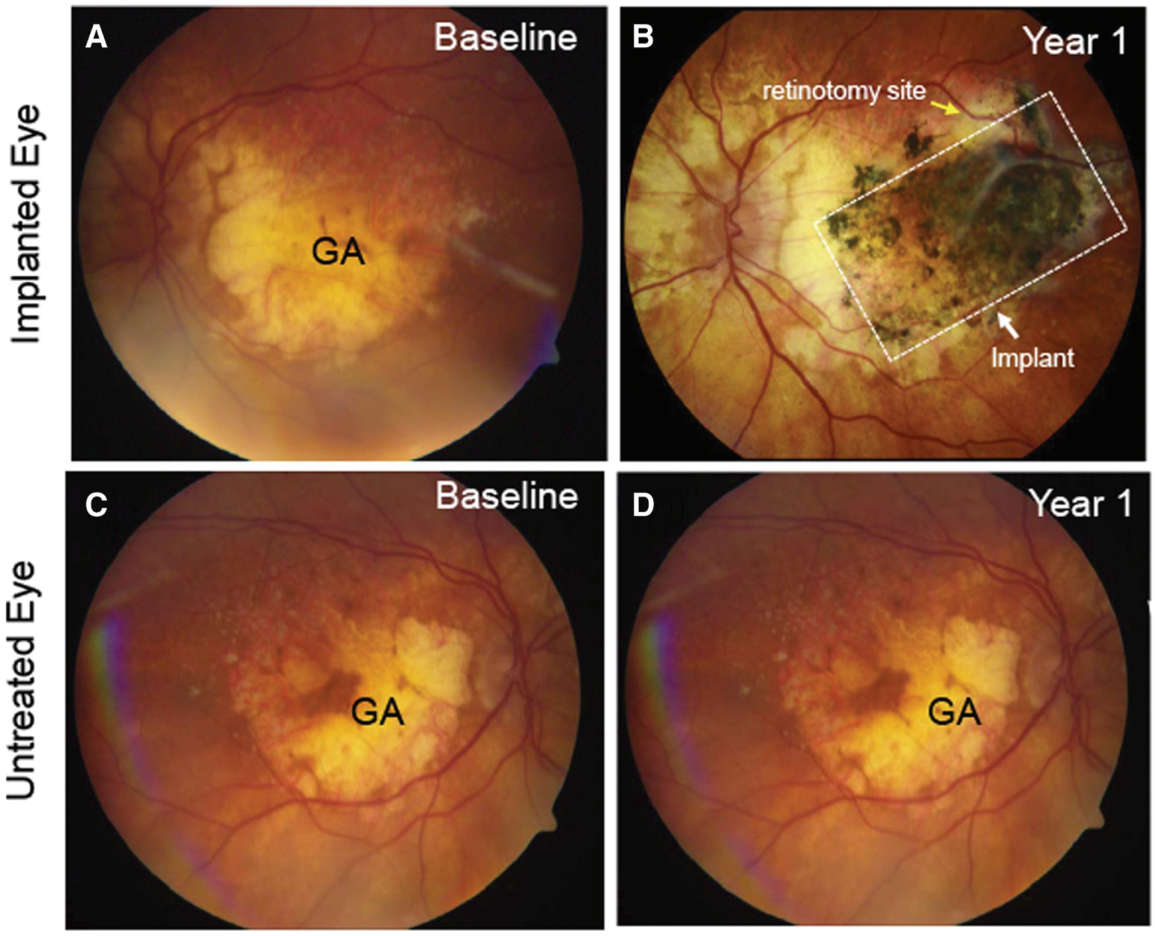

Figure 2. Color fundus photographs of subject 125 at baseline and 1 year after CPCB-RPE1 surgical implantation into the subretinal space (**A**) Preoperative photograph demonstrates variable areas of depigmentation in the central macula consistent with geographic atrophy (GA) in advanced dry age-related macular degeneration. (**B**) Postoperative fundus photographs of the same region at 1 year after CPCB-RPE1 implantation demonstrates the presence of the implant and its associated pigmented cells covering a large portion of the GA lesion. One edge of the pigmented implant is denoted by an arrow inferiorly for reference. The retinotomy site is denoted by an arrow superiorly. (**C**) Fundus photograph of the nonimplanted eye at baseline. (**D**) Fundus photograph of the nonimplanted eye 1 year later. Reproduced with permission from Kashani et al.[43]

specific HLA class I and II antigens to determine whether subjects in the trial developed an immune response to donor-specific HLA antigens on the implant. One of 13 tested subjects had pre-existing antibodies to a single donor HLA antigen, and 6 had pre-existing antibodies to non-donor HLA molecules at baseline. These pre-existing antibodies remained detectable during follow-up. Twelve of 13 subjects never developed detectable antibodies to any donor HLA antigen through year 1 after implantation. One subject developed a "weak" antibody response to an HLA antigen expressed by donor RPE cells (DQB1) at 6 and 12 months after surgery. Thus, there is evidence of immune surveillance of the transplant, but this surveillance was consistent with survival and apparent functionality of a substantial majority of the transplanted cells by year 2 after surgery. Although there was histologic evidence of immune surveillance, there was no clinically detectable inflammation such as vasculitis, retinitis, choroiditis, or vitritis, based on the routine clinical exams as well as special studies that can be conducted in living patients (eg, fluorescein angiography, OCT). An important finding is that there was no evidence of gliotic encapsulation of the implant although some limited subretinal fibrosis was associated with the implant (Fig. 2B). The presence of CD34-positive cells subjacent to the implant may indicate some degree of integration with the host rather than isolation.

These clinical findings in a single patient stand in contrast to results obtained from animal models that predict immune rejection of subretinal allogenic RPE transplants, including experiments involving nonhuman primates (NHPs). Some preclinical models, however, do indicate that differentiated monolayers of allogenic human fetal RPE and human ESC-RPE can avoid immune rejection after ocular transplantation.[46,47] If one can generalize based on findings from this single patient, it may not be necessary to develop autologous RPE transplants in the setting of AMD-GA. Of note, it is important to minimize surgical trauma, which can initiate an immune response.[48] While the generation of autologous tissue is costly and time-consuming, sustained pharmacological immunosuppression in elderly patients is generally not well tolerated as has been demonstrated in several ophthalmic studies.[27,49,50]

RPE Transplant Immunology: Preclinical Results

A number of approaches have been considered to address the immune response to subretinally transplanted tissue (Table 2).

Table 2. Approaches to manage the immune response to subretinal transplantation.

Management of immune response		
Allogenic cells	Pharmacological	Systemic Local
	HLA-matched stem cell	
	HLA-deficient stem cell	
Autologous cells	Induced pluripotent stem cells	
	RPE stem cells	
	Native RPE cells	

Please see text for details.

Preclinical studies of RPE transplant immunology have yielded results that, in some cases, may seem to be in conflict with those reported by Kashani et al.[43] McGill et al,[51] for example, reported that allogenic iPSC-RPE transplants in NHPs were rejected. However, these transplants involved cell suspensions rather than scaffold-based cells, and the transplant surgery was not simply transretinal. Retinal blebs were created via a transvitreal approach, but cell delivery was done through a transscleral-transchoroidal approach. In addition, the transplanted cells were labeled with green fluorescent protein, which may have toxic properties and could initiate an immune response.[52] Nonetheless, this study provided information that may be quite relevant to human experiments. First, although transplant rejection was documented histologically, these findings were not evident on clinical exams nor with modern imaging technology (Fig. 3). Second, the inflammatory response to the allogeneic transplant changed over time. Initially, the inflammatory response was mediated by microglia, macrophages, and T cells. By 3 weeks after surgery, however, the inflammatory response, involved microglia and B cells. The inflammatory response was restricted to locations of the transplant cells. Most importantly, by 7 weeks after transplantation, the inflammatory response had resolved based on histological study. Localized choroidal thickening with a dense infiltrate of mononuclear cells and microglia was evident at all time points, though. Finally, systemic peripheral lymphocyte activation was below detectable levels. Thus, human transplant tissue obtained 2 years after surgery may fail to real reveal the full extent of an inflammatory response, if any occurred, and the absence of systemic evidence for immune surveillance may not signify tolerance of the transplant. Survival of allogenic iPSC-RPE following subretinal transplants in pigs has yielded similar results.[53]

A subpopulation of adult human RPE cells can be activated in vitro to a self-renewing, multipotent RPE-stem cell that loses RPE markers, exhibits extensive proliferative capacity, and can be redifferentiated into stable RPE monolayers.[54] Liu et al[55] found that human RPE stem cell-derived RPE[54] on polyethylene terephthalate (PET) scaffolds seemed to survive well and exhibited some signs of integration with the host retina. These animals were treated with systemic immunosuppression using sirolimus. Host RPE was debrided after subretinal bleb formation to allow the transplanted RPE to come into direct contact with host choriocapillaris. Abrogation of the outer blood-retinal barrier (induced by RPE debridement) compromises the immune privilege of the subretinal space.[56] There was no evidence for epithelial-mesenchymal transition or frank gliosis associated with the transplants. Because this study involved xenografts, its relevance to allogeneic transplants is not clear. The authors noted reduction of outer nuclear layer (ONL) thickness overlying the RPE xenograft, which may be a sign of graft vs. host disease. These cells are being studied in a phase I/II trial involving patients with AMD-GA (Safety and Tolerability of RPESC-derived RPE Transplantation in Patients With Dry Age-related Macular Degeneration. https:// clincaltrials.gov/ct2/show/NCT04627428).

Transplant studies using immunodeficient RCS rats do not necessarily eliminate the possibility of transplant immune rejection. The immunodeficient RCS rat is T-cell-deficient, but these animals possess bone marrow-dependent B cells and NK cells. Rajendran Nair et al,[44] for example, performed iPSC-RPE transplants in immunodeficient RCS rats. The RPE were derived from healthy adult human fibroblasts, and the cells were transplanted as a monolayer on parylene sheets. Eleven months after transplantation, iPSC-RPE survival and phagocytic function were observed in less than 50% of the transplanted rats. The authors posited that the loss of transplanted RPE over time and the alteration in the monolayer structure may be a manifestation of an immune reaction to the xenograft. CD68 was used to identify reactive microglia in the transplanted eyes. Whereas CD68 expression was not observed at 1 month after transplantation, some CD68 expression was observed in the implants and in areas adjacent to them by 11 months after transplantation. Thus, consistent with the results reported above, there can be a time-dependent activation of different components of the immune response to transplanted tissue, and microglia and macrophages can play a role in the loss of transplanted RPE at later time points.

Genetic modification of cells has been undertaken to reduce the immunogenicity of the transplant. Petrus-Reurer et al[57] created human ESC-RPE lacking HLA class I and/or HLA class II antigens. In preclinical models, xenografts of these cells exhibited reduced and delayed donor rejection. Hu et al[58] generated hypoimmune pluripotent stem cells by depleting HLA class I and II molecules and overexpressing CD47. The expression of CD47 allows cells to avoid detection and destruction by NK cells. From such iPSCs, one can generate a variety of cell types (eg, RPE, photoreceptors) for transplantation. Hu et al have provided convincing evidence that these cells (and their progeny, eg, pancreatic islet cells) escape immune surveillance in NHPs and allogeneic humanized mice.[58,59] If there is no cell migration outside the eye, risks inherent to this approach (ie, uncontrolled cell proliferation) might be mitigated by routine fundus screening exams. Jang et al[60] used CRISPR/Cas9 to knockout HLA-B from iPSCs, and these cells showed reduced immunogenicity. If off-target effects are induced by these methods, then resistance to immune surveillance could pose a risk of tumor formation, but these risks are inherent when using genetically modified cell products. In any event, the exclusion of genomic instability (via G-band karyotyping) and persistence of reprogramming systems (via quantitative PCR) are essential aspects of product characterization for cell-based therapies.[61]

It may be that the best method to manage immune surveillance of allogeneic RPE transplants (eg, local vs. systemic treatment; duration of treatment before surgery and treatment after surgery; combination of medications used) will

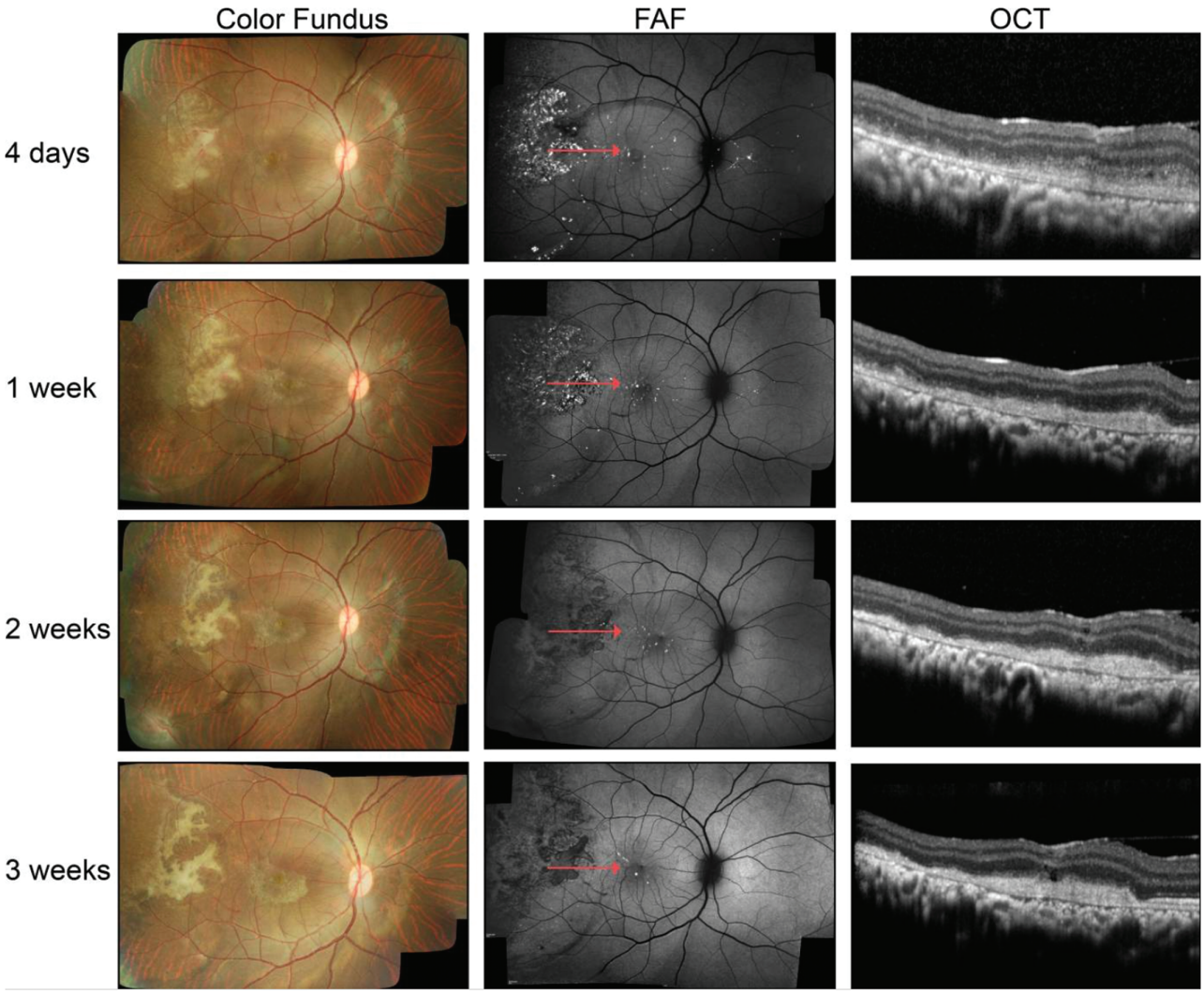

Figure 3. In vivo imaging of green fluorescent protein (GFP)-labeled RPE cells transplanted into the subretinal space of non-immune-suppressed rhesus monkeys using color fundus photography, fundus autofluorescence (FAF), and optical coherence tomography (OCT) at 4 days and 1, 2, and 3 weeks posttransplantation. In the representative color fundus images, white subretinal material was present at 4 days and evolved in shape and appearance over subsequent weeks. The GFP fluorescence of the transplanted cells was evident at 4 days but extinguished by 2 weeks. Subretinal debris, fibrotic scarring, and mononuclear cells, as confirmed by histology, resulted in OCT images that showed material in the subretinal space that without confirmation through histologic study could be misinterpreted as the transplanted RPE cells. Reproduced with permission from McGill et al.[51]

vary according to (1) the cell source (ESC vs iPSC); (2) the method of stem cell differentiation; (3) the stage of cell passage prior to preservation; (4) the method of cryopreservation; (5) the route of cell delivery (transvitreal vs transscleral); (6) the method of cell delivery (scaffold vs suspension); and (7) and the disease state (eg, AMD-GA vs neovascular AMD).

Scaffolds vs Cell Suspensions

Surgical trauma can activate the innate immune system.[48] A variety of surgical approaches have been explored for cell delivery (Table 3).

At this time, delivery of cell suspensions to the subretinal space requires less trauma than delivery of scaffold-based cell preparations. In addition, the preparation of cell suspensions is simpler than scaffold preparation. In view of these observations, one may question the need for scaffolds. In part the need arises from the fact that Bruch's membrane acquires a number of biochemical and morphological abnormalities with aging and in AMD.[62] Probably as a result, transplanted RPE do not adhere well to aged Bruch's membrane in organ culture studies.[63] Scaffolds might thus establish a surface that separates newly transplanted RPE from death signals in Bruch's membrane (eg, advanced glycation end products[64]) and provides ligands that foster RPE adhesion and differentiation (eg, laminin[27,65]). Scaffolds can be composed of a variety of materials that vary in structure, biodegradability, and modulus.[66-68] Scaffolds can be nanoengineered with pores and to micron-level thickness so as not to interfere with diffusion of substances between the RPE and subjacent choriocapillaris yet retain handling properties consistent with surgical delivery to the subretinal space.[69-71] Development of subretinal fibrosis, which has been observed following some cases of RPE-scaffold delivery,[27,65] for example, has not been reported after subretinal RPE cell suspension delivery. Nonetheless, scaffolds have several critical advantages over suspensions. A well-differentiated monolayer demonstrates better photoreceptor rescue and better survival in the subretinal space.[47] Scaffold-based RPE preparations also demonstrate better resistance to oxidative damage.[72] Since scaffolds can deliver

Table 3. Surgical approaches to cell delivery for retinal disease therapy.

Approach	Disadvantages	Advantages
Transvitreal subretinal injection	• Maximally invasive • Requires creation of retinal detachment	• Established surgical technique • Delivers cells directly to target photoreceptors • Transplanted cells can interact directly with host photoreceptors (eg, outer segment phagocytosis) and subjacent choroid (eg, choriocapillaris maintenance)
Suprachoroidal injection	• Invasive • Requires creation of retinal detachment	• May be less invasive than transvitreal approach • May be more demanding technically than transvitreal approach if target is subretinal space • If Bruch's membrane penetrated, then transplanted cells can interact directly with host photoreceptors (eg, outer segment phagocytosis) and subjacent choroid (eg, choriocapillaris maintenance)
Intravitreal injection	• Does not deliver cells directly to subretinal space which precludes direct interaction with host photoreceptors and choroid • May be best adopted for photoreceptor rescue approach based on provision of trophic factors	• Minimally invasive
Systemic injection	• Requires cells to migrate to eye and establish presence in a location (eg, vitreous cavity, choroid, subretinal space) compatible with host photoreceptor rescue and host choriocapillaris maintenance	• Least invasive approach

Table 4. Materials used for scaffolds in human RPE transplants.

Material	Biodegradable	Use in human trials
Parylene C[66,67]	No	Yes
Polyethylene terephthalate[25,56]	No	Yes
Poly(lactic-co-glycolic acid)[68,69]	Yes	Yes

well-differentiated RPE cells whereas cell suspensions require 1-2 weeks to re-establish full differentiation on a supportive surface, scaffold-based RPE can more rapidly produce factors that not only rescue overlying photoreceptors and subjacent choroidal endothelial cells, they can also more rapidly produce factors, such as transforming growth factor (TGF)-beta, that contribute to the immune suppressive environment of the subretinal space. Although a number of different materials have been used as scaffolds (reviewed by Jha and Bharti[73] and Hotaling et al[66]), just a few materials have been used thus far in clinical studies (Table 4).

If RPE suspensions could be induced to attach and differentiate rapidly as a polarized monolayer on host Bruch's membrane, then the manufacturing and surgical advantages of cell suspensions might dominate over the benefits of scaffold-based delivery approaches. In this context, it is noteworthy that Croze et al[74] reported that Rho associated kinase (ROCK) inhibition promotes attachment, proliferation, and wound closure of human ESC-RPE in cell culture. These authors suggested that pharmacological approaches such as this one might even be used to stimulate in situ RPE to resurface areas of AMD-GA. Ishida et al[75] studied the effects of the pan-ROCK inhibitor, Y-27632, on iPSC-RPE in vitro and in vivo. In vitro, these investigators found that ROCK inhibition promoted proliferation and cell-cell and cell-substrate adhesion in human iPSC-RPE. Y-27632 treatment also inhibited human iPSC-RPE apoptosis, enhanced cell pigmentation, and increased tight junction formation compared to untreated cells. Furthermore, Y-27632 treatment suppressed the production of inflammatory cytokines (eg, IL-6, CCL2/MCP-1, CXCL22/I-TAC) and HLA class II molecules (HLA-DR, -DQ, -OP) in iPSC-RPE. Y-27632-treated iPSC-RPE induced less proliferation of CD4 and CD8 cells suggesting that these RPE cells had lower immunogenicity than untreated iPSC-RPE. Treated and untreated iPSC-RPE were similar regarding outer segment phagocytosis and expression of VEGF, RPE65, bestrophin, Pax6, and tyrosinase. Y-27632-treated iPSC-RPE produced more pigment epithelial derived factor (PEDF) than untreated RPE. These investigators carried out allogeneic iPSC-RPE suspension transplants in immunosuppressed (oral cyclosporin A) NHPs and found that Y-27632 treatment was associated with establishment of a 5-fold greater graft area with better monolayer formation.

Transplantation of scaffolds and cell suspensions both require creation of a localized retinal detachment. We have found that localized iatrogenic retinal detachment is associated with damage to rod-bipolar and cone-bipolar synapses, which are associated with changes in the scotopic rod and photopic cone electroretinogram (ERG), respectively.[76] This damage can be mitigated via intravitreal or subretinal administration of ROCK inhibitors at the time of retinal detachment.[77-79] Thus, regardless of the cell delivery approach, combining cell transplantation with ROCK inhibition may improve the outcome of RPE transplantation.

RPE Cell Manufacturing

In order for cell-based therapy to proceed to clinical application, investigators must: (1) develop a prototype product that can be manufactured according to GMP; (2) conduct preclinical studies to demonstrate the safety and efficacy of the product; (3) submit an investigational new

Table 5. Human Clinical Trials Involving RPE Transplantation.

Title	Clinicaltrials.gov	Cell type	Disease target	Trial design	Results
A phase I/IIa, open-label, single-center, prospective study to determine the safety and tolerability of subretinal transplantation of human ESC-derived RPE (MA09-hRPE) cells in patients with advanced dry AMD	NCT01674829	hESC-RPE	AMD (GA)	Phase I/IIa	
Feasibility of production of iPSC-derived RPE fulfilling regulatory requirements for human transplantation in dry AMD	NCT02464956	iPSC (skin, blood cells)	AMD (GA)	Cohort prospective	
A phase I/II, open-label, multi-center, prospective study to determine the safety and tolerability of sub-retinal transplantation of human ESC-derived RPE (MA09-hRPE) cells in patients with Stargardt's macular dystrophy	NCT01345006	hESC-RPE (MA09-hRPE)	STGD	Phase I/II	Trend toward improved VA in treated eye (12 letters)
A phase I/II, open-label, multi-center, prospective study to determine the safety and tolerability of subretinal transplantation of human ESC-derived RPE (MA09-hRPE) cells in patients with advanced dry AMD	NCT01344993	hESC-RPE (MA09-hRPE)	AMD (GA)	Phase I/II	Median VA increase treated versus untreated eye (14 letters versus 1 letter, $P = .0117$)
Phase I/IIa dose escalation safety and efficacy study of human ESC-derived RPE cells transplanted subretinally in patients with advanced dry-form AMD (GA)	NCT02286089	hESC-RPE	AMD (GA)	Phase I/IIa	
Clinical study of subretinal transplantation of human ESC-derived RPE in treatment of macular degeneration diseases	NCT02749734	hESC-RPE	AMD, STGD	Phase I/II	
Open-label, single-center, prospective study to determine the safety and tolerability of subretinal transplantation of SCNT-hES-RPE cells in patients with advanced dry AMD	NCT03305029	hESC-RPE (SCNT-hES-RPE)	AMD (GA)	Phase I	
A phase I/IIa safety study of subretinal implantation of CPCB-RPE1 (human ESC-derived RPE cells seeded on a polymeric substrate) in subjects with advanced, dry AMD	NCT02590692	hESC-RPE on parylene membrane	AMD (GA)	Phase I/IIa	
Treatment of AMD by fetal RPE transplantation	NCT02868424	fRPE	AMD (GA)	Phase I	
Treatment of dry AMD disease with RPE derived from human ESCs	NCT03046407	hESC-RPE	AMD (GA)	Phase I/II	
A phase I/II, open-label, multi-center, prospective study to determine the safety and tolerability of subretinal transplantation of human ESC-derived RPE (hESC-RPE) cells in patients with Stargardt's macular dystrophy	NCT01469832	hESC-RPE	STGD	Phase I/II	VA, microperimetry—no evidence of benefit
A safety surveillance study of events of special interest occurring in subjects with macular degenerative disease treated with human ESC-derived RPE cell therapy	NCT03167203	hESC-RPE	STGD	Phase I/II	
A phase Ib, multicenter, dose escalation, evaluation of safety and tolerability of ASP7317 for GA secondary to AMD	NCT03178149	hESC-RPE (ASP7317)	AMD (GA)	Phase Ib	
Safety and efficacy of subretinal transplantation of human ESC-derived RPE in treatment of AMD diseases	NCT02755428	hESC-RPE	AMD (GA)	Phase I/II	
STREAM: a phase I/2, open-label, safety, tolerability and preliminary efficacy study of implantation into one eye of hESC-derived RPE in patients with RP due to monogenic mutation	NCT03963154	hESC-RPE (Patch ISTEM-01)	RP monogenic mutation	Phase I/II	
A phase I/IIa trial for autologous transplantation of iPSC-derived RPE for GA associated with AMD	NCT04339764	iPSC-RPE (somatic cells) on PLGA scaffold	AMD (GA)	Phase I/IIa	

Table 5. Continued

Title	Clinicaltrials.gov	Cell type	Disease target	Trial design	Results
Phase I, open-label, safety and feasibility study of implantation of Pf-05206388 (human ESC-derived RPE living tissue equivalent) in subjects with acute wet AMD and recent rapid vision decline	NCT01691261	hESC-RPE on polyester membrane	AMD (GA)	Phase I	
Safety and efficacy of autologous transplantation of iPSC-derived RPE in the treatment of macular degeneration	NCT05445063	iPSC-RPE	AMD (GA)	Phase I	
A phase1/2a, open-label study to evaluate the safety and tolerability of RPE stem cell-derived RPE (RPESC-RPE) transplantation as therapy for dry AMD	NCT04627428	RPESC-RPE (RPESC-RPE-4W)	AMD (GA)	Phase I/IIa	
Long term, open-label, safety follow-up study following transplantation of Pf-05206388 (human ESC-derived RPE) in subjects with acute wet AMD and recent rapid vision decline	NCT03102138	hESC-RPE	AMD (wet)	Cohort prospective	
Safety and efficacy of subretinal transplantation of clinical human ESC-derived RPE in treatment of RP	NCT03944239	hESC-RPE	RP	Phase I	
Follow-up to 5 years of a phase I/II, open-label, multi-center, prospective study to determine the safety and tolerability of subretinal transplantation of human ESC-derived RPE (hESC-RPE) cells in patients with Stargardt's macular dystrophy	NCT02941991	hESC-RPE	STGD	Phase I/II	
This study is a phase I/II, open-label, non-randomized, prospective study to determine the safety of human ESC-derived RPE (hESC RPE) subretinal injections versus hESC RPE seeded on a polymeric substrate implanted in the subretinal space	NCT02903576	hESC-RPE	AMD (GA)+ AMD (wet)+ STGD	Phase I/II	
Autologous induced stem-cell-derived retinal cells for macular degeneration*	UMIN000011929 (UMIN-CTR)	iPSC-RPE (skin fibroblast)	AMD	NS	
A study of transplantation of allogenic iPSC-derived RPE cell suspension in subjects with neovascular AMD*	JPRN-UMIN000026003	iPSC-RPE	AMD (wet)	NS	
A study of transplantation of autologous iPSC-derived RPE cell sheet in subjects with exudative AMD*	JPRN-UMIN000011929	iPSC-RPE	AMD (wet)	NS	
A clinical study that will evaluate the safety and efficacy of subretinal administration of a suspension of iPSC-derived RPE cells in patients with retinal pigment epithelium tear*	JRCT2073230077	iPSC-RPE	RPE Tear	Interventional	
Clinical research of allogeneic iPSC-RPE cell strip transplantation for RPE impaired disease*	JRCTa050210178	iPSC-RPE	RPE impaired disease	Interventional	
Clinical research of allogeneic iPSC-RPE cell suspension transplantation for RPE impaired disease*	JRCTa050200122	iPSC-RPE	RPE impaired disease	Interventional	

*Trial is being conducted outside the US.
Abbreviations: AMD, age-related macular degeneration; ESC, embryonic stem cell; GA, geographic atrophy; iPSC, induced pluripotent stem cell; NS, not stated; RP, retinitis pigmentosa; RPE, retinal pigment epithelium; STGD, Stargardt disease; VA, visual acuity.

drug (IND) application that includes the manufacturing process, preclinical data, and human study protocol; and (4) if approved by the relevant regulatory agency, initiate a phase I trial to assess the product's safety in the target patient population.[80] If the product is demonstrated to be safe in a phase I study, an IND application for follow-up trials assessing safety and efficacy is submitted. The data from these larger trials are then compiled and submitted for a biologic license application (BLA) in order to obtain commercial approval for the cell-based therapy product. At this time, none of the ESC-RPE or iPSC-RPE therapies have reached the stage of BLA submission although there are a number of FDA-approved clinical trials in phase I/II in the US using ESC- and iPSC-derived products (Table 5). There are 6 additional trials using iPCS-RPE-derived products outside the US (Table 5).

Challenges involved in the development of stem cell-based products have been well described[80] and will not be repeated

here. It is interesting to note, however, that although iPSCs can be generated by reprogramming somatic cells of any variety, the majority of ongoing clinical trials have used skin fibroblast or peripheral blood CD34 cells. CD34 cells evidently have a higher reprogramming efficiency as compared to terminally differentiated blood cells possibly because the cells are already in a stem cell state with chromatin more easily reprogrammed into a fully pluripotent state.[81] Initial iPSC manufacture involved exposure to 4 transcription factors (Oct 3/4, Sox2, Klf4, and c-Myc) all delivered using retrovirus, which integrates reprogramming factors into the transduced cell genome.[82,83] Fortunately, it is now possible to generate iPSCs without integrating strategies (eg, episomal plasmids, micro RNA, chemical reprogramming).[84-92] Because teratoma formation can occur with as few as 2 human ESC colonies spiked into feeder fibroblasts, very careful purification is required in the manufacturing of stem cell-derived clinical products.[93] Native RPE stem cells also are a potential source of therapeutic tissue.[94] Methods to obtain RPE rapidly from human ESCs and iPSCS have been reported.[32,95] Nonetheless, the production of ESC and iPSC products is time and cost intensive (compare for example Sugita et al[24] and Mandai et al[20]). Croze et al[96] have identified 2 benefits of ROCK inhibitors in this regard: (1) ROCK inhibitors allow extended passage of human ESC-RPE and iPSC-RPE (increasing from 5 passages before epithelial to mesenchymal transition in untreated cell lines to 13-14 passages in treated cell lines); (2) ROCK inhibitors increase the rate of RPE proliferation (allowing an average of 30 doublings in treated cell lines vs 9 doublings in control cultures). The effects on proliferation cease once exposure to the ROCK inhibitor is eliminated. Thus, ROCK inhibitors allow one to use less starting material and passage cells for a longer period. These investigators concluded that ROCK inhibition with Y-27632 should allow human ESC-RPE and iPSC-RPE to be seeded at one-quarter the normal density and grown for up to 13 passages, which could lead to faster production of cells for treatment at lower cost. Once stem cell-derived RPE has been manufactured, a remaining technical issue involves storage of the product prior to transplantation. Determining the optimal stage for cryopreservation of stem cell-derived RPE is complex and may vary with the stem cell line used, the cryopreservative used, and the cooling rate.[97-99]

Lee et al[100] have designed a novel poly(glycerol sebacate) scaffold that permits simultaneous delivery of RPE and overlying photoreceptors. The scaffold's wells have a honeycomb-shaped hexagonal perimeter and are 40 μm deep to enable the retention of both RPE and photoreceptors. The base is perforated with 4 μm diameter pores to enable fluid and chemical exchange with the subjacent choroid. The scaffold wells are laminin-coated. Scaffolds are first seeded with human iPSC-RPE to create an RPE monolayer at the base of each well. Dissociated retinal organoid cells are then seeded onto the RPE monolayer. If integration with the host retina can be achieved, this device could be used to deliver sight-restoring, not just sight-preserving, therapy as areas of AMD-GA are largely devoid of functional photoreceptors. As is the case for any scaffold, in vivo as well as in vitro studies will be necessary to validate the safety and tolerability of this device.

In addition to their role in cell delivery, scaffolds may also play a central role in enabling efficient large-scale production of stem cell-derived RPE for therapy. Faynus et al[101] reported the use of Cytodex microcarriers as scaffolds on which iPSC-RPE can be grown for harvest. The Cytodex (C) microcarriers are biologically inert, cross-linked dextran matrices that can be coated with Matrigel, vitronectin, or denatured type 1 porcine collagen to promote cell attachment. These investigators showed that human ESCs can be directly differentiated into progenitor RPE on C1 Matrigel carriers. (C1 microcarriers exhibit a positive charge, and C3 microcarriers are functionalized with denatured porcine type 1 collagen.) Human iPSC-RPE cultured on C1-Matrigel, C1-vitronectin, or C3 microcarriers express mature phenotype (eg, pigmentation, hexagonal morphology, polarization, RPE marker genes), secretory profile, and phagocytic function. The microcarrier-RPE can be separated from its substrate readily using a xeno-free dissociation reagent (TrypLE enzyme). Microcarrier-RPE secrete more VEGF and PEDF than RPE grown on cell-culture plates, which may render this culture system an efficient source of neurotrophic factors. Faynus et al noted several advantages of microcarrier production systems over conventional culture systems: (1) greater available surface area; (2) greater scalability; (3) reduced production cost/cell. So, manufacturing space and cost of goods can be optimized using microcarrier scaffolds for RPE production. Microcarriers are particularly suited to automated manufacturing comprising closed, modular, and scalable components. Artificial intelligence screening using quantitative bright-field absorbance microscopy and deep neural networks enables prediction of iPSC-RPE transepithelial resistance and polarized VEGF secretion (both useful barometers of RPE differentiation).[102,103] Combining closed automated, modular microcarrier production systems with artificial intelligence screening of the final therapeutic product may enhance efficiency, increase scale, and reduce the cost of iPSC-RPE production.

Future Directions

Better methods to monitor host immune surveillance of the graft might improve allogeneic transplant survival. Clinical evaluation with fundus examination, fluorescein angiography, and currently available OCT technology are important but are not likely to detect the initial stages of rejection-a time when intervention with more aggressive immunosuppression might be indicated. Laboratory studies monitoring activation of host peripheral blood mononuclear cells probably should be done at regular intervals since the nature of the immune response to the graft seems to vary over time. It might be helpful to develop imaging technology that permits the identification of immune cells (eg, by labeling host immune cells with markers that can be visualized with OCT or with fundus autofluorescence), which could facilitate early detection of an immune reaction to the graft. Most elderly patients do not tolerate sustained immunosuppressive agents well, and these approaches might minimize exposure to these agents. In addition, it might be useful to systematically compare different methods of donor tissue preparation, as described in the Cell Manufacturing section, to identify the method that is least immunogenic while maximizing the likelihood of donor-host integration, if such a manufacturing process exists. In the case of scaffold-based cell delivery, it may be possible to incorporate anti-inflammatory molecules into the scaffold (ie, scaffold as a sustained delivery device) to minimize the host response to the graft.

One might wish to provide adjunctive therapy to facilitate success. Could co-administration of ROCK inhibitors improve transplanted RPE suspension survival and, perhaps more importantly, reduce host retina damage associated with the iatrogenic detachment required to deliver RPE and photoreceptors to the subretinal space?

Additional developments in scaffold technology may further increase the chance for RPE transplant success. As mentioned above, the scaffold might serve 2 purposes: (1) a platform to deliver an organized layer of cells that will cover a precisely defined surface area; (2) a drug delivery system to promote cell survival, integration, and immunosuppression.

Medical and gene therapy for AMD-GA are under development and, once adequate efficacy is demonstrated, are likely to serve as the primary therapies for AMD-GA. However, effective medical and gene therapy probably will not render cell-based surgical treatment for AMD-GA irrelevant. Chronic diseases tend to require a spectrum of interventions with medical therapy being most important in early stages and surgical therapy being useful in selected cases with relatively late-stage disease. While medical therapy remains the primary and highly effective treatment for patients with diabetes mellitus, for example, there continue to be patients who, for various reasons, progress to advanced stages of diabetic retinopathy and who can only be treated effectively with surgical intervention. A similar situation may occur for AMD-GA. In addition, there is some evidence that the efficacy of photoreceptor transplantation may be augmented by co-transplantation with RPE as discussed in the accompanying review. Thus, efforts to develop RPE transplants for retinal blindness are likely to have value in the future and should continue.

Conflict of Interest

Marco A. Zarbin: Consultant for Apellis, Boehringer Ingelheim, EdiGene, Genentech/Roche, Illuminare, Life Biosciences, Novartis Pharma AG, Perfuse Therapeutics, Seeing Medicines, Tamarix Pharmaceuticals, Tenpoint Therapeutics; Equity: NVasc. The other authors declared no potential conflicts of interest.

Author Contributions

V.K.: conception and design, manuscript writing, data analysis, final approval of manuscript. O.G.G.M.: conception and design, manuscript writing, data analysis, final approval of manuscript. M.A.Z.: conception and design, manuscript writing, data analysis, final approval of manuscript.

Data Availability

No new data were generated or analyzed in support of this research.

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