

# Chapter 11

## Stem Cell-Based Therapeutics in Ophthalmology: Application Toward the Design of Clinical Trials

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**Abstract** Stem cell-based therapeutics have been proposed as a technology for restoration of anatomic structure and visual function for retinal degenerative diseases. Success in animal studies and preliminary trials may offer hope for patients afflicted by a variety of retinal degenerations. However, as clinical trials expand and advance to latter phases, it is important to address key study design issues. This chapter discusses the parameters for research into stem cell-based therapeutics. Efficacy endpoints for studies can be defined along objective physiologic, psychofunctional, anatomic, and functional living axes. Pupillometry, electroretinography, and radiologic tools are discussed as objective tools for the assessment of treatment outcome. Additionally, optical coherence tomography(OCT), fundus autofluorescence (FAF), and other imaging tools may be used. Psychofunctional tests may be less reliable among a pediatric population. Finally, improvements in functional living may be reported by patients and assessed by various measures.

### Introduction

The normal human corneal epithelium is composed of flat stratified squamous epithelial cells. Goblet cells, which populate the conjunctival epithelium and are important as a source of mucin production for the tear film, are normally absent in the corneal epithelium. Normal corneal epithelium overlies a cuboid basal layer lying on the avascular corneal stroma. The population of epithelial cells that are

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located at the corneal limbus are commonly referred to as limbal stem cells and are responsible for the continued renewal of the cornea's epithelium [1, 2]. Various ocular pathologies that affect the limbal stem cells, such as chemical injuries, contact lens abuse, cicatricial pemphigoid, or Steven–Johnson syndrome, may lead to vision-threatening corneal compromise.

The human retina is a rich and complex neurosensory structure that depends on tight integration with other ocular structures for proper function. In particular, the neurosensory retina (composed of the retinal ganglion cells, the inner nuclear layer, and the photoreceptor layer) depends on healthy apposed retinal pigment epithelium (RPE) and interposed retinal vasculature. Although the retina does not have an intrinsic regenerative capacity, retinal stem cells are located in the RPE of the pars plana and pars plicata, and retinal progenitor cells are found in the ciliary margin zone. Diseases that compromise retinal vasculature, such as diabetes mellitus, may lead to loss of retinal neurosensory structures, and diseases that affect the RPE, such as age-related macular degeneration (AMD), may lead to secondary loss of photoreceptors.

Repopulation for unilateral or incomplete bilateral limbal stem cell deficiency has been achieved via autologous sources [3, 4]. Recent improvements in autologous limbal stem cell transplantation include the development of a temperature-sensitive culture dish and the use of amniotic membrane that may improve viability of the transplanted cells. In cases of bilateral limbal stem cell deficiency, various strategies have been utilized, including allogenic transfer combined with immunosuppression and transfer of cultured autologous cells from stratified epithelia of other areas of the body.

Targets for stem cell therapy in the retina include the vascular endothelial cells, the RPE, and the photoreceptors. RPE cell replacement has been studied in clinical trials, mostly with limited results [5, 6]. The generation of a spontaneous immortalized RPE cell line and successful retinal cell transplantation into rodent models of retinal degeneration offer promise for retinal repair.

## Stem Cell-Based Therapeutics

Stem cell-based therapeutics have been proposed as a technology for restoration of anatomic structure and visual function for retinal degenerative diseases. Success in animal studies and preliminary trials may offer hope for patients afflicted by a variety of retinal degenerations. However, as the clinical trials expand and advance to latter phases, it is important to address key study design issues that will be explored below.

The following material was adapted from the Federal Drug Administration (FDA) Cellular, Tissue and Gene Therapies Advisory Meeting held on June 29, 2011 [7], in particular the discussion led by Dr. J. Timothy Stout (Casey Eye Institute) [also Stout and Francis [8]]. Key questions were posed regarding study design, in particular: (1) the definitions of efficacy endpoints; (2) safety concerns; and (3) drug administration. In the following sections, we will review each of these three components.

## Efficacy Endpoints

Efficacy endpoints can be defined along objective physiologic, psychofunctional, anatomic, and functional living axes.

Objective physiologic endpoints are not dependent on patient feedback, and thus are not subjective. One such test is pupillometry, for which there are available commercial testing units that are well validated, sensitive, and reliable; they are a good modality for use in both adult and pediatric patients. Pupillometry can be helpful for diseases affecting the entire retina, but may be difficult to use in cases of nystagmus. Another objective physiologic endpoint can be measured by nystagmography, which is similar to pupillometry, is commercially available, sensitive and reliable, and provides a good modality for both adults and children. However, most ophthalmologists do not routinely use this test, and its use would likely be limited to those severe disease cases associated with nystagmus.

Electrophysiology is another available objective physiologic modality. The electroretinogram (ERG), visually evoked potential (VEP), and multifocal ERG (mfERG) have been implemented by commercial systems for adult and pediatric patients, but complications may include imperfect standardization between centers as well as issues of reliability and validity. Moreover, these techniques have not been commonly used for endpoint analysis. Below, we discuss the features of this type of imaging in evaluating the outcome of treatment.

Alternatively, radiologic tools such as functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) may be useful technologies to incorporate, but their availability may be limited to certain centers and their use in the pediatric population may be constrained. In addition, their use for ophthalmic protocols has not been validated and therefore will need further study.

Unlike physiologic endpoints, psychofunctional endpoints require patient feedback for the interpretation of results. Visual acuity is the most fundamental measurement. Although often considered a “gold standard,” visual acuity testing may be a less reliable measure in the pediatric population.

Color vision testing is readily commercially available, but the validity and reliability as a measure of disease progression or therapeutic response remain to be studied. As with visual acuity testing, reliability will be reduced when testing children. In addition, a small percentage of the population may have preexisting color vision deficits, thus potentially excluding this test from that patient subset.

Contrast sensitivity is another psychofunctional technique that is readily available and has previously been validated for optic nerve disease trials. Its use, however, may be limited in the pediatric population and in adults that are affected by ocular media issues such as cataract.

Lastly, visual field testing is a commonly used psychofunctional test that is implemented by many commercial systems. It has been well validated and can discriminate between central versus peripheral disease-specific patterns. However, its utility is limited by poor reliability with patient fatigue or in patients too young to accurately complete the test.

Anatomic endpoints are largely based on well-established and recently developed imaging techniques. Digital fundus photography and fluorescence angiography are widely available and validated, although their use may be limited in cases of nystagmus or in the pediatric population.

Optical coherence tomography (OCT) has become a more widely used and validated imaging modality. However, some centers may not have access to the newest high-resolution and high-speed spectral domain systems. In addition, patient cooperation difficulties due to age or nystagmus may limit the utility of OCT.

Fundus autofluorescence (FAF) is becoming a more commercial imaging technique that can potentially provide quantitative characterization of autofluorescence patterns in retinal diseases. Although some commercial spectral domain OCT systems have integrated FAF imaging, some centers may lack access to this imaging modality. Moreover, its utility may be limited in cases of nystagmus or young children.

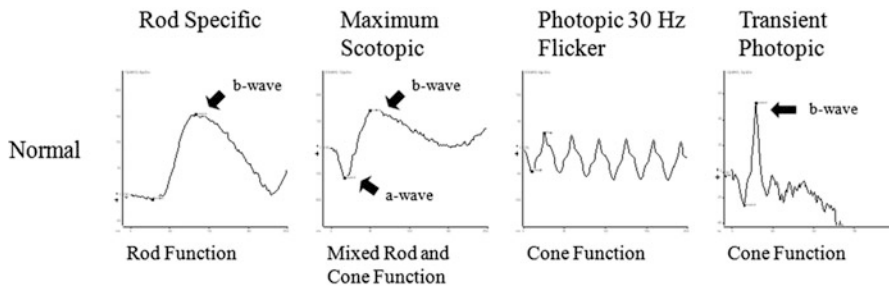
Adaptive optics (AO) offers a powerful technique to measure retinal morphology, including cone density and spacing, and to potentially discern differences in various retinal diseases or to measure responses to therapy. Its limitations, however, are that there are few commercially available systems available and that poor fixation in cases of nystagmus or poor patient cooperation severely limit its utility.

Functional living endpoints include patient reported outcomes (PROs), mobility testing, and reading performance metrics. PROs, such as the VFQ51, VFW25, and Visual Function Index, are validated but subject to patient bias and would have limited use in the pediatric group. Mobility testing is not standardized or commonly used, but may be a useful test for monitoring outcomes. Reading performance metrics are validated tests that are commercially available but may be affected by patient fatigue. This test is probably best suited for adults affected by macular disease.

### ***The ERG in Electrodiagnostic Imaging***

Electroretinography is a tool for measuring the electrical impulses of neurons. Photoreceptors and downstream neurons in the retina maintain a non-neutral electrical “resting potential” by manipulating the intracellular and extracellular concentrations of positive sodium, potassium, and calcium ions and negative chloride ions, as well as larger electronegative molecules.

Human rod cells present a model system of phototransduction. The chromophore, or light-sensing pigment, in rods is 11-*cis*-retinal, which is bound to an apoprotein called opsin, forming rhodopsin. When a photon strikes 11-*cis*-retinal, the added energy causes it to isomerize into all-*trans*-retinal [10, 11, 12, 13]. This conformational change causes rhodopsin to activate transducin, a heterotrimeric G protein [14, 15, 12]. Activated transducin binds to the inhibitory subunits of phosphodiesterase 6 (PDE6), thereby de-inhibiting it. The newly active PDE6 hydrolyzes cyclic guanosine monophosphate (cGMP), reducing intracellular cGMP levels and closing cGMP-gated cationic channels (CNG) in the rod cellular



**Fig. 11.1** Normal electroretinogram (ERG) Tracings. The a-wave and b-wave are noted where applicable

membrane [16, 11, 17, 13]. This reduces the influx of  $\text{Na}^+$  and  $\text{Ca}^{2+}$  into the cell, thereby hyperpolarizing it.

The hyperpolarization of the cell causes it to cease transmitting glutamate across synapses to bipolar cells, inducing changes in their polarization. Bipolar cells transmit this signal either directly to ganglion cells, each of which has an axon proceeding out of the orbit along the optic nerve, or to amacrine cells, which then activate ganglion cells or alter the output of other bipolar cells. Photoreceptors, bipolar cells, and amacrine cells operate via graded potentials, but ganglion cells generate action potentials in response to incoming signals from bipolar and amacrine cells; these action potentials help to propagate the information along the optic nerve. The function of each of these cell types can be measured using precise electroretinographic techniques.

### Wave Components Explanation

The typical ERG waveform (see Fig. 11.1, Maximum Scotopic) is the sum result of activity in the photoreceptors and bipolar cells, with some contribution from Müller cells. The initial negative deflection, known as the a-wave, is the result of early signals from the rod and cone photoreceptors. The subsequent rise toward the positive peak, known as the b-wave, is created primarily by slower signals from the rod and cone bipolar cells. The ascending slope from the a-wave to the peak of the b-wave typically shows several small oscillations; these are called the oscillatory potentials, or OPs, and reveal the function of the amacrine cells. Other components that become apparent only under certain conditions are beyond the scope of this chapter.

### Safety Concerns

The normal human eye is generally considered an immune-privileged organ, but one of the concerns associated with the intraocular administration of a potential gene or stem cell-based therapy is the development of an immune-mediated response after repeat or contralateral eye dosing. Preclinical studies in animals

may help to predict the immune response, but may be problematic because response may vary with animal species, specific therapy administered, site of administration, injection technique, host immune response, timing of the contralateral dose, use of immunosuppressive agents, and intra-subject eye disease.

The risks associated with repeat or contralateral dosing can be possibly minimized. Suggested strategies include general safety and adverse reaction surveillance, specific monitoring for an immune response, staggering patient enrollment, adjusted administration intervals, and immunosuppressive regimens targeted toward reducing risk, although none of the suggested strategies have been well established or validated.

Preliminary investigation of readministration of recombinant adeno-associated virus (AAV) carrying the *RPE65* gene in three patients with Leber congenital amaurosis 1.7–3.3 years after they had received their initial subretinal injection indicate that readministration is both safe and efficacious after previous exposure to the vector [9]. Further work is warranted to characterize the safety and efficacy of readministration of gene products.

## Drug Administration

Many varied delivery methods for gene therapy or stem cell-based therapeutics exist. In this section, we will review the following methods: systemic, topical, transscleral, anterior chamber, intravitreal, subretinal, and suprachoroidal.

Systemic delivery has the advantage of being minimally invasive; however, it has an multiplicity of infection. some viral inactivation may occur, and safety concerns such as promoter control and widespread integration may exist. Systemic delivery is inappropriate for cell-based therapies.

Topical delivery is minimally invasive, but also minimally effective due to the very low levels of transduction through conjunctival and corneal epithelia and the lack of transduction to the posterior pole. The use of collagen shield may aid enhancement. Topical delivery is inappropriate for cell-based therapies.

Transscleral and transcorneal deliveries are minimally invasive as well, but suffer from low transduction efficiency. Iontophoresis is an established methodology in drug delivery that is likely to be less effective for viral vectors. Transscleral and transcorneal deliveries are inappropriate for cell-based therapies.

Delivery via the anterior chamber is minimally invasive with some transduction effect on the trabecular meshwork endothelium, corneal endothelium, ciliary body endothelium, and iris epithelium. No transduction to the posterior segment is achieved by this delivery method, rendering it inappropriate for cell-based therapies.

Intravitreal delivery is minimally invasive and has become a standard of care in the vast majority of retinal practices with the introduction of anti-VEGF intravitreal injections. Good transduction of the ciliary body epithelium is achieved. Stem cells introduced by this delivery method may proliferate, possibly leading to epiretinal membrane formation.

Standard subretinal delivery is an invasive procedure, requiring a vitrectomy with a posterior retinotomy. Excellent transduction of photoreceptors and RPE can be achieved, and this methodology is the current standard of care for gene delivery. Disadvantages of this delivery method include unpredictable bleb development and unpredictable efflux of product with a posterior retinotomy, as well as possibly higher complication rates.

Subretinal delivery can also be accomplished via an ab externo entrance. No vitrectomy or retinotomy is required. This method has a steeper surgical learning curve compared to the standard subretinal delivery method and is not validated at this time.

The suprachoroidal delivery method is minimally invasive and conceivably can be performed as a procedure in the clinic. Excellent transduction of the choriocapillaris and choroid can be achieved. Though this method is appropriate for gene and cell-based therapies, its validity remains to be investigated further.

## References

1. Pellegrini G, Golisano O, Paterna P, Lambiase A, Bonini S, Rama P et al (1999) Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. *J Cell Biol* 145(4):769–82
2. Dua HS, Azuara-Blanco A (2000) Limbal stem cells of the corneal epithelium. *Surv Ophthalmol* 44(5):415–25
3. Nishida K, Yamato M, Hayashida Y, Watanabe K, Yamamoto K, Adachi E et al (2004) Corneal reconstruction with tissue-engineered cell sheets composed of autologous oral mucosal epithelium. *N Engl J Med* 351(12):1187–96
4. Nakamura T, Kinoshita S (2003) Ocular surface reconstruction using cultivated mucosal epithelial stem cells. *Cornea* 22(7 suppl):S75–80
5. Algreve PV, Gouras P, Dafgard Kopp E (1999) Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. *Eur J Ophthalmol* 9(3):217–30
6. Kaplan HJ, Tezel TH, Berger AS, Del Priore LV (1999) Retinal transplantation. *Chem Immunol* 73:207–19
7. Food and Drug Administration Center for Biologics Evaluation and Research. Cellular, Tissue and Gene Therapies Advisory Committee. June 29, 2011. Transcript proceeding by: CASET Associates, Ltd.
8. Stout JT, Francis PJ (2011) Surgical approaches to gene and stem cell therapy for retinal disease. *Hum Gene Ther* 22(5):531–5
9. Bennett J, Ashtari M, Wellman J, Marshall KA, Cycjowski LL, Chung DC et al (2012) AAV2 gene therapy readministration in three adults with congenital blindness. *Sci Transl Med* 4(120):120ra15
10. Burns ME, Baylor DA (2001) Activation, deactivation, and adaptation in vertebrate photoreceptor cells. *Annu Rev Neurosci* 24:779–805
11. Stryer L (1991) Visual excitation and recovery. *J Biol Chem* 266(17):10711–14
12. Tsang SH, Gouras P, Yamashita CK, Kjeldbye H, Fisher J, Farber DB et al (1996) Retinal degeneration in mice lacking the gamma subunit of the rod cGMP phosphodiesterase. *Science* 272(5264):1026–29
13. Yarfitz S, Hurley JB. Transduction mechanisms of vertebrate and invertebrate photoreceptors. *J Biol Chem.* 1994;269(20):14329–32.

14. Arshavsky VY, Lamb TD, Pugh EN, Jr (2002) G proteins and phototransduction. *Annu Rev Physiol* 64:153–87
15. Fung BK, Hurley JB, Stryer L (1981) Flow of information in the light-triggered cyclic nucleotide cascade of vision. *Proc Natl Acad Sci, USA* 78(1):152–6
16. Lagnado L, Baylor D (1992) Signal flow in visual transduction. *Neuron* 8(6):995–1002
17. Tsang SH, Gouras P (1996) Molecular Physiology and Pathology of the Retina. In: Tasman W, Jaeger EA (eds) *Duane's Clinical Ophthalmology*, JB Lippincott, Philadelphia