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Pluripotent Stem Cell-Based Organoid Technologies for Developing Next-Generation Vision Restoration Therapies of Blindness

AU2 ▶

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Abstract

Blindness, associated with death of retinal cells at the back of the eye, is caused by a number of conditions with high prevalence such as glaucoma, age-related macular degeneration, and diabetic retinopathy. In addition, a large number of orphan inherited (mostly monogenic) conditions, such as retinitis pigmentosa and Leber Congenital Amaurosis, add to the overall number of patients with blinding retinal degenerative diseases. Blindness caused by deterioration and loss of retina is so far incurable. Modern biomedical research leveraging molecular and regenerative medicine approaches had a number of groundbreaking discoveries and proof-of-principle treatments of blindness in animals. However, these methods are slow to be standardized and commercialized as therapies to benefit people losing their eyesight due to retinal degenerative conditions. In this review, we will outline major regenerative medicine approaches, which are emerging as promising for preserving or/and restoring vision. We discuss the potential of each of these approaches to reach commercialization step and be converted to treatments, which could at least ameliorate blindness caused by retinal cell death.

Keywords: blindness, retina, organoids, photoreceptors, vision, retinal degeneration, cell therapy

Introduction

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REGENERATIVE, CUTTING-EDGE surgical and molecular medicine treatments, and personalized medicine approaches are viewed as new wave of therapies to treat **un-
durable** diseases and even aging.¹⁻⁴ Among those therapies are stem/cell and gene therapy approaches,^{2,5-8} monoclonal antibodies (MABs),⁹ RNA, microRNA (miRNA), and DNA-focused therapies for suppressing dominant negative alleles, and aberrant splicing,^{10,11} neuroprotective, and immunomodulatory treatments for controlling inflammation and cell death,¹²⁻¹⁴ whole-eye transplantation,¹⁵ fetal retina transplantation,¹⁶ optogenetics,¹⁷ genome editing *in vivo*,¹⁸⁻²⁰ and even induced tissue regeneration.²¹ These methods are emerging as very promising and even revolutionary ways of rebuilding and restoring degenerating human retina in the near future. However, none is being used yet as an established reliable therapy for restoring vision. Retinal

organoids provide yet another promising approach for rebuilding retina in patients with advanced retinal degeneration and also serve as replenishable source of retinal progenitors for replacement and neuroprotective strategies.^{23,24} In this study, we will outline and review this work, focused on commercialization of retinal organoid technologies, and compare with other approved and emerging vision restoration technologies.

Restoring vision caused by cell death of retinal neurons, including retinal pigment epithelium (RPE), photoreceptors (PRs), and retinal ganglion cells (RGCs) is a highly unmet and urgent clinical need, requiring new ideas and approaches.²⁵ The goal is to design new drugs, biologics, and ocular delivery devices to restore or preserve vision in millions of people by leveraging new and promising regenerative medicine therapy findings.²⁶

Retinal degeneration has many causes, which are mostly genetic but sometimes systemic [eg, diabetic retinopathy (DR)

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FI ► AU5 ► and potentially dry AMD] (Fig. 1). Only few technologies/treatments have been commercialized so far to address this need.

Established technologies approved for investigational or commercial clinical use. There are only few promising commercialized therapies, which are helping people with retinal degenerative diseases. Among them is application of anti-vascular endothelial growth factor (VEGF) MABs, or MABs (for wet form of macular degeneration) to suppress neovascularization in the eye.²⁷⁻²⁹ Several companies produce anti-VEGF MABs (drugs) [Avastin (bevacizumab), Lucentis (ranibizumab), and Eylea (aflibercept)]. Monthly intraocular injections of these drugs work well to suppress neovascularization and loss of vision. In addition, in the last 5-7 years, gene therapy technologies for monogenic retinal degenerative conditions gained prominence mostly due to successful work

in RPE-65 patients (Leber's Congenital Amaurosis).³⁰⁻³³ This work was successfully tested in clinical trials³⁰ and commercialized as Luxturna, or voretigene neparvovec-rzyl, product (Spark Therapeutics) based on the work of Drs. Jean Bennett, Albert Maguire, and their team.^{34,35} However, compared to anti-VEGF MAB injections (which are relatively cheap, between \$100 and \$2,000/injection) and can be used in all AMD and DR patients (not individualized therapy), gene therapy treatments require development of individualized costly therapy for each small cohort of patients. Therefore, although both approaches are promising, one of them (MABs) shows straightforward commercialization potential, while the other is very expensive (\$425,000 per eye, or \$850,000 per patient), yet, very promising as well. Similar or higher cost may be expected for other diseases, which are potentially good candidates for gene therapy treatments. While these therapies have been already commercialized and reimbursement or at least the approximate cost worked out, a number of stem cell-based treatments are at the investigational stage. These therapies will face the challenges of developing novel reimbursement strategies for commercialization as products.^{36,37} These therapies include human pluripotent stem cell (hPSC)-derived RPE transplantation for dry form of AMD (NCT01345006, Ocata/Astellas; NCT02286089; Biotime/LCTX),³⁸⁻⁴¹ adult RPE-derived RPE,⁴²⁻⁴⁴ RGX-314 gene therapy drug for wet AMD (NCT03066258),⁴⁵ epiretinal grafts of fetal retinal progenitors for retinitis pigmentosa (RP) patients⁴⁶ (NCT03073733; jCyte),

RPE patch technologies⁴⁷ (eg, NCT02590692; Regenerative Patch Technologies), and a number of other therapies.^{2,7} Cell replacement, tissue/ocular niche repair, and immunomodulatory/neuroprotective mechanisms were proposed as mechanisms behind the efficacy of these treatments.^{2,12,40} RPE cells (the "drug") can be mass-produced, stored, and injected in large cohorts of patients with dry AMD, which in turn makes this therapy potentially easier to commercialize than gene therapy for RPE-65. Likewise, manufacturing of retinal progenitors for delivery into the epiretinal space has been worked out.⁴⁸ Compared to biomufacturing retinal cells, scaling up biomufacturing of three-dimensional (3D) biologics such as RPE sheets^{47,49} is more challenging. The sheet seems clinically very promising, yet, hard to transplant into the subretinal space without specialized skills and impossible to store and inject like MAB-based drugs. The injectable biologics, delivered intravitreally, clearly requires less skills, which contributes to the cost of therapy as well as reproducibility. Commercializing 3D biologics is expected to be more expensive and presents more challenges for developing reimbursement strategies. At the same time, both therapies (injectable biologics for RP and subretinally delivered 3D sheets for RP/AMD) have their unique therapeutic niches.² The only other vision restoration approach, which is neither biologic nor small-molecule-based, that has been commercialized is represented by several neuroprosthetic devices (eg, ARGUS-II and similar devices).^{50,51} The projected reimbursement cost for ARGUS-II therapy is about \$150,000 per patient, which is costly for insurance companies but is within the reimbursement range and enables straightforward commercialization. The low resolution of such devices, the need for precise surgical placement (mandatory for positive outcomes), and sophisticated design leave a lot of room for improvements, but with advances of new biomaterials and electronics placing of more pixels/inch (to enable much better resolution of vision) seems feasible.^{52,53} In the market, where hardly anything works for people suffering from devastating blindness, this is already a big leap forward.

Emerging technologies

There are a large number of emerging technologies, which are promising in animal studies, but have not found a path to the clinic yet. The efficacy, safety, and the likely average

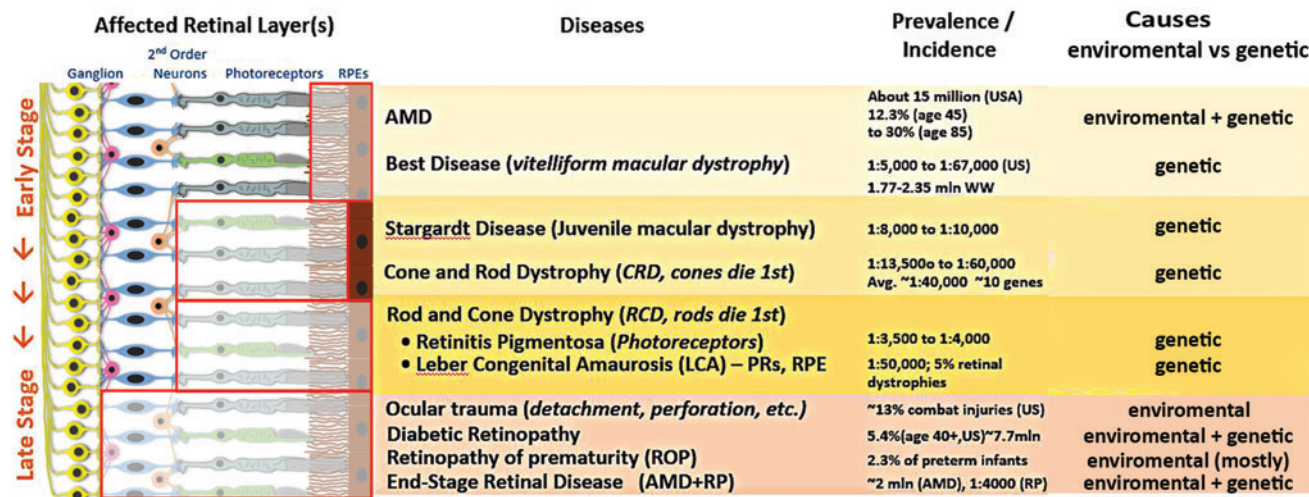


FIG. 1. Conditions associated with retinal degeneration and loss of vision. Color images are available online.

selling price (ASP)^{36,54} are the main determining factors, which impact the decision for commercializing these therapies. The ASP is directly impacted by the cost of biomanufacturing^{55,56} and the number of patients, among other things, who can benefit from this therapy, to make these treatments sustainable (cost-effective) and remove commercialization barriers.^{36,57} For example, if a therapy shows signs of promise but the ASP per patient is close to \$1,000,000 and the number of patients with this condition is very small, commercialization of such technology and converting it to a “product” for treating these patients may be challenging. This is because it may not find a reimbursement strategy to cover the high cost of producing this biological drug. The path to investigational new drug (IND) approval by Food and Drug Administration (FDA) is long and costly,⁵⁸ and if the number of patients for an approved IND is small, the treatment may not be sustainable.

Injectable and storable therapies aimed at suppressing dominant negative alleles and aberrant splicing,^{59–62} also neuroprotective compounds and immunomodulatory treatments,^{12–14,63–66} aimed at abating inflammation seem to be more feasible for commercialization. Therapies similar to Spinraza (Nusinersen, from Biogen) for suppressing spinal muscular atrophy in young children (incidence ~1:6,000 to 1:10,000 children) and delivered via the intrathecal injection are needed in the ocular space⁶² because of its simplicity, reproducibility, and storable/replenishable nature of biologic drug. The drug is an antisense oligonucleotide and modulates the alternative splicing of the *SMN2* gene, functionally converting *SMN2* (paralogous gene) into *SMN1* gene (mutant in SMA patients), enabling translation of functional SMN1 protein in spinal motor neurons. The same logic of drug development and delivery (injection into the vitreous space) may be applied to developing therapies for patients with autosomal dominant retinitis pigmentosa (adRP). Injection into the vitreous leads to little-to-no systemic exposure because of the blood-ocular barrier, and is easy to do because anti-VEGF injections became a routine procedure. adRP is a heterogeneous group of RD diseases, with more than 25 genes known to cause adRP.⁶⁷ While the prevalence of RP is ~1:4,000, 25%–30% of RP cases are caused by adRP,⁶⁷ which is a lot of patients. Allele-specific suppression of dominant-negative (gain-of-function) rhodopsin mutation with allele-specific oligonucleotides (ASOs) targeting mutant rhodopsin messenger RNA (mRNA) with P23H mutation is feasible, slows PR degeneration, and preserves PR function.⁶¹ Furthermore, position-dependent chemical modifications to the ASO enable selectivity between the mutant and the wild-type alleles, making this a reliable and viable therapy.⁶⁸ Even with a small size of cohort of patients, the storable injectable nature of the biologic drug, combined with ease, cost, and reproducibility of biomanufacturing (oligonucleotide, off-the-shelf drug) make the development of this therapy to the market feasible from the investment point of view and also due to straightforward reimbursement strategies.

Cell-based injectable therapies (RPE transplantation,³⁸ epiretinal grafts⁴⁶) may have easier path to commercialization as “off the shelf” storable treatments aimed at large cohorts of patients (AMD and RP, respectively). Yet, even injectable biologics face with commercialization challenges.⁶⁹ *In vivo* genome editing^{70–72} seems promising and may be injectable, yet, the projected cost of such therapy is hard to estimate. In addition, such therapy needs to be ad-

ministered very early and before the onset of RD and the onset of gliosis. Modulating miRNAs *in vivo* may be productive for ameliorating RD (discussed in Baker and Flannery⁷³) and companies are doing preclinical R&D work demonstrating the feasibility of using miRNAs or miRNA inhibitors as injectable therapies.⁷⁴ There are 2 interesting retinal therapies, which (although seem to be at very early discovery stage) have a potential to revolutionize the way how we treat blindness. These are whole-eye transplantation¹⁵ and induced tissue regeneration.²¹ Whole-eye transplantation promises to introduce a totally new human eye without mutations carrying blinding retinal mutations. This approach is suitable for monogenic recessive RP and Leber Congenital Amaurosis diseases, and for slowly progressing AMD, but not systemic diseases such as DR, unless in combination with other drugs addressing DR. Connectivity of newly introduced eye with the brain areas responsible for processing of the visual information needs to be reestablished. However, promising preclinical work on RGC axonal elongation makes this task potentially feasible.^{74–78} The second approach is focused on inducing retinal tissue regeneration, which includes PRs, other retinal neurons, RPE, and RGCs.^{22,79,80} This approach promises to regenerate the lost retinal cells *in vivo* and without transplantation. The approach is based on intraocular injection of small molecules causing partial dedifferentiation of remaining retinal cells *in situ*. This is expected to induce the controlled exit of cells (without inducing tumorigenesis) from postmitotic state back into mitotic state to replenish the cells lost due to RD or trauma. The induced tissue regeneration may be a promising approach for a number of slowly progressing retinal degenerative diseases (eg, RP, AMD), yet, the safety question must be thoroughly addressed to prevent inducing tumorigenesis with genes known to control chromatin plasticity.^{81,82} Those RD diseases, which require short-distance connectivity for retinal repair (eg, RPE and PR regeneration) seem to be more amenable to treatments at the moment because of the challenges of restoring long-distance connectivity. Neither of these approaches has been commercialized yet.

Optogenetic techniques (channelrhodopsin and similar approaches^{17,73,83–85}) seem very promising for vision restoration. They are based on introducing the light-sensing molecules into RD retina with completely degenerated PRs and can be injectable (intraocular or subretinal delivery). Optogenetics carries a promise of restoring light sensitivity in patients with advanced RD by enabling the surviving retinal cells other than PRs to respond to light. Among all other therapies of blindness, optogenetics stands apart as one of the truly vision restoration therapies, promising to restore light sensitivity in retina with no surviving PRs. The quality of vision, which may be regained after this therapy, is yet unknown and remains to be tested. PR-less retina (with only 2nd order neurons or/and RGCs responding to light) may provide signals, which may or may not be interpreted as vision to brain. However, recent progress in development of this approach is encouraging.⁸⁶

Retinal organoids technologies and the ability to derive human retinal tissue (resembling human fetal retinal tissue) in a dish from hPSCs brought a lot of promise to regenerative medicine experimental therapies focused on restoring vision.⁸⁷ Retinal organoids undergo self-formation when hPSCs are induced to differentiate (with various techniques and methods)

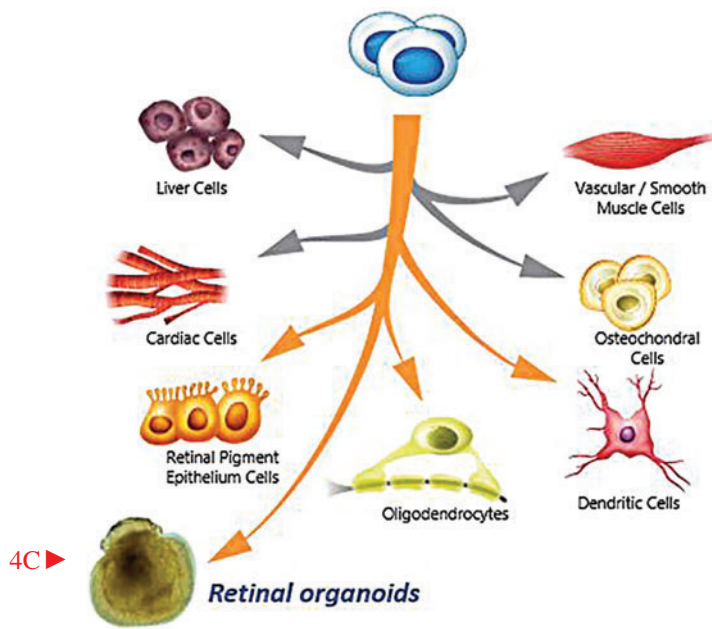


FIG. 2. Differentiation and self-determination of retinal organoids from hPSCs in a dish. hPSC, human pluripotent stem cell.

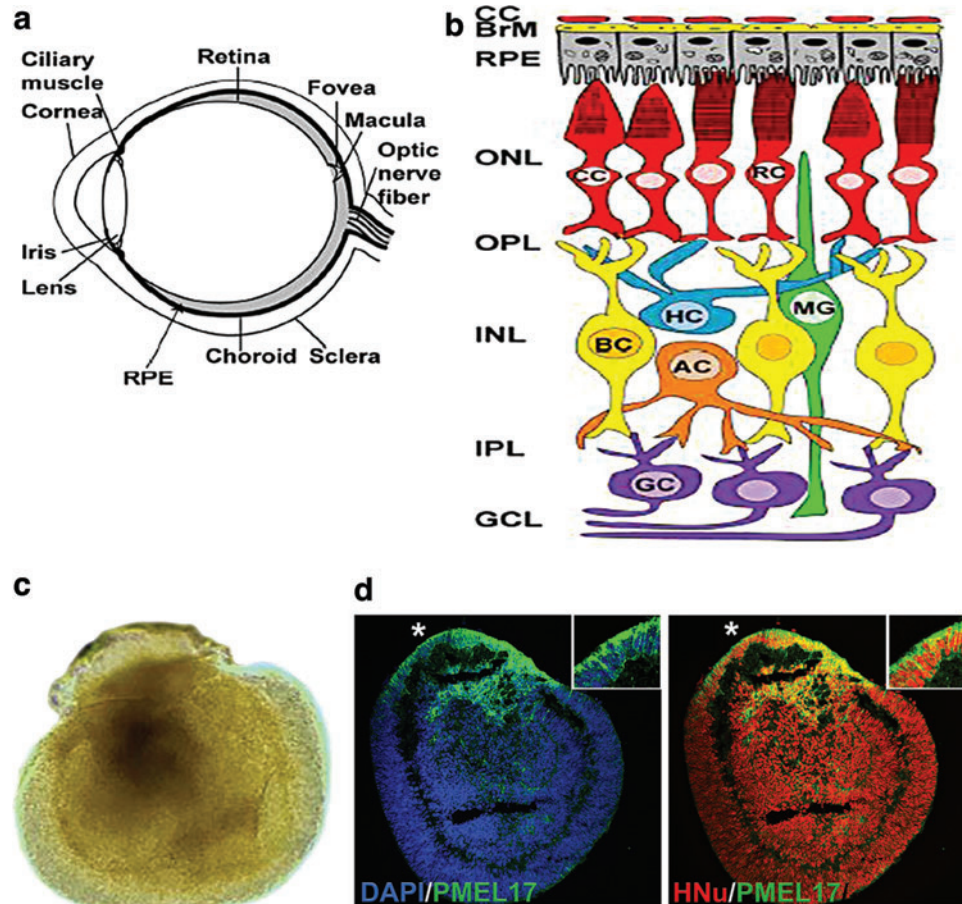
F2 ▶ toward neural and retinal lineage (Fig. 2).^{24,88–91} Large-scale biomanufacturing of retinal organoids can be developed at a relatively inexpensive cost.^{24,92,93} Young retinal organoids (weeks 8–12 after induction) carry all types of retinal cells and layers, typical for developing human fetal retina. Just like in

human fetal retinal tissue, retinal organoids carry a developing layer of PRs (which quickly separates in a separate, outer neuroblast-like layer), also 2nd order neurons/progenitors of retinal interneurons, and RGCs (which, together, separate into inner neuroblast-like layer).²⁴ The only exception seems to be RPE, which is always present in developing human fetal retina, but may be either completely absent⁹¹ or present as patches²⁴ (depending on the method) and still does not cover the whole neural retina (Fig. 3d', d''). Compared to human fetal retina, hPSC-derived retinal organoids carry no ethical baggage associated with clinical application of human fetal retina or retinal cells, and provide replenishable source of human retinal cells and retinal tissue for retinal therapies aimed at rebuilding degenerated retina and slowing down vision loss.

In the market niche (blindness caused by retinal cell death) (Fig. 1), which urgently needs new safe, effective, and commercializable technologies, hPSC-derived retinal organoids may facilitate the development of treatments to address both early and late stages of RD and vision loss (Fig. 4). Lineage Cell Therapeutics, Inc. [supported by National Eye Institute (NEI) funding] and other teams⁹² have recently demonstrated the ability of human retinal tissue derived from hPSC-retinal organoids to cause vision improvements in blind immunocompromised rats, developed by Dr. Seiler.⁹⁴ (Fig. 5). This technology is a logical continuation of a 30 years work pioneered by Drs. Aramant, Dr. Seiler^{16,95–99} and independently by other groups (eg, Mark Humayun),¹⁰⁰ focused on introducing retinal tissue, rather than dissociated retinal cells, into the subretinal space of an eye with advanced RD and complete PR cell death. Similar to neuroprosthetic and optogenetic approaches,

◀F3
◀F4
◀F5

AU19 ▶ **FIG. 3.** hPSC-retinal organoid similarity with developing human fetal retina. (a) Schematic diagram of a human eye. (b) Schematic wiring diagram of the mammalian retina. (c) hPSC-derived retinal organoid (10-week-old). (d', d'') Immunohistochemistry analysis of human retinal organoid stained with melanin-specific antibody (PMEL-17, green)/counterstained with nuclei stain (DAPI (d'), and with PMEL-17+human nuclei-specific antibody (HNu, red) (d''), demonstrating accumulation of RPE cells mostly on one side of retinal organoid, with occasional stretch of PMEL-17[+] RPE cells on the side. RPE, retinal pigment epithelium. Color images are available online.



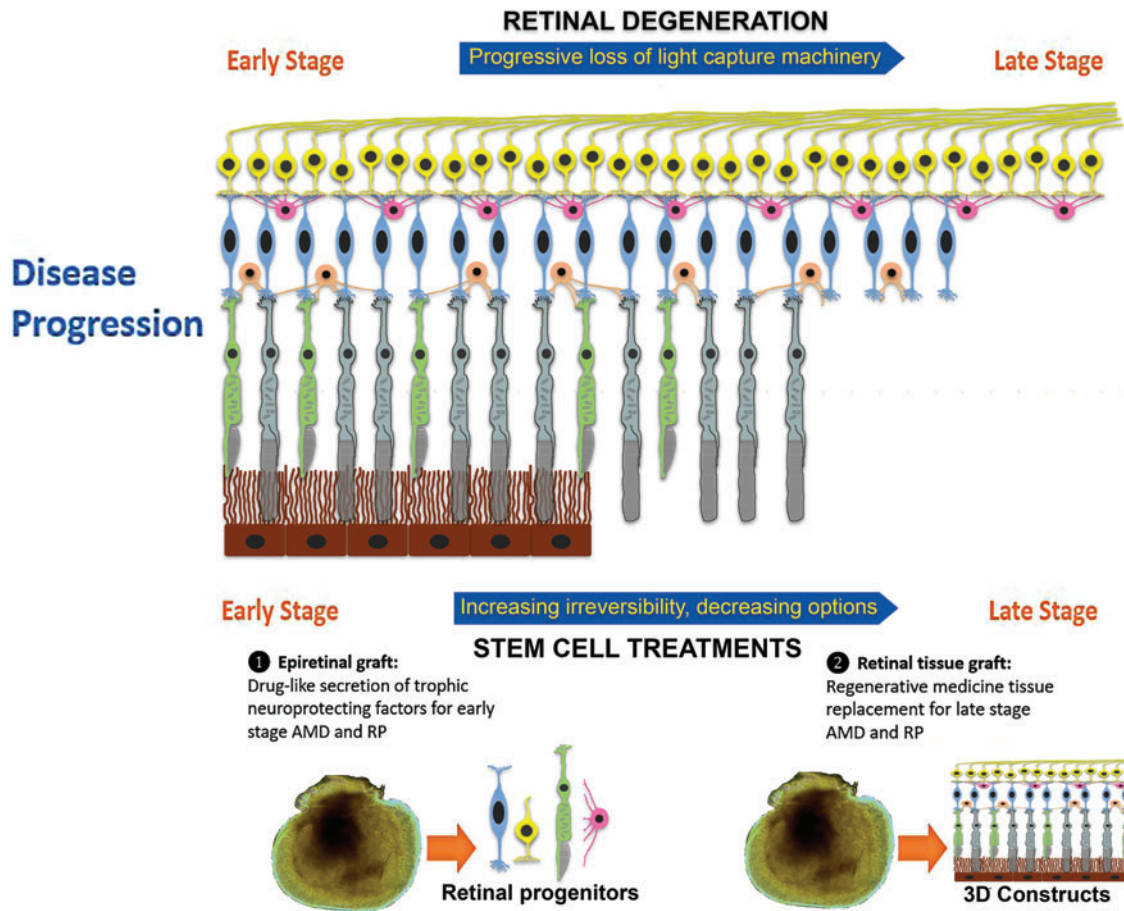


FIG. 4. Early- and late-stage retinal diseases, which may be amenable to treatments, designed from hPSC-retinal tissue. Color images are available online.

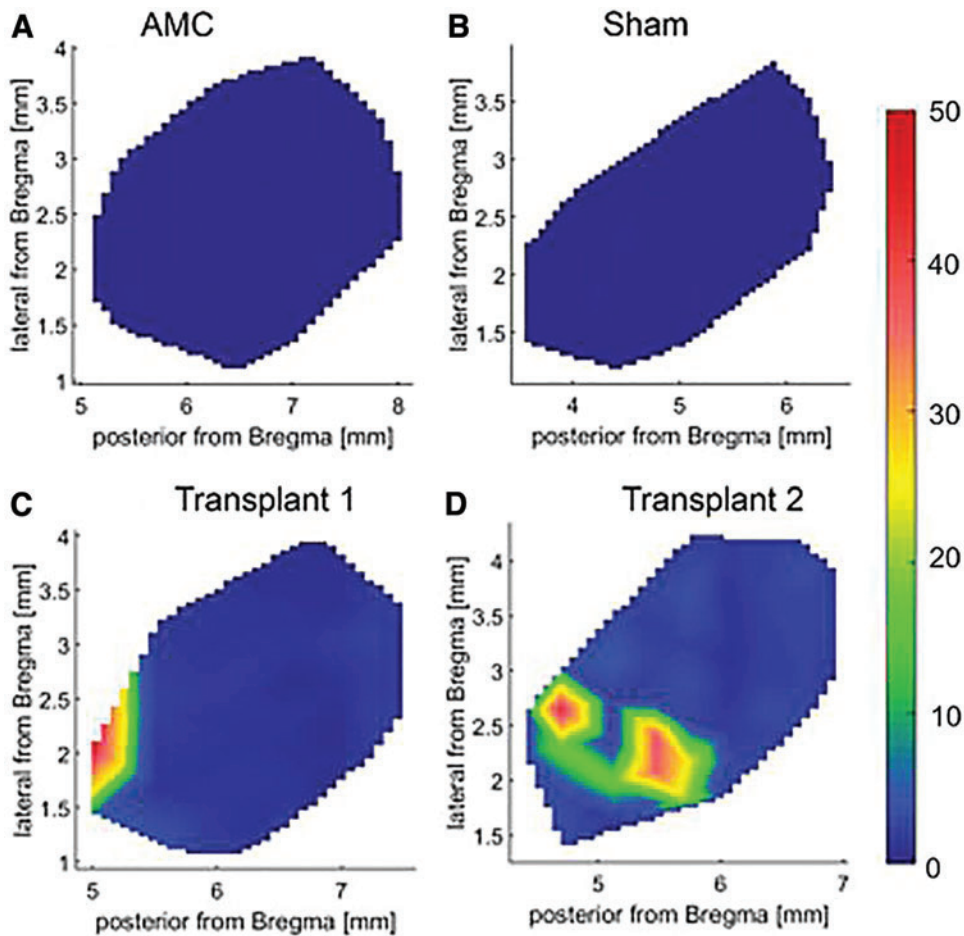


FIG. 5. The recording from the superior colliculus after transplantation of hPSC-retinal tissue into the subretinal space of blind immunodeficient rats (6 months after the surgery). Color images are available online. ◀AU20

transplanting human retinal tissue is one of the few emerging technologies, which enable restoration of visual perception/rudimentary vision in an eye with profound/complete blindness. This technology addresses the need of patients with advanced RD conditions, whose retina is already beyond the stage of repair. The transplantation methods in a large eye (cat)⁹³ demonstrate feasibility of establishing axonal and synaptic connectivity between the graft (hPSC-retinal organoids) and the recipient retina, and may be a good start for developing this technology toward eventual clinical applications in patients with severe vision loss and terminal RD stage. Although clearly further preclinical work is needed, one may expect this technology to make a difference for people with terminal blindness, especially if larger hPSC-retinal grafts are introduced into subretinal space in a large eye. In relation to developing larger flat sheets of hPSC-retina the work from Dr. Larry Rizzolo is especially noteworthy, as it outlines the potential path forward to solving the spherical geometry of retinal organoids, preventing efficient coculture with RPE.^{101,102} The size of such bioprosthetic graft is clearly less of an issue in experimental animal model with much smaller eye size such as a rat.⁹²

Another potentially promising application of retinal organoid technologies is the ability of developing neuroprotection strategies in the ocular space similar to that, which is already being tested successfully in clinical trials by^{103,104} company. jCyte used human fetal retinal cells derived and expanded from procured human fetal retinal tissue as a starting material for demonstrating promising efficacy and safety⁴⁶ data in patients with RP, which enabled it to enter into licensing and commercialization agreement with Santen Pharmaceutical. Retinal tissue derived from hPSC does not have the strict ethical and supply restrictions of aborted fetal retinal tissue and therefore may be a good alternative to procured human fetal retina for delivering neuroprotection into the eye. To this point, we tested the safety of this approach in 3 large eyes of animal models [normal cats without RD (5 weeks),⁹³ *CRX*^{+/-} cats (3 months in the ocular space), and *PDE6A*^{-/-} dog (2 months in the ocular space)]. **SF1 ▶** Supplementary Fig. S1, which is a RetCam image, shows the presence of hPSC-retinal organoids in the vitreal space of a *PDE6A*^{-/-} dog. No retinal inflammation was observed, which is critical and enables further development of this approach toward potential clinical applications. Although another useful application of hPSC-retinal organoids (disease modeling) was not discussed here because of our focus on biologic therapies, young retinal organoids (~weeks 6–16) present a useful model for interrogating early steps of human retinal development.^{90,105–108} As retinal organoids mature in culture, we and others reported loss of RGC and INL neurons (inner retina lamination) by about 6 months in culture,^{109–111} while PR layer is preserved. This enables modeling certain but not all aspects of retinal biology and RD diseases,^{112,113} which are not/less dependent on RPE. Developing long-term planar cocultures of 3D retinal organoids and RPE will enable screening for drugs modulating degeneration of PR-apical RPE niche, as many, if not most, RD diseases originate in the outer segments-apical RPE.

Conclusions

In the large number of new and emerging technologies based on molecular and regenerative medicine, retinal

organoid-derived therapies could potentially address both early (epiretinal graft neurotrophic effect) and late stage (cell replacement) diseases. As with other promising approaches, further preclinical work and refinement of retinal organoid technologies are needed to develop them toward clinical trials and commercialization.

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Author Disclosure Statement

No competing financial interests exist.

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Supplementary Material

Supplementary Figure S1

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