Age-related macular degeneration (ARMD) is the leading cause of blindness in subjects older than 50 years of age in the developed world. There are two types of ARMD, neovascular (NV) and nonneovascular (NN). While anti-VEGF–based therapies have significantly decreased the visual morbidity associated with NV-ARMD, there are no effective treatments for NN-ARMD. A detailed discussion of NV-ARMD and related therapies is the topic of another section of this special supplement. This review will focus mainly on NN-ARMD. Vision loss in non-neovascular ARMD is highly correlated with the loss of RPE cells and areas of geographic atrophy (GA). Pilot studies using subretinal transplantation of autologous or allogeneic RPE during the past 20 to 30 years have demonstrated that stem cell–derived RPE have the potential to rescue photoreceptor function and restore vision. New methods of differentiating RPE from human embryonic stem cells (hESC) and induced pluripotent stem cells (iPSC) have created a potentially unlimited supply of RPE cells to meet the demands of future commercially viable stem cell products. Thanks to fundamental advances in stem cell biology, vitreoretinal surgery, and noninvasive retinal imaging, stem cell–based therapies for NN-ARMD are emerging and several clinical trials are in progress. However, there are major regulatory, safety, and technical challenges that remain. This review will focus on summarizing the most promising aspects of stem cell–based therapy for NN-ARMD and highlighting areas that require further research.

Keywords: nonneovascular, macular, degeneration
central vision despite relatively large regions of GA due to sparing of the central fovea and preferential peripheral enlargement of GA lesions. Therefore, diagnostic methods for estimating the rate of progression and identifying the regions of greatest risk for progression are critical to maximize the risk-benefit ratio of any therapy. Geographic atrophy is characterized clinically by sharply demarcated hypopigmented areas in the macula. Due to the decreased pigmentation and loss of choriocapillaris, areas of GA are usually apparent by prominence of the underlying choroidal vessels. Drusen are found in the majority of sites that go on to develop GA, but they generally fade in areas where GA develops. The presence of large, soft confluent drusen is a significant risk factor for developing choroidal neovascularization (NV-ARMD) as well as GA (NN-ARMD). Histopathology of GA confirms RPE cell death, atrophy of the outer neurosensory retina and choriocapillaris. \(^{11}\) Longitudinal studies show variable progression rates of GA with a mean growth rate of approximately 1.2 to 2.8 mm\(^2\) per year and as high as 6 mm\(^2\). \(^{6,12,13}\) The Beaver Dam Eye Study showed that eyes with multifocal disease had larger increase in area of GA and progressed to foveal involvement more frequently than eyes with single foci of disease over 5 years (12 vs. 2.24 mm\(^2\)). \(^{6}\) Spectral-domain optical coherence tomography (SD-OCT) and fundus autofluorescence (FAF) are relatively new clinical tools that are commonly used in the clinical assessment of ARMD. Both diagnostic tests demonstrate that the clinical findings described above are a result of RPE loss and atrophy of overlying neurosensory retina. \(^{11,14}\) Spectral-domain OCT of GA typically shows enhancement of the underlying choroid and loss of the RPE hypereffectivity with variable involvement of the overlying neurosensory retina. \(^{14}\) Fundus autofluorescence demonstrates a reduction in the autofluorescence signal that originates largely from RPE and is absent in the region of the GA. \(^{11,14}\) Spectral-domain OCT and FAF are now commonly used in quantitation of GA in clinical trials. Functional tests such as microperimetry\(^{15}\) and multifocal electoretinography are less commonly performed but increasing in importance for our understanding of retinal function as discussed later in this review.

**Pathophysiology of NN-ARMD and Potential Targets for Therapeutic Intervention**

As mentioned above, ARMD is characterized by degenerative changes that are thought to primarily occur in the RPE. These changes are accompanied by degenerative changes of Bruch’s membrane, the choriocapillaris as well as the overlying photoreceptor layer. The exact etiology of ARMD is unknown largely due to lack of good animal models but it is thought that a combination of genetic predisposition and environmental factors predispose to the degenerative changes. \(^{7}\) Genetic, \(^{16}\) clinical, \(^{17,18}\) and pathologic studies\(^{19,20}\) have suggested several factors contribute to ARMD including oxidative damage, \(^{21}\) accumulation of lipofuscin, \(^{18}\) impaired chorioidal perfusion, \(^{21}\) and chronic inflammation. \(^{7}\)

A number of findings support the role of oxidative stress in the retina and RPE. These include high light exposure, generation of reactive oxygen intermediates during phagocytosis of photoreceptors, accumulation of lipofuscin, and high levels of polyunsaturated fatty acids in the outer retina. \(^{21}\) In addition, elevated levels of antioxidant enzymes in the RPE of subjects with AMD, advanced glycation end-products in drusen and basal laminar/linear deposits, lipoperoxidation, and DNA strand breaks in eyes with GA all suggest that oxidative stress contributes to AMD. \(^{22}\) Lastly, epidemiologic studies that show smoking is a significant risk factor for AMD and that a diet rich in antioxidants decreases the risk of AMD. \(^{23}\) It is therefore likely that any stem cell-based treatment will have to survive the same oxidative stress of the native RPE and specific considerations for this are discussed later in this article.

Individual pharmacologic therapies aimed at reducing oxidative stress have been limited in efficacy suggesting additional pathological mechanisms are at play. Nevertheless, it is enticing to speculate that these therapies may be useful adjuncts that may enhance the survival or efficacy of stem cell-based therapies by reducing the oxidative burden in the subretinal environment. An NIH-funded, multicenter, randomized study, AREDS2, assessed the efficacy of lutein, zeaxanthin, and/or long chain omega-3 fatty acids, docosahexanoic acid and eicosapentaenoic acid, in addition to the original AREDS formulation and did not significantly reduce the progression to advanced AMD. \(^{10}\) Another phase 2 study investigating the safety and preliminary efficacy of a disubstituted hydroxylamine with antioxidant properties for treatment of GA did not show significant benefit. \(^{24}\) Additional studies are underway assessing the safety and efficacy of omega-3 fatty acids. The Alzheimer’s amyloid beta protein has also been colocalized with activated fragments of complement C5 in drusen and is a potential activator of complement system in humans. \(^{25}\) The presence of these findings as well as the activation of alternative complement pathways supported by the CFH polymorphism supports a role for inflammation in the pathophysiology of AMD. Lastly, recent evidence suggests that abnormal processing of long double-stranded Alu RNA sequences by Dicer1 may lead to activation of the inflamma-some but the exact role of this process in the pathophysiology of ARMD is still under investigation. \(^{22}\)

Unfortunately, isolated therapies aimed at reduction of inflammation in NN-ARMD have not been fruitful, but these therapies may be helpful adjuncts to stem cell-based therapies by minimizing the inflammatory state of the host environment. The use of a low-dose, sustained release formulation of fluorocinolone acetonide (Iluven; Alimera Sciences, Alpharetta, GA, USA) that is delivered as a nonbioeradical intravitreal implant has not been successful in phase II study of patients with bilateral GA (study terminated as reported on ClinicalTrials.gov). Immumomodulators such as glatiramer acetate (Copaxone; Teva Pharmaceuticals, Irvine, CA, USA) and sirolimus (Rapamycin; Pfizer, New York, NY, USA) have similarly not been successful at preventing progression of GA in early phase 1 and 2 studies. \(^{22,26}\) While a number of studies using complement inhibitors such as Eculizumab (Soliris; Alexion Pharmaceuticals, Cheshire, CT, USA) have not been efficacious, a phase 2 study showed that intravitreal administration of a protein factor D blocker, Lampalizumab, was able to reduce progression of GA although final results have not been published (NCT02288559). However, it is encouraging that a phase 3 study investigating the efficacy and safety of intravitreal injections of Lampalizumab in patients with GA is underway (NCT02247479). In addition, there are a number of early phase 1 studies using complement factor blockers that are underway. These studies provide hope that treatment of the inflammatory component of ARMD may decrease the rate of progression, but they are not designed to reverse or completely prevent vision loss. Therefore, there is a very significant need for treatments that can restore vision loss in the large population of elderly people currently afflicted with NN-ARMD.
**Rationale for Stem Cell Therapy for Nonvascular AMD**

The RPE is a monolayer of cells located between the choriocapillaris and the neurosensory retina. It has a critical role in the survival and function of the overlying photoreceptors and the underlying choriocapillaris. The function and characteristics of the RPE have been reviewed extensively elsewhere.\(^27\) Among these critical functions, the RPE secretes pigments, epithelial derived factor (PEDF), VEGF as well as the extracellular matrix, which may have an antiangiogenic function. Abnormalities in RPE occur in a number of conditions including mutations in RPE65.\(^{28}\) merTK,\(^{29}\) Bestrophin,\(^{30}\) and lecithin retinol acyltransferase31 but the most prevalent disease resulting from RPE dysfunction is ARMD. Therefore, there is ample motivation to pursue a therapy that can preserve or repopulate this important cell layer with the goal of restoring vision loss.

Any attempt at replacement of the RPE cell layer should address several fundamental concerns. First, will the donor RPE survive in the host for a significant amount of time to justify the risks of implantation and cell-based therapy (e.g., immunosuppression)? Second, will the donor RPE maintain their polarity and function as normal RPE would do? Third, can the donor RPE reverse or prevent further degeneration associated with the disease process? Fourth, are there sources of such donor RPE that are plentiful enough and ethically available for widespread commercial use? Fifth, what is the best technique to deliver the RPE into the subretinal space?

The earliest attempts at RPE transplantation occurred over 20 years ago in animals and provided proof-of-principle that it could work.\(^{32-34}\) These studies were complemented by the efficacy of macular translocation surgeries for GA.\(^{35,36}\) Macular translocation surgery demonstrated that translocating the neurosensory retina such that the fovea was placed over an apparently normal region of RPE allowed short-term visual gains. However, long-term follow-up demonstrated high recurrence rates of GA lesion in the new subfoveal RPE region.\(^37\) Because of these initial studies, various sources of cells have been used as donor RPE in many human and animal studies. Most of these studies have focused on NV-ARMD although several studies have been performed on small cohorts with GA associated with NN-ARMD. These include homologous,\(^{38}\) heterologous,\(^{39}\) or allogeneic\(^{40-44}\) adult RPE transplantation as well as fetal RPE transplantation.\(^{42-45}\) Retinal pigment epithelial that have been genetically modified\(^47\) or spontaneously transformed\(^48\) have also been used as donor cells. Lastly, RPE substitutes including iris-derived pigment epithelium,\(^{49}\) schwann cells,\(^{50}\) bone marrow-derived stem cells,\(^{51}\) umbilical-derived cells,\(^{52}\) and embryonic stem cells\(^{53,54}\) have all been suggested and used as donor cells. In general, the attempts at human RPE transplantation in GA using autologous\(^{55-57}\) and allogeneic\(^{45,58,59}\) RPE transplants have had similar success as those with NV-ARMD. The one notable difference has been a lower incidence of cases with immunologic rejection in subjects with NN-ARMD. This has been associated with less vascular compromise in this disease phenotype compared with NV-ARMD.\(^{45}\) It is encouraging that a phase 2 study is currently underway using a subretinal injection of suspensions of human embryonic stem cell derived RPE (hESC-RPE) in subjects with GA and NN-ARMD.\(^{60,61}\) These studies collectively support the safety and potential efficacy of subretinal RPE transplantation for GA associated with NN-ARMD. In addition, the quality-of-life benefit and improvement in reading ability after macular translocation have been demonstrated although largely in the setting of NV-ARMD.\(^62\) While it has to be determined whether similar quality-of-life measures will be observed in subjects with NN-ARMD there are no compelling reasons that similar benefits should not translate to successful RPE transplantation in subjects without long-standing vision loss from GA and NN-ARMD.

Because the initial report of human homologous and autologous RPE transplantation in 1991, almost 30 additional RPE grafts have been performed and reported in the literature and there are likely more currently in progress.\(^63\) It should be noted that some studies are also using non-RPE stem-cell populations, such as bone marrow–derived stem cells, to design treatments for NN-ARMD using either intravitreal or intravenous administration.\(^{64,65}\) In general, these methods take advantage of the nonspecific trophic effects of stem cells to support retinal and RPE function in degenerative diseases but there is some suggestion that cell repopulation may occur. A detailed discussion of this methodology is the subject of another section of this special supplement. Although there is evidence of RPE repopulation by systemic administration of some bone marrow–derived cells lines in animal models,\(^66\) there are significant safety and efficacy hurdles to be overcome for the systemic administration of stem cells when local delivery methods are also viable. Therefore, among the numerous sources of donor RPE, hESC and induced pluripotent stem cells (iPSCs) have presented the most compelling options for several reasons. First and foremost, they are a source of almost endless RPE donor cells that can undergo strict quality control testing and forgo the often complicated process of harvesting autologous or allogeneic grafts. Second they can be fully differentiated into RPE either as cell suspension or monolayers. Third, they present the opportunity for genetic manipulation via ex vivo gene transfer that may be useful in suppressing immunogenic properties of the cells or introducing novel functionality to supplement in vivo function of the cells. None of the other categories of donor cells meet all of these criteria.

Retinal pigment epithelial cells are exquisitely sensitive to local extracellular substrates for anchoring and survival.\(^67\) A healthy and intact Bruch’s membrane, which is the natural RPE basement membrane has been shown to improve the survival, repopulation, and confluence of RPE cells.\(^67\) Retinal pigment epithelial cell phenotype is also critical for normal RPE function.\(^68\) Both the composition and permeability of Bruch’s membrane change with increasing age and lipid accumulation.\(^69,70\) While donor RPE in suspension have been shown to attach to exogenous Bruch’s, it is more common for RPE cells in suspension to aggregate in multiple layers and assume an abnormal phenotype.\(^69\) In addition, dissociated hESC-RPE can dedifferentiate and may not redifferentiate appropriately. While it may be possible to rehabilitate the endogenous host Bruch’s membrane, this has not been demonstrated to date. Therefore, it is very likely that transplanted RPE cells will require some form of substrate to support implantation. Such a substrate must support RPE attachment and differentiation. It must also be amenable to surgical manipulation and implantation. Lastly, it must be compatible with the host immune system and be immunologically silent.

Multiple groups are developing scaffolds, for RPE transplantation. Two general types of substrates are possible for scaffolds to support the RPE before, during and after implantation. First, a biodegradable scaffold has been designed to provide temporary support for the implanted RPE without providing a long-term target for immunogenic responses.\(^71\) Limitations of a biodegradable scaffold include the possibility of toxic by-products resulting from degradation. Also it may be difficult to design such a scaffold to be rigid and durable enough for surgical placement although at least one group has demonstrated some success with such a method.\(^72\) A second category of substrates are biologically inert and nondegradable. Examples include polyester membranes,\(^72\) plasma polymers,\(^73\)
polyimide,74 and parylene.54,75 Our group has observed that subretinal transplantation of monolayers of hESC-RPE on a parylene substrate have improved survival in comparison to cell suspensions.54,76 In vitro studies suggest that a monolayer of hESC-RPE cells on a parylene substrate are phenotypically and functionally more similar to endogenous RPE and are more resistant to oxidative stress-induced apoptosis.77 These findings suggest that monolayers of hESC-RPE may have improved survival after implantation in the highly oxidative environment of the subretinal space. Overall, it is very encouraging that studies using both cell suspensions and monolayers with substrates have been demonstrated to be safe in initial clinical studies. A recent Phase 1 and 2 study of subretinal injection of hESC-RPE cell suspensions was demonstrated to be safe and with some preliminary efficacy.60 Phase 2 and 3 studies are currently underway.

**SOURCES OF STEM CELLS**

There are two viable sources of stem cells for deriving RPE, iPSCs and hESCs, which are reviewed in detail elsewhere.78 Induced pluripotent stem cells are derived from fully differentiated adult somatic cells that are reprogrammed in vitro to differentiate into RPE.78,81 These cells have been demonstrated to perform phagocytic functions, demonstrate RPE like gene expression profiles and promote photoreceptor survival.71,80 There remains unanswered questions regarding the host immune response to iPSC-derived cells as well as their epigenetic profile.82 Clinical trials are underway to test the safety of iPSC cells in humans.71 Human embryonic stem cells are derived from the inner cell mass of blastocysts and also have been programmed to differentiate into RPE.78,83 Clinical trials using hESC-derived RPE have demonstrated safety60 and additional trials are underway to demonstrate efficacy in NN-ARMD. Both of these sources of RPE can provide potentially limitless quantities of RPE to support clinical trials and commercial development of RPE implantation technology.

**CRITERIA FOR STEM CELL IMPLANTATION AND SUBJECT SELECTION**

Subject selection is critical for the operational success of RPE transplantation as well as functional success associated with visual improvement. In general, results from macular translocation and cataract studies, allogeneic and autologous RPE grafts suggest that donor RPE with similar histocompatibility profiles and host retina with preserved photoreceptor anatomy and function are critical for success. Current diagnostic imaging methods allow very detailed assessment of both macular structure and function. Specifically, SD-OCT allows very detailed assessment of photoreceptor structure in NN-ARMD and may allow prediction of GA progression.14 Spectral-domain OCT has demonstrated that not all regions of GA are equal and the anatomic state of the overlying neurosensory retina can vary significantly from almost no change to severe atrophy of the outer retinal structures.15 Autologous RPE transplantation studies suggest that subjects with recent loss of visual function may benefit most from RPE transplantation.55 These findings suggest that the visual potential of neurosensory retina over areas of long-standing RPE atrophy is poor. Subjects with such severe anatomical changes may not show significant improvement in visual function under any circumstances. However, it is possible that RPE transplantation in this population may preserve the remaining RPE and neurosensory retina at the borders of GA lesions or at least slow the progression of disease through a trophic effect. Nevertheless, SD-OCT data from autologous RPE transplantation studies has demonstrated preservation of outer retinal structures overlying the graft up to 3 years post surgery.57 This demonstrates that RPE can rescue overlying neurosensory retina. In order to maximize the benefit of RPE transplantation, future studies will have to more closely identify the anatomic correlates of good visual potential using SD-OCT.

An enticing possibility for stem-cell therapy as a treatment of degenerative disease is the replacement of neurosensory retina, specifically photoreceptor cells, either alone or in addition to RPE transplantation. Because most cases of severe NN-ARMD ultimately involve loss of photoreceptors, this method seems the most rationale for severe disease but requires more research to address additional challenges. Retinal transplantation has the added complexity of requiring neural integration of transplanted tissue with the host in addition to the other challenges associated with cell-based therapy discussed elsewhere in this review. It is promising that multiple animal studies have demonstrated functional and anatomic integration of donor neural retinal tissue into animal hosts.84 For example, transplantation of human fetal retina into nude adult rat retina resulted in histologically detectable synaptic connections.85 Adult retinal transplantation in human subjects has been demonstrated to be safe in subjects with end-stage retinitis pigmentosa and ARMD but gains in visual function have not been demonstrated.86–89

Measures of photoreceptor function, such as microperimetry (MP) and multifocal electroretinography (mERG) are also providing an increasingly useful assessment of visual function in areas of GA.15,90 Microperimetry provides an image-guided visual field that coregisters visual field deficits onto a fundus image. Therefore, microperimetry allows detailed correlation of visual function with anatomic location of the retina.91,92 In addition, microperimetry can demonstrate foveal fixation or the location of preferred retinal loci in cases of extrafoveal fixation.93 In early phase clinical trials that target subjects with long-standing GA and severe outer retinal degeneration, fine changes in microperimetry thresholds are unlikely to be detected. Nevertheless, in these severe cases, gross changes in fixation preferences would be very meaningful.57 In less severe stages of NN-ARMD, retinal threshold mapping using MP will be critical for assessment of visual potential preoperatively and early subclinical responses postoperatively. For example, multifocal studies of subjects with GA have demonstrated that deterioration of the outer retinal structures such as the inner segment/outer segment (IS/OS) band do not always correlate with loss-of-function. In at least some cases, subjects maintain useful vision or fixation patterns even in atrophic areas.90 Macular microperimetry and fixation analysis have some predictive value in macular translocation surgery.90–94 but studies in RPE transplantation have not been conducted yet.

Multifocal ERG allows objective measurement of outer retinal function but has been infrequently used in advanced ARMD because of technical limitations in testing patients with poor vision. Multifocal ERG studies in subjects with early or mild ARMD have demonstrated preferential loss of both rod and cone function at that time point.95,96 By extrapolation, these studies suggest that mERG response density in the border zone of GA lesions may provide an early assessment of photoreceptor health and may also serve as a prognostic marker of potential for visual recovery. A few studies have been able to demonstrate diffusely depressed mERG changes in subjects with advanced ARMD.90,97 One study reporting mERG responses in subjects with NV-ARMD demonstrated transiently improved responses at 3 months post-RPE transplantation.98 These findings are preliminary but careful mERG studies in current and future clinical trials of NN-ARMD may allow us to detect improvement in photoreceptor health.
before any visual recovery is noted subjectively. Most current studies using stem cell–based therapy of NN-ARMD are performing mERG but results have not been reported yet.

Fundus autofluorescence provides a gross measure of RPE function and has been used to demonstrate presumptive degenerating RPE near GA lesions as well as the viability of autologous RPE grafts over time. The exact nature of FAF signal is unclear but studies suggest that it is primarily related to the bis-retinoid, A2E, that is associated with RPE lipofuscin, nondegradable material within intracellular lysosomes and melanolipofuscin. Decreased FAF correlates with reduced nondegradable material within intracellular lysosomes and melanolipofuscin. Measurement of GA size has been well documented with multiple imaging modalities including SD-OCT, FAF, and FA and there is no clear consensus about the best method. It is critical for assessments of growth rates to be standardized using well-calibrated and consistent testing methodologies. Such a consensus on diagnostic testing has not yet been reached, and it is necessary to better understand the anatomic and functional correlates of GA size, MP, and mERG because each seems to measure a different but very important aspect of retinal function and health. A comprehensive battery of diagnostic tests will be necessary to evaluate and understand the preoperative status of the retina and RPE, the postoperative response of the retina and RPE to the implantation and the patient’s subjective perception of visual improvement.

Considerations for Surgical Implantation

Harvesting of autologous RPE from the peripheral retina and reimplantation has demonstrated proof-of-principle that RPE replacement can improve vision in GA. However, in general, this strategy has had limited success due to higher complication rates inherent in the surgical harvesting process. Studies that have used cadaveric allogeneic RPE are not subject to the same complications of harvesting procedures but have shown variable rates of immune-mediated rejection, suggesting the need for local or systemic immunosuppression. The use of cadaveric allogeneic RPE is also complicated by the lack of appropriate quality control mechanisms and poor characterization of the donor cells genetic and physiologic features before implantation.

We have already discussed the surgical demands of various stem-cell replacement strategies tangentially but a direct discussion of this topic is worthwhile. Nonstem cell–derived RPE allografts and autografts have been delivered as suspensions or sheets in subjects with both NN-ARMD and NV-ARMD, as described above. In general, use of nonstem cell–derived allografts or autografts imposes similar surgical limitations with the main difference being the lack of an immune response from autografts. First, nonstem cell–derived allografts and autografts require harvesting from available cadaveric donor tissue or host tissue, which places severe limitations on availability of tissue. Second, the harvesting process is inherently damaging and suboptimal due to unpredictability of harvesting time and unknown condition of the RPE. Nevertheless a significant number of studies have demonstrated the use of RPE allografts and autografts in the past with variable success as described above. These studies were critical for demonstrating proof-of-principle that RPE transplantation can work but the significant risks of the surgeries have prevented widespread acceptance.

Regardless of the source of RPE, the delivery techniques are generally limited to subretinal injection of cell suspension of RPE or subretinal placement of a sheet of tissue containing RPE. The former is advantageous in that the delivery of a cell suspension does not require a large retinotomy and is relatively fast and simple. Major limitations of this method include the reflux of RPE cells into the vitreous, relatively poor adherence to Bruch’s membrane and failure to form an effective monolayer. Alternatively, the delivery of subretinal sheets containing RPE has also been demonstrated by multiple groups but requires larger retinotomies, takes significantly longer to implant, and is also prone to incorrect implant orientation and postoperative proliferative vitreoretinopathy. The main advantage of implanting RPE sheets with a scaffold is that the orientation, polarization and function of the RPE is more likely to consistently replicate that of the native RPE. Despite the limitations of both methods, there are promising advances in both methods that are being implemented in current clinical trials.

Other Challenges of Stem-Cell Therapy

There remain a number of additional challenges in the development and ultimate implementation of stem cell–based therapy in the treatment of NN-ARMD as well as other diseases. These include cost as well as regulatory and quality control challenges that are distinctly different from those for devices or biologics treatments. For example, stem cell–derived products need to have established standards of sterility, purity, identity, tumorigenicity, and potency to ensure the safety and efficacy of the final product. These standards must be employed in current good manufacturing practice (cGMP) settings that ensure comprehensive testing for pathogens and contaminants, especially undifferentiated cells that may increase the tumorigenicity of the implant. In addition, the production of stem cell–derived products can take 6 to 12 months and require highly trained personnel employing labor intensive cell culture methods. While these methods may have been practical in the past for small scale production, commercial scale production will require the development of novel methods with scalability. Therefore, additional research and resources are needed to develop clinical grade cell lines, differentiation protocols, and drug master files that can provide the common framework on which large scale stem cell–based therapies can be built. Another major challenge of stem-cell therapy in the eye in particular is assessment and treatment of host immune-regulatory responses. A number of studies have suggested that despite the immune-privileged status of the subretinal space, immune-mediated rejection can and does occur. Methods to address this include the genetic manipulation of hESC or iPSC to minimize the immunogenic potential. In addition, banking of hESC lines with known major histocompatibility complex antigens is possible and would facilitate host-donor matching just like other major organ transplants, although lifelong immunosuppression may still be needed. Lastly, pharmacologic immunosuppression before and after transplantation are feasible and have been shown to be at least partly efficacious but not without potentially serious adverse effects. Additional research is needed to improve our understanding of the immune-mediated response in the subretinal space. A detailed review of the immunologic considerations in subretinal surgery are discussed in a very recent review as well.

Lastly, unlike traditional biologic or pharmaceutical treatments, cell-based treatments will likely require novel in vivo monitoring methods that can assess the health of the implanted cells as well as measure their functional impact in...
real time. In cases where scaffolds are used, it is likely that traditional measures of retinal structure and function may not work well or in the same manner as in nonimplanted eyes. For example, investigators have shown that poly lactic-co-glycolic acid (PLGA) subretinal implants create artifactual increases in mERG signal and may not allow standard mERG testing to assess the overlying retinal function.\textsuperscript{108} Because RPE transplantation procedures are typically on the order of several hundred-thousand cells, very sensitive tests must be developed for this purpose. The application of SD-OCT, FAF, mERG, and microperimetry are a major step forward in this direction but it is likely that additional tests of RPE health will be necessary to understand the biochemical and pharmacologic function of the cells after transplantation. For example, quantitative FAE\textsuperscript{22} in vivo, real-time spectroscopy,\textsuperscript{109,110} and a host of SD-OCT–based measures of tissue composition\textsuperscript{111–113} present promising technology that can resolve cellular and molecular changes in retinal tissue.\textsuperscript{113} Identification and quantification of outcome parameters in cell-based therapies will also be challenging. In the case of bilateral, advanced dry AMD investigators will have the benefit of using the contralateral eye as an internal control for both anatomic and functional progression. However, this may be confounded if subjects learn to use previously nonfunctional retinal loci for fixation as has been reported in the past.\textsuperscript{91} In fact, visual acuity in general may not be a useful measure of efficacy or function and alternative efficacy measures must be carefully included in the trial designs. This kind of testing will be critical to assess the long-term impact and survival of the implanted cells. More importantly, real-time assay of the health of implanted RPE can serve as a guide for pharmacological interventions that may improve the survival of the implants (e.g., steroids and immunosuppression) as well as guide potential genetic modifications in future stem cell–derived products to improve survival.

**Conclusions**

During the past 20 to 30 years, studies have clearly demonstrated that RPE transplantation can restore at least some aspects of retinal structure, function, and subjective vision in animals and humans with NN-ARMD. Advancements in basic science and translational fields such as stem-cell biology, retinal surgery, noninvasive retinal imaging, retinal physiology, and vision science have poised the field on the edge of human clinical trials that can offer vision restoring therapy to millions of people affected by NN-ARMD. Several early phase human clinical trials are in progress around the world and the results will no doubt be exciting but continued research and collaboration are needed among funding sources, academic labs, and industrial partners to ensure success. The continued support of private agencies as well as public agencies like the NIH and National Eye Institute as well as state agencies such as the California Institute of Regenerative Medicine and the New York Stem Cell Foundation are critical for the successful implementation of stem cell–based therapiesth. Funding from these agencies should be aimed at the major current needs and opportunities for advancing stem cell–based therapy. These include the following: (1) development of novel, noninvasive diagnostic tests to assay RPE and retinal function at the molecular and cellular level, (2) development of novel transplantation tools and surgical methods for optimal delivery of RPE to the subretinal space, (3) expansion and advancement of stem-cell science for the purpose of understanding host immune response in the subretinal space, and (4) developing clinical grade methods to genetically modify stem cell–derived RPE.

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