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MINIREVIEWS

# Age-related macular degeneration treatment in the era of molecular medicine

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## Abstract

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the developed world. The quality of life of both patients and families is impacted by this prevalent disease. Previously, macular degeneration had no known effective treatment. Today, vitamins for non-exudative AMD and intravitreal injection of medications for its exudative form are primary forms of current treatment. Modern advances in molecular science give rise to new possibilities of disease management. In the year 2003 the sequencing of the entire human genome was completed. Since that time, genes such as complement factor H, high-temperature requirement factor A1, and age-relateed maculopathy susceptibility 2 have been discovered and associated with a higher risk of AMD. A patient's genetic make-up may dictate the effectiveness of current or future therapeutic options. In addition, utilizing genetic data and incorporating it into new treatments (such as viral vectors) may lead to longer-lasting (or permanent) VEGF blockade and specific targeting of complement related genes. There have also been considerable advances in stem cell directed treatment of AMD. Retinal pigment epithelial (RPE) cells can be derived from human embryonic stem cells, induced pluripotent stem cells, or adult human RPE stem cells. Utilizing animal models of RPE and retinal degeneration, stem cell-derived RPE cells have been successfully implanted into the subretinal space. They have been injected as a cell mass or as a pre-prepared monolayer on a thin membrane. Visual recovery has been demonstrated in a retinal dystrophic rat model. Preliminary data on 2 human subjects also demonstrates possible early visual benefit from transplantation of stem cell-derived RPE. As more data is published, and as differentiation and implantation techniques are optimized, the stabilization and possible improvement of vision in individuals with non-exudative macular becomes a real possibility. We conclude that the technologic advances that continue to unfold in both genetic and stem cell research offer optimism in the future treatment of AMD.



**Key words:** Age-related macular degeneration; Stem cell therapy; Anti-vascular endothelial growth factor; Gene therapy; Complement factor H; High-temperature requirement factor A1; Age-relateed maculopathy susceptibility 2; Pharmacogenomics; Genetics

**Core tip:** New therapies for age-related macular degeneration (AMD) such as stem cell transplantation and viral vector delivery are currently under intense investigation. Possible new treatments for both non-exudative and exudative AMD are on the horizon. Human embryonic stem cell derived retinal pigment epithelial cells have been transplanted into the subretinal space in human subjects. Viral vectors that encode proteins with a strong affinity for vascular endothelial growth factor are in clinical trials. In light of these exciting advances in both genetic and stem cell therapy, the future of AMD treatment shows substantial promise.

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## INTRODUCTION

Age-related macular degeneration (AMD) is the leading cause of blindness in the developed world, surpassing cataracts which were the leading cause in 1990[1]. In the United States, current prevalence of advanced AMD defined as geographic atrophy or exudative macular degeneration is estimated to be at 1.75 million. By 2020, it is predicted that 3 million patients will suffer from advanced AMD[2]. At present, there are effective treatments for the exudative form of AMD[3-7], however, when faced with advanced non-exudative AMD visual loss there is little to offer. Research efforts focused on the biological mechanisms of the disease, utilization of stems cells, and genetically based treatments are currently underway to develop novel human therapies.

## RISK FACTORS

AMD is a disease that presents with a wide spectrum of severity - from early small drusen with no visual impact to geographic atrophy or choroidal neovascular membrane formation causing severe visual impairment. Multiple non-modifiable and modifiable risk factors have been implicated.

### Age

AMD increases significantly in prevalence, incidence, and progression with increasing age. The Beaver Dam eye studies found that by age 75, 7.1% of patients had late AMD compared to 0.1% in those aged 43-54 and 0.6% in age group 55-64[8,9]. Another, more recent study demonstrated that 57.4% of patients over age 85 had signs of AMD[10].

### Genetic risk

One study found that 14% of patients with AMD reported a parental history and 21% reported a sibling history of AMD compared to 1% and 2% respectively in control patients. Examined siblings of affected patients showed a 16% prevalence of intermediate disease and a 23% prevalence of advanced disease[11].

### Inflammation

Several studies have shown the presence of complement factor byproducts and complement regulatory proteins in drusen and juxtaposed RPE cells. In one study, RPE cells adjacent to drusen exhibited a phenotype consistent with cellular response to complement attack[12]. The role of the complement pathway has been strengthened by the discovery of complement factor gene associations with AMD[13,14].

### Oxidative stress

Retinal pigment epithelium (RPE) cells are prone to damage from oxidative stress due to toxin or light exposure[15-17]. The decreased risk of progression with antioxidant supplementation as seen in the AREDS studies yields further evidence of this important mechanism[18,19].

### Other risk factors

Some additional non-modifiable risk factors include female sex, hyperopia, and Caucasian race[8,20,21]. Modifiable risk factors include smoking, elevated HDL cholesterol, atherosclerosis, and obesity[10,22].

## HUMAN GENOME PROJECT

In April 2003, at an estimated cost of 2.7 billion dollars, the sequencing of the human genome was completed. The project mapped 3 billion base pairs and is estimated to contain approximately 20500 genes. These genes only make-up 1%-2% of the entire sequence. Human to human variation is approximately 0.1% and is more commonly found in non-coding DNA. The past 10 years have led to numerous discoveries including the identification of about 5000 disease producing genes. With technological advances in gene sequencing, today's cost to sequence the human genome has dropped considerably to approximately 1000 dollars (as compared to 2.7 billion 10 years ago). In addition, what used to take years to decades of collaboration among institutions to discover one gene can be completed by one lab within days to weeks[23].

### Genome-wide association studies

Genome-wide association studies (GWAS) involve analyzing variations in single nucleotide polymorphisms (SNPs) in patients with a particular disease compared to controls[24]. Coupled with the International HapMap project[25,26] that is responsible for mapping SNP's, GWAS studies today can evaluate a growing number of genetic loci. The overall goal of GWAS is to identify at risk genetic markers for individuals with multifactorial diseases in which a familial component has been identified. Accurate mapping may provide information about disease phenotype, predict genetic markers associated with disease progression, and lead to tailored risk reduction techniques and treatment options. Some limitations of GWAS include: the discovery of many SNPs that are not related to disease, expense, and the requirement of large studies in order to identify a modest risk association[24,27].

## AMD GENETICS DISCOVERY

### Complement factor H

In 2005, the first GWAS for AMD was published in which a major susceptibility locus was identified. A SNP rs1061170 in the complement factor H (*CFH*) gene in chromosome 1 was shown to have a high association with AMD. SNP rs1061170 encodes a tyrosine to histidine change at the 402 position of the gene (*Y402H*). Complement Factor H inhibits the conversion of C3 to its C3a/C3b components and competes with Factor B to prevent activation of C3b to C3bB[13,14]. A meta-analysis

of eight studies showed that a single allele (heterozygous for risk *Y402H* allele, CT genotype) confers a 2.5-fold increased risk of AMD, while those homozygous for the risk allele (CC) had a 6 fold increased risk. Predicted population attributable risk (PAR) in the same meta-analysis for the risk genotype (CC or CT) was 58.9%[28]. Meta analyses in Asian and Chinese subjects demonstrated a similar increase in risk of AMD per C allele, but a lower PAR[29,30].

## OTHER ASSOCIATED GENES

In a meta-analysis in 2005, Fisher *et al*[31] showed a significant link between the locus 10q26 and AMD. Age-related maculopathy susceptibility 2 (*ARMS2*), and high-temperature requirement factor A1 (*HTRA1*) are genes in this locus (10q26) and variations confer a significant risk for AMD that may be higher than with CFH[32,33]. Conversely, polymorphisms of the genes for complement factor B and complement component 2 seem to confer a protective effect to the development of AMD[34-36].

## POTENTIAL IMPACT ON MANAGEMENT

Commercial AMD genetic testing has been available for screening at risk patients for several years. However, the value of screening is limited due to the lack of understanding of the association between genetic mutations, modifiable risk factors, and the current therapeutic options. It would be useful to target specific lifestyle modifications in patients depending on their genetic make-up. In addition, if disease progression could be predicted by analyzing the genetic profile of an individual, decisions about the frequency of monitoring and the institution of early intervention could be tailored accordingly[37]. The data however remain conflicting and limited.

## GENETIC IMPACT ON CERTAIN RISK FACTORS

### *Smoking*

Several studies show a strong association between smoking and advanced AMD in patients with at risk genes. DeAngelis *et al*[38] showed 144 fold increased risk of CNV in patients who had a 10 pack-year smoking history and were homozygous for the at risk CFH variant compared to individuals who smoked less than 10 pack-years and were heterozygous for the at risk variant, or those who carried the non-risk gene[38]. Similar results were noted for patients with variant at the 10q26 locus[39,40]. Since smoking is an independent risk factor for progression of disease, the effect of smoking in individuals with the risk genotype yields a multiplicative effect[38].

### *Obesity*

AREDS showed a body mass index > 25 did not increase the risk of AMD in patients with a non-risk CFH genotype, however, those who were heterozygous or homozygous for the risk variant showed increased risk with an odds ratio of 2.2 and 5.9 respectively[41].

### *Genetic impact on risk of progression*

The Beaver Dam Eye study patient cohort looked into the association of the CFH and ARMS2 risk alleles and the natural history of AMD. Persons aged 45 years with no evidence of AMD who had low, intermediate, and high AMD genetic risk groups were estimated to develop early AMD at 33.0%, 39.9%, and 46.5% by age 80 years. Late AMD was estimated at rates of 1.4%, 5.2%, and 15.3%, respectively, for these same groups[42]. In addition, a Spanish study showed significant associations of the rate of progression and growth of geographic atrophy with genetic polymorphisms *CFH Y402H*, *CFH-62Ile* and *CFB-32Gln*[43]. Several other studies showed underlying genotype was associated with development of geographic atrophy but had no implication on progression of disease[44,45].

### *Pharmacogenomics: Targeting/Tailoring treatment*

Therapeutic interventions available for the treatment of macular degeneration have been limited to risk modification, anti-oxidants, laser, photodynamic therapy (PDT) and anti-vascular endothelial growth factor (VEGF) agents. With the discovery of the anti-VEGF agents for the treatment of exudative AMD, choices of regimen, dosing, and frequency have been largely dictated by medication cost, physician preference, and large studies that leave a lot to interpretation by the prescribing physician. Tailoring and targeting treatment to individual patients based on calculated susceptibility of the disease to specific intervention(s) is certainly in the future for medicine. Knowing which anti-VEGF medication would be most effective, ideal injection frequency, and the individual risk of complications (such as drug toxicity or RPE tears) prior to initiating treatment would revolutionize our current approach to anti-VEGF treatment and may reduce unnecessary costs[46].

Genetic studies in AMD therapeutic responses have mixed results thus far.

### *Antioxidants*

Klein *et al*[47] studied 876 patients from the AREDS trial who had category 3 and 4 AMD and showed that AREDS vitamin supplementation resulted in a 68% reduction in the rate of progression in the subgroup with the homozygous non-risk genotype compared to a reduction of only 11% in the subgroup with the homozygous risk genotype of *CFH Y402H*. Further analysis revealed the interaction to be explained by zinc. Interaction was noted in the groups taking zinc when compared to those not taking zinc. However, the authors did not feel their results justified routine screening. No genetic treatment interaction was noted for patients with LOC387715/ARMS2[47].

Awh *et al*[48] studied 989 patients from the AREDS trial and showed that patients with a 1 or 2 CFH at risk alleles benefited from antioxidants and not zinc and that

patients with ARMS2 at risk alleles benefited from zinc only regimens[48]. Patients with at risk ARMS2 and CFH alleles showed no benefit to any of the AREDS supplementation. The authors recommended that patients with moderate AMD would benefit from selective nutritional supplementation based on genetic profile[49].

Both studies derived their cohort from the AREDS trial and yet the results are different. However, the AREDS trial was a prospective study that wasn't designed to look into a genetic treatment interaction. Statistically, these studies were too underpowered to achieve statistical significance. In 2012, a task force from the American academy of ophthalmology advised against genetic testing for AMD and that recommendation is unlikely to change with the current evidence[50].

### Photodynamic therapy

Initially, 2 small studies have suggested a possible association between CFH and PDT response, but their results were conflicting. In 2008, Goverdhan *et al*[49] published a study of 27 patients and showed that patients carrying 2 CFH at risk alleles responded poorly to PDT compared to other groups. This study was limited by the small sample size. Brantley *et al*[51] looked at a group of 69 patients and showed that in patients with classic CNV, response to PDT was worse for patients with no risk alleles compared to those with one or two at risk alleles[51].

Additional studies with larger sample sizes did not show any association between CFH variants and response to PDT. The largest study of 273 Australian patients looked into this association and found no significant difference in CFH genotypes and response to PDT[52].

Chowers *et al*[53] looked at the association of response to PDT and CFH in an Israeli population of 131 patients and showed no significant association between the genetic variants and response to PDT and number of PDT sessions needed[53]. The same team evaluated the association of PDT response to ARMS2/HTRA1 and showed no significant association between the different genetic variants and the response to PDT or the number of PDT sessions required[54].

### Anti-VEGF

Anti-VEGF agents have also been explored. Initially, several studies showed a potential association CFH genotype and response to anti-VEGF response. One group found that intravitreal bevacizumab was associated with worse visual outcomes in patients with the *CFH Y402H* risk genotype[55]. The same group found in a different study that patients with the high risk genotype may require more injections of ranibizumab, but that there was no impact on visual acuity[56]. Hagstrom *et al*[57] recruited 834 patients participating in the Comparison of AMD Treatment Trials (CATT). Each patient was genotyped for rs1061170 (*CFH*), rs10490924 (*ARMS2*), rs11200638 (*HTRA1*), and rs2230199 (*C3*). The study showed no significant difference in patients with high risk alleles (*CFH*, *ARMS2*, *HTRA1*, *C3*) compared to low risk allele in final vision, change in vision, anatomical outcomes (OCT, FA, thickness, lesion size) or number of injections whether bevacizumab or ranibizumab was used[57].

Association between polymorphisms in VEGFA and VEGFR and the response to bevacizumab and ranibizumab were explored. Several studies showed a treatment benefit to anti-VEGF agents, however these studies were limited by the small sample sizes and the limited standardization of their outcomes[58-60]. Hagstrom *et al*[61] showed a different conclusion in a cohort of patients from the Comparison of AMD Treatment Trials. They recruited 835 patients participating in CATT and the patients were genotyped for 7 SNP's in *VEGFA* gene and 1 SNP for *VEGFR2* gene. Results showed no association between the studies genotypes and vision or number of injections[61].

Despite the limited results with AMD, successful examples of genotype directed interventions do exist in other areas of medicine. For example, the drug Abacavir, a reverse transcriptase inhibitor has been associated with an uncommon but potentially fatal and unpredictable hypersensitivity reaction in Human Immunodeficiency Virus patients. In a study published in the New England journal of Medicine, patients with HLA B5701 allele have a 50% chance of developing hypersensitivity while patients without the allele have no risk of developing the hypersensitivity reaction. It is now the standard of care to test patients for this allele prior to starting Abacavir[62].

## GENE THERAPY

An emerging therapeutic option is to deliver anti-angiogenic genes using a viral vector. This could allow a sustained delivery of the desired peptide. The challenges here lie in the engineering of a vector that will both target a specific cell (retinal pigment epithelial cell) and allow effective translation of the desired protein.

Pigment epithelial derived factor (PEDF) is a potent anti-angiogenic compound. Campochiaro and colleagues completed a phase I clinical trial in individuals with advanced neovascular AMD using Adenoviral vectors expressing human PEDF (AD-PEDF-11). Twenty eight patients received a single intravitreal injection of low or high dose AD-PEDF-11. Results at 6 and 12 mo showed that the median size of the lesion increased by ½ and 1 disc area respectively in the low dose group compared to no change in the lesion area for the high dose group at 6 and 12 mo. A phase Ⅱ is planned in the future[63].

Another potential VEGF blocker is soluble fms-like tyrosine kinase (sFLT), an endothelium specific receptor tyrosine Kinase. It binds to VEGF with high affinity. A phase I trial of 6 patients who were treated with intravitreal ranibizumab on day 0 and day 30 and received a subretinal injection of a high or low dose sFLT-1 integrated into adeno-associated virus serotype 2 (AAV-sFLT) on day 7. Patients were followed monthly with strict ranibizumab retreatment criteria. By day 380, the high dose group gained 12.5 letters, low dose 8.7 and the control untreated group lost 3.5 letters. Re-treatment with ranibizumab was also less frequent in the treatment group than control. Patients treated with AAV-sFLT showed no

significant adverse events[21]. AAV2-sFLT01 is another sFLT viral vector designed to block VEGF function and is currently in a phase 1 trial[64].

## BRIEF HISTORY OF STEM CELL RESEARCH

The terminology of "stem cells" - regenerative precursor cells - was first postulated in 1909 by Alexander Maksimov, a Russian histologist[65]. Since that time there have been many advances in our understanding of stem cells and our ability to isolate them. There are several different approaches to obtaining these progenitor cells; some are derived from adult tissues and others from embryos (embryonic stem cells). Embryonic stem cells are totipotent, meaning they can differentiate into any cell type from any of the three germ layers. In addition, embryonic stem cells have the capacity of unlimited, undifferentiated proliferation *in vitro*. Embryonic stem cells express a high level of telomerase activity, which yields a prolonged replicative life span. Human embryonic stem (hES) cells were first successfully isolated from human blastocysts in 1998[66]. The hES cells were initially grown on a mouse embryonic fibroblast feeder layer[66,67]. Further progress allowed the hES cells to be grown on human feeder layers, and avoid the risk of exposure to animal retroviruses[68]. Now techniques are available to grow hES cells using a serum free, sterile generated protein cocktail that avoids the use of human or animal serum, or human feeder layers[69]. Recent studies also demonstrate that hES cells can be successfully isolated from human blastocysts produced by *in vitro* fertilization rather than from ex-utero embryos[66,70].

Inevitably, ethical and moral issues exist in the use of embryonic stem cells for research purposes. In an effort to avoid such controversy, much work has been done to develop pluripotent adult stem cells that are also capable of differentiating into virtually any tissue in the body. These cells are referred to as induced pluripotent stem (iPS) cells. Such iPS cells have been derived successfully both in mice and in humans[71]. In addition to avoiding the many of the ethical concerns, these adult stem cells can be derived from the patient for whom they would be used, potentially minimizing complications such as rejection of donor tissue.

## METHODS OF DIFFERENTIATION INTO RPE CELLS

The RPE has become a primary target for stem cell therapy of AMD. The RPE functions include phagocytosis photoreceptor outer segments, supply of nutrients to the retina, absorption of stray light that passes through the photoreceptors, and formation of the blood retinal barrier[72-74]. Dysfunction of this vital cellular layer in macular degeneration ultimately leads to photoreceptor loss and decreased visual acuity. Differentiation of hESC to RPE was first described in 2004; these cells were compared to fetal RPE cells and were found to express RPE specific markers[75]. RPE cells have been successfully derived using various methods such as co-culture on inactivated mouse embryonic fibroblasts coupled with stromal cell-derived inducing activity, utilizing embryoid body formation, as well as exposing the cells to several signaling pathways[72,75-78]. One group utilized to NIC and Activin A to differentiate hES cells to RPE and successfully transplanted them into the Royal College of Surgeons (RCS) rat and demonstrated rescue of retinal function[71].

In addition to differentiating hES cells into RPE, human induced pluripotent stem cells (iPSCs) have also been described[79-81]. RPE derived from iPSCs (iPS-RPE) expressed cell markers similar to RPE from hESCs. A retinal outer segment phagocytosis assay demonstrated similar efficacy in iPS-RPE and hESC-RPE to fetal retinal pigment epithelium[79]. Another group was able to grow iPS-RPE as a monolayer without an artificial membrane and successfully implant it into the RCS rat with restoration of ERG responses[80].

Adult human RPE stem cells (hRPESC) are another source of RPE cells. These cells are derived from elderly donor eyes and expanded to differentiate into RPE cells. One study found that a single donor may be able to provide enough cells to cover hundreds of patients using stem cell proliferation techniques[82]. Further study is needed to determine if these cells are suited for intraocular transplantation.

One study evaluated the essential role of RPE in the proper structural formation of photoreceptors, including the outer segments. Therefore, the ability to create polarized RPE cells may aid in the differentiation of functional photoreceptors for possible therapeutic use in ocular diseases causing photoreceptor dysfunction or death[83]. Overall, a key to developing successful stem cell therapies for AMD is establishing a high-yield, accurate, and reproducible method of differentiating stem cells to RPE that can survive *in vivo*, integrate properly into the host retina, and perform the essential RPE functions.

In addition to the above advances in RPE derivation from stem cells, hESC derived photoreceptors can be successfully implanted into $Crx^{-/-}$ mice with improved ERG responses compared to controls[84]. This may allow for not only photoreceptor rescue but potentially replacement of dead/degenerated photoreceptors in the future.

## METHODS OF INTRAOCULAR IMPLANTATION

After isolation of retinal pigment epithelial cells from either hRPESC, iPSC or hESC, the next challenge becomes creating an effective method of intraocular implantation to maximize the therapeutic effect. Two primary techniques have been employed: injection of a cell mass into the subretinal space, or implantation of a monolayer prepared on a thin membrane.

### *Cell suspension injection techniques*

In nude rats a 1.2 mm scleral incision was made 1.5 mm posterior to the limbus and then a local retinal detachment was created by injecting 5 microliters of balanced saline solution. Next, *via* a 32 G blunt-end injection cannula, 2 microliters of phosphate-buffered saline solution containing the hESC-RPE was injected into the subretinal space *via* the sclerotomy site[85]. A different group also utilized a trans-scleral technique in rats, however, no subretinal bleb was created prior to cell suspension injection[86,87].

### *Thin membrane implantation techniques*

Given that the RPE is a single layer of polarized cells it is presumed that the best functional outcome of transplantation would yield a monolayer of new RPE. Injecting a cell mass can lead to clumping of RPE cells rather than the formation of a monolayer. In search for a solution, groups have cultured RPE cells on thin membranes as a monolayer followed by implantation of the membrane-RPE complex into the subretinal space[82,85,88-90].

One group, utilizing chinchilla rabbits, performed a two-port core vitrectomy, then created a small bleb retinal detachment with 25-30 mL of balanced saline solution using a 41 G Teflon cannula. Conventional infusion caused the bleb to flatten during the procedure, therefore a custum-made infusion with 2 side ports was utilized to minimize disturbance of the bleb[88]. The retinotomy was enlarged with scissors and then a polyester membrane lined with hESC-RPE was inserted using a custom-made subretinal shooter instrument[82,88]. Electrostatic adhesions between the implant and the delivery device often required additional maneuvering (with subsequent retinal damage) to successfully place the thin membrane into the subretinal space. Utilizing a hydrogel encapsulation of the implant significantly decreased these hydrostatic forces and improved the success of subretinal placement[88]. Other customized injection instruments have been designed, including one that gently delivers a parylene membrane lined with a monolayer of hESC-RPE using an infusion of balanced salt solution through small holes in the device[90]. A previous group had used a similar technique using an injection instrument and mircoforceps. This group also utilized perfluorocarbon to create an intraocular tamponade to prevent reflux of fluid through the retinotomy site[89].

Another group, utilizing nude rats, compared the injected RPE cells cultured on a parylene membrane into the subretinal space *via* a trans-scleral incision. First, a 1.2 mm scleral incision was made 1.5 mm posterior to the limbus and then a local retinal detachment was created by injecting 5 microliters of balanced saline solution. Next, the choroid was cut, taking care to avoid retinal damage, and the hESC-RPE membrane substrate was inserted into the subretinal space utilizing forceps[85].

In addition to hESC-RPE, hRPESC have also been used and proliferated on a polyester membrane and implanted into rabbit eyes and survive with maintenance of cellular polarity up to 4 wk after graft implantation[82].

## IMPACT ON VISUAL ACUITY

Comparison of injection hESC *via* a cell suspension vs hESC cultured on a parylene membrane demonstrated increased cell survival in the polarized monolayer group. In this same study, the rats were observed for up to 12 mo with persistent cell survival and there was no evidence of teratoma or ectopic tissue formation[85]. Another group injected the RCS dystrophic rats with different doses of a subretinal hESC mass (either with 50000 or 100000 hESCs) and found that treated rats demonstrated better spacial acuity when compared to sham and untreated rats. Once again, this study demonstrated no evidence of teratoma or tumor formation (up to 220 d)[87]. Also, utilizing the RCS rats and the cell suspension technique, Lund *et al*[91] demonstrated improvement in vision in the RCS rats that achieved spacial acuity up to 70% of the non-dystrophic rats[91]. Transplantation of iPSCs have also been performed in RCS rats and demonstrated slowed visual decline, however, the transplanted cells were lost to immune response despite immunosuppressive therapy[92].

### *Human trials*

The preliminary results of 2 human subjects, one with Stargardt's macular dystrophy and one with dry AMD are now available. The patients were given low dose tacrolimus and mycophenolate mofetil 1 wk prior to the procedure. HESC MA09 cells were used for implantation. The procedure entailed a pars plana vitrectomy with induction of a PVD from the optic disc followed by the injection of approximately 50000 hESC-RPE (150 microliters) into the subretinal space at a predetermined location based on OCT findings consistent with an area of RPE and photoreceptor compromise. The induced bleb had flattened by 4 h after the surgery in both patients. The patient with Stargardt's disease improved from hand motion to 20/800, and the patient with AMD improved from 20/500 to 20/320. However, it is unclear whether the mild improvement was due to the transplanted cells, immunosuppressive medications, or to placebo effect. In the future as more data are collected we will have a better understanding of the effect of implanted hESC-RPE in the human subretinal space[93].

## CONCLUSION

AMD affects millions of people worldwide and is the leading cause of blindness in the United States[1]. As our understanding of molecular medicine has expanded so has our approach to disease treatment. The severe vision loss that almost always accompanied exudative macular degeneration in years past is now being widely treated with anti-VEGF injections[4-7]. Current therapies, including viral vector delivery of VEGF binding proteins, may allow for prolonged VEGF blockage allowing for a significantly reduced number of intravitreal injections[63]. Also, understanding the complex array of genes associated with AMD may allow us to make tailored therapeutic decisions and/or lifestyle recommendations depending

on a patient's genetic make-up[47,52,57,62].

Currently there is no treatment to offer patients with advanced non-exudative AMD, however, stem cell therapy may provide a future solution. Techniques to differentiate hESC (or iPSC and hRPESC) into RPE cells allow for a potentially numberless supply of cells to utilize in transplants to treat not only macular degeneration but also other disorders such as Stargardt's macular dystrophy, retinitis pigmentosa, cone-rod/rod-cone dystrophies, *etc*. More data is required to determine if a single origin of RPE is more effective than the others in creating fully functioning RPE cells. Once the RPE cells are successfully derived, there must be safe, reproducible, and effective method(s) to surgically implant the new cells. There are currently different techniques and instruments under investigation, whether it be by subretinal injection of a cell mass or implantation of a pre-RPE cultured thin membrane, and further study is necessary to determine which technique will yield the best visual outcomes with the least degree of surgical complications. In addition to RPE, studies have also been performed demonstrating that hESC derived photoreceptors can be successfully implanted into mice[85]. This may allow for not only photoreceptor rescue but also potential replacement of dead photoreceptors in the future. In light of these exciting advances in both genetic and stem cell therapy, the future of AMD treatment shows substantial promise.

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