Autologous stem cell therapy for inherited and acquired retinal disease

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The mammalian retina, derived from neural ectoderm, has little regenerative potential. For conditions where irreversible retinal pigment epithelium or photoreceptor cell loss occurs, advanced techniques are required to restore vision. Inherited retinal dystrophies and some acquired conditions, such as age-related macular degeneration, have a similar end result of photoreceptor cell death leading to debilitating vision loss. These diseases stand to benefit from future regenerative medicine as dietary recommendations and current pharmacologic therapy only seek to prevent further disease progression. Cell-based strategies, such as autologously derived induced pluripotent stem cells, have come a long way in overcoming previous technical and ethical concerns. Clinical trials for such techniques are already underway. These trials and the preceding preclinical studies will be discussed in the context of retinal disease.

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Degenerative eye disorders caused by inherited gene mutations lead to progressive and irreversible vision loss. Dietary recommendations for some conditions may slow disease progression but fail to completely halt vision loss or restore lost sight. Vision is essential for most activities of daily living and degeneration of the retinal pigment epithelium (RPE) or photoreceptor cell layer can lead to extreme visual impairment and blindness. As such, the elderly were found to fear blindness more than any illness besides cancer [1].

The neurosensory retina is made of multiple cell layers that together transduce light stimuli into action potentials [2–4]. The outer nuclear layer (ONL) is predominantly comprised of rod and cone cell bodies. These synapse into intermediate cells that reside in the inner nuclear layer. The visual signal is then transmitted to neuronal cells, whose cell bodies reside in the ganglion cell layer. The ganglion cell layer axons relay the visual signal to the brain through the optic nerve. The RPE is a monolayer of hexagonally shaped cells that lie adjacent and posteriorly, to photoreceptors. The RPE is necessary for maintaining the blood–retina barrier and plays an essential role in the phagocytosis of photoreceptor outer segments and recycling of vitamin A derivatives. Therefore, loss of RPE can lead to subsequent photoreceptor degeneration.

A variety of monogenic changes are known to cause inherited retinal dystrophies, with a similar end pathway of photoreceptor cell death [5]. For these conditions, stem cell transplantation offers a promising therapeutic modality. Embryonic stem cell (ESC)-derived RPE has been demonstrated to be transplantable and functional in animal models of retinal degeneration [6]. However, this method poses ethical concerns. Induced pluripotent stem cells (iPSCs) are unique as they are reprogrammed from adult somatic cells, allowing investigators to cater the development of cell lines towards a desired cell type. Thus, these autologously derived cells, in principle, should not elicit an immune response, as the patient's own cells are used, potentially eliminating the need for

immunosuppression [7–13]. However, the disease-causing mutation is still present, so in order to generate healthy cells, gene-editing techniques can be used to repair monogenic, patient-specific molecular defects. These modified cells are then differentiated to generate a particular cell type, and subsequently seeded into their respective anatomic niche.

Like ESCs, iPSCs self-propagate and are pluripotent, and thus can be used for tissue-specific cell regeneration. Originally, iPSCs were created by Yamanaka through retroviral transduction of mouse dermal fibroblasts with only four transcription factors *Oct4*, *Sox2*, *Klf4* and *c-myc* [14]. Since then, iPSCs have been generated for many types of tissues and species. There are well-described culture protocols to induce iPSC cells that can be created from adult cells through the viral transduction of transcription factors [15,16]. Since Yamanaka's method of creating iPSCs, different teams have established protocols to induce iPSCs through an excisable single lentiviral vector [15,17], a single multiprotein expression vector [18], and a nonintegrating episomal vector [15,19]. Another method to induce iPSCs from primary human fibroblast cultures is through *in vitro* transcribed mRNA of the four reprogramming factors originally used by Yamanaka [20]. Other teams reprogrammed somatic cells with direct transfection of recombinant proteins [21] or chemically induced mouse somatic cells with small-molecule compounds [22]. There are methods to induce iPSC cells that are xeno-, virus-, c-Myc- and feeder-free [15,23–24]. Also, several laboratories created differentiation methods and protocols able to differentiate iPSC cells into retinal ganglion cells, retinal pigment epithelial cells and photoreceptor cells [2,25–28].

The eye is relatively immune privileged and the inherent immunosuppressive properties of iPSC RPE cells may contribute to their successful transplantation [11]. Normal RPE cells can suppress T-cell activation of CD4⁺ and CD8⁺ T cells as part of their role in maintaining the immune privilege site of the eye. This ability can also be demonstrated in iPSC RPE cells. Like normal RPE cells, cultured iPSC-derived RPE cells are also shown to elaborate TGFβ2, thrombospondin-1 (TSP-1) and other soluble inhibitory factors that inhibit the function of immune cells [11].

Currently, vision research is at the forefront of stem cell research. Ability to monitor *in vivo* changes with noninvasive imaging of the eye, its relative immune-privileged status and accessibility are major reasons for success. This favorable environment in addition to the advantages of autologous-based techniques, make iPSC derived cells a potentially great source of treatment. This review will discuss the applications of autologous stem cell therapy in the context of retinal disease, specifically in preclinical and clinical studies, and the challenges that persist.

At the bench: *in vitro* & *in vivo* approaches

Human fibroblasts can be transduced with lentiviral vectors into undifferentiated iPSCs. Pluripotency of the human iPSC cell line was demonstrated via immunochemistry in teratoma assays: all three germ layer tissue types were present. iPSCs can then undergo standard culture techniques to enhance differentiation into retinal ganglion cells, photoreceptor cells and RPE cells depending on how they are stimulated [16,29–32]. Patient-specific iPSCs that undergo differentiation into RPE cells provide an arena for the clinical translatability of this strategy to retinal disease. iPSCs-derived RPE cells were analyzed with electron microscopy and showed typical features such as apical microvilli on their outer surface. RPE-specific markers were expressed in the cultured iPSC RPE cells [33]. These cells also maintained their phagocytic ability, an important function for RPE cells which allows them to phagocytose the outer segments of photoreceptor cells. Carr *et al.* demonstrated that iPSC-derived RPE cells can be safely injected in the subretinal space of Royal College of Surgeons (RCS) rats [33]. The RCS rat is a model of *MERTK*-associated RP, a gene predominantly expressed in the RPE and important for phagocytosis [3,34]. While the graft cells remained in a differentiated state and exhibited morphological and functional features of RPE cells, they failed to maintain a monolayer configuration 8 days after transplantation [33]. However, the injected dystrophic RCS rats maintained better visual acuity compared with sham injected rats, and maintained the ONL. This study demonstrated ability of the RPE cells *in vitro* and *in vivo* by measuring phagocytic function of the cells after treatment using immunohistochemistry and by measuring retinal function with optokinetic head-tracking in the RCS dystrophic rat, respectively.

Li *et al.* [8] found that iPS-RPE injected into the subretinal space of one eye of a mouse model of retinitis pigmentosa, using the fellow eye as a control (*Rpe65^{rd12}/Rpe65^{rd12}* mice), demonstrated anatomic acceptance and electrophysiological improvement after transplantation. These mutant mice have a defect in RPE65, which impairs regeneration of 11-cis-retinal after phototransduction. Human fibroblasts were transduced with lentiviral vectors into undifferentiated iPSCs which then underwent standard culture techniques to enhance differentiation into RPE

lines. Pluripotency of the human iPSC cell line was demonstrated via immunochemistry in teratoma assays: all three germ layer tissue types were found. After the iPSCs differentiated to RPE cells, they were analyzed with electron microscopy and showed typical features such as apical microvilli on their outer surface. RPE specific markers were expressed in the cultured iPSC RPE cells. RPE cells have a phagocytic property which allows them to phagocytose the outer segments of photoreceptor cells. This is an important function of the RPE and its malfunction leads to disease state. These iPSC were shown via fluorescent microscopy to phagocytose outer segments. The RPE cells were then injected as a suspension into the mice at postnatal day 2 and followed for 6 months. When the injected mice were studied sequentially for 6 months, electroretinogram (ERG) responses showed that retinal function could be restored as evidenced by improvement in the ERG amplitudes when compared with the control eyes [8]. This effect was noted 6 weeks after transplantation. Of note, no tumor was detected in any of the mice injected.

Defects in the *MFRP* gene cause autosomal recessive retinitis pigmentosa in humans [35]. *MFRP* forms a membrane receptor on the RPE cell. *MFRP* mice have a deletion on intron four which leads to a defect in the amino acid present in the *MFRP* protein. These mice lose photoreceptors throughout life and are another preclinical animal model of retinitis pigmentosa. Patient-specific iPSC-RPE cell lines were made from fibroblasts of two patients with *MFRP* defects [35] and, with delivery of human *MFRP* via recombinant adeno-associated virus (AAV) gene therapy, showed recovered pigmentation. Suspensions of treated iPSCs were injected into the subretinal space of *MFRP* mice. Treated mice showed anatomic restoration of the RPE pattern comparable to the morphologic pattern seen in the normal control mice. These transplanted RPE cells maintained tight intercellular tight junctions, as determined by the transepithelial resistance. These junctions are crucial for the maintenance of the blood–retinal barrier.

Another function of the RPE is to help maintain the blood–retinal barrier through intercellular tight junctions. The measurement of transepithelial resistance reflects the integrity of the RPE tight junctions. Functional improvement was noted after injection with these treated iPSCs as shown by an increase in transepithelial resistance measurements as compared with untreated cells. Quantitative autofluorescence in *MFRP* mice shows an increase in punctate and generalized lipofuscin accumulation whereas mice receiving subretinal injections of the adeno-associated virus–*MFRP* vector cells showed significant decrease in the accumulation of lipofuscin and an improved ERG response.

As an alternative to cells in suspension, Kamao *et al.* produced sheets of autologous human iPSCs-RPE [31]. These sheets of human induced pluripotent stem cells (hiPSC)-RPE cells were shown to display a reproducible monolayer with functional tight junctions and a basement membrane structure as assessed by immunohistochemistry. They maintained a transepithelial electrical gradient and produced growth factors such as PEDF and VEGF.

Integration into neural circuitry

Cell-based approaches to studying and potentially treating inherited ocular disease face a variety of technical challenges in addition to ethical issues. One of the main challenges for all types of cell-based interventions will be ensuring functional integration into host retina [3]. Retinal dystrophies, for example, may require photoreceptor cell replacement depending on disease etiology and stage. Transplanted cells in this case must integrate into the existing neural circuitry for proper function. This includes the transplanted cell assuming the correct orientation in the neurosensory retina, with the outer segments juxtaposed to RPE. Complicating this issue, disease processes may alter synaptic modeling which leads to abnormal connections and furthermore, the environmental cues needed for graft cell integration may be altered by the disease process [36].

Despite these obstacles, several encouraging studies documented functional improvement and/or integration with host circuitry [37–42]. Cell transplantation/fusion attempted in various mouse models, such as *Gnat1*^{−/−} [39], the RCS rat [38] and the *rd1* mouse model [37], shows evidence of incorporation into neuronal synapses and improvement in retinal function. These are all rodent models of inherited retinal dystrophies, mutations in *GNAT1* can cause congenital stationary night blindness, and mutations in *MERTK* (RCS model) and *RP1* cause RP in patients. Transplanted *Nrl*-GFP+ rod photoreceptor precursors in *Gnat1*^{−/−} even showed evidence of light responsiveness and projection of information to the visual cortex [39]. Treated animals in this study performed better on a functional task under scotopic conditions that required cognitive processing, namely the visually guided water maze. In a similar fashion, utilizing the RCS rat model, human retinal progenitor cells from fetal neural retina (hRPCs) were delivered by subretinal injection and improved the optokinetic response, which is a surrogate for visual acuity. Superior ONL thickness and corresponding improvements in ERG response suggest that the cells integrated into host retina [38]. For the *rd1* mouse, functional photoreceptors and formed synapses were seen in Eye-wall

c-kit⁺/stage-specific embryonic antigen 1 (SSEA1)-treated retina and resulted in increased a- and b-wave ERG amplitudes [37]. Interestingly, donor postmitotic photoreceptor precursor cells transplanted into murine eyes were found to undergo material transfer of RNA and/or protein between donor and host cells [43].

Specific to iPSCs, Zhong *et al.* found that cultured human iPSCs recapitulated the main steps of retina development that occur *in vivo*, forming 3D retinal cups that contained the major retinal cell types and showed evidence of outer-segment disc formation and photosensitivity [28]. Lamba *et al.* similarly showed that photoreceptors derived from iPSC survived when injected into the subretinal space. Some cells were even detected at the level of ONL, indicating their ability to migrate and integrate into the retina [44]. In a study which compared iPSC- and ESC-derived photoreceptor progenitor cells, a similar efficacy was found. Not surprisingly, the number of engrafted cells correlated with retinal function [45]. Of note, recent attempts to develop clinically compatible methods to manufacture iPSC-derived photoreceptors from adults are reported. Clonally expanded iPSCs were differentiated into photoreceptor precursor cells and injected into severe combined immune deficiency (SCID) mice (immunocompromised), showing no abnormal proliferation or tumor potential in 10 months [46]. Other aspects of the retinal circuitry can be targeted as well, an example being the development of iPSC-derived retinal ganglion cells to approach conditions such as optic neuropathy [47–49].

Host-graft connections are reported in large animal models as well, suggesting clinical feasibility [50,51]. Human ESCs differentiated into a variety of retinal cell types in primates and the resulting photoreceptors even show structured ONLs. Synaptic connections between host and graft cells were identified with immunohistochemistry [51]. Additionally, cultured retinal progenitor cells of pigs were transplanted into a laser-injured allogeneic pig [50]. Expression of transducin, recoverin and rhodopsin in the subretinal space suggested successful differentiation into photoreceptors, with some migration into the retina.

There have been multiple approaches aimed to increase the integration of transplanted cells into host retina [52–54]. Hyaluronan-based injectable hydrogel was found to increase retinal stem cell-derived rod photoreceptor survival in comparison to artificial cerebral spinal fluid, as assessed by histology. The prosurvival effect was via interactions with the CD44 receptor and as such, was not seen in *CD44*^{-/-} mice [52]. Another biodegradable and biocompatible material studied is nanofibrous scaffolds. Nadri *et al.* [53] used conjunctiva mesenchymal stem cells that were combined with a poly-L-lactic acid nanofibrous scaffold and subsequently induced to differentiate. Electron microscopy determined that transplanted cells were spindle shaped and properly oriented in the nanofiber. In an earlier study, Yao *et al.* used poly(lactic-co-glycolic acid) microspheres to deliver MMP2 and retinal progenitor cells to the subretinal space of a *Rho*^{-/-} mouse model. MMP2 breaks down cell adhesion molecules that disrupt graft integration. As a result, it increased the number of graft cells migrating into the degenerated retina [54].

Despite successes seen in small and large animal models, new challenges have been identified. For example, some of the injected graft cells in one study were found to remain in the subretinal space and were detrimental as an immune reaction ensued [40]. It was reported that in the tg(Cpfl1;*Rho*^{-/-}) mouse, which exhibits a paucity of endogenous photoreceptors, grafted rods remain in the subretinal space. These cells undergo differentiation but do not integrate in the neural retina or increase visual function as measured by ERG. This contrasted with *Prom1*^{-/-} mice, wherein mES-derived rods matured into rod photoreceptors, expressed rhodopsin, and partially integrated into neurosensory retina. A corollary to this may be that patients with advanced stages of retinal degeneration, in which little or no photoreceptors are left, may have less potential for incorporating the administered cells.

At the bedside: clinical trials

The translational application of stem cell technology to clinical Phase I/II human trials after over a decade of preclinical research is currently underway [55]. Recent ocular stem cell human trials focus on transplantation of RPE derived from various types of stem cells, including ESCs and hiPSC. Previous RPE transplantation clinical trials set the stage for current stem cell investigations. Allogeneic transplants of RPE cells derived from fetal tissue were first attempted in the 1990s and resulted in frequent graft rejection [56,57]. Autologous RPE cell sheets harvested from the periphery of the eye were subsequently attempted clinically in the early 2000s and long-term vision preservation observed in some patients was at the expense of complex and invasive procedures that carried a high incidence of hemorrhage or retinal detachment [58,59]. These preliminary trials helped to validate the concept of RPE replacement and developed strategies that laid the groundwork for current stem cell technologies.

Autologous iPSC trials

The Riken Center for Developmental Biology in Japan described the first and only clinical report of a patient to receive a subretinal transplant of iPSC-RPE cell sheets [60]. The patient presented with neovascular membranes from polypoidal choroidal vasculopathy and received an autologous hiPSC-RPE cell sheet in September 2014, administered subretinally. Surgical removal of the neovascular membrane was performed prior to the transplant. Notably, serious adverse events were not identified at 25 months of follow-up. The patient did not receive immunosuppressants and showed no signs of rejection. One year after the transplant, best corrected visual acuity was unchanged. Expansion of the graft occurred radially during the follow-up period which suggested graft survival. Furthermore, cross-sectional images of the retina using optical coherence tomography identified areas of high density, possibly indicative of photoreceptor recovery.

It is important for ophthalmologists and physicians to be aware of unregulated stem cell treatments that are currently being offered for vision loss due to retinal degenerations, for which serious complications have been noted. In 2016, we reported a case of severe vision loss due to central retinal artery occlusion in a patient with retinitis pigmentosa following a peribulbar injection of autologous bone marrow stem cells. The patient underwent this procedure in a nonregulated clinical trial center [61]. Several mechanisms are possible in this reported case. Vascular occlusion may have occurred due to an unanticipated injection into a small artery, leading to bone marrow stem cells entering the ophthalmic artery via retrograde flow. The peribulbar injection may also have increased either the ocular and orbital pressure. When this increase exceeds the pressure of the central retinal artery pressure entering the globe, a cessation of blood flow to the retina can occur. Direct contact between the injection needle and the central retinal artery is also possible, but seems to be the least likely [61]. Kuriyan *et al.* [62] reported three patients with age-related macular degeneration who underwent bilateral intravitreal ‘stem cell’ injections at the same clinic and sustained severe vision loss [62]. All six injected eyes in this series developed retinal detachments with proliferative vitreoretinopathy during the observation period. The authors hypothesize that the injected cells may have transformed into myofibroblasts leading to detachment. Additionally, all patients were found to have lens subluxation with zonular weakness, which could possibly be due to trypsin contamination of the preparations or inadequate injection technique. It is important to note that these centers did not adhere to US FDA-regulated scientific procedures and reporting. It is unclear from the reported procedure as to whether these patients even had stem cells injected or simply a mixture of mesenchymal tissue. Unfortunately, such incidents may shake the public’s confidence in the potential of stem cell therapy. It is necessary for all centers to adhere to strict regulatory measures. To reduce severe complications that result from stem cell clinics would require preparations to be based on strong preclinical evidence, safety studies in mammalian models, proper patient counseling and FDA approval. It is also imperative to compare these treatments to the standard of care.

Conclusion & future perspective

Despite significant challenges, autologous cellular therapies are reaching clinical applications in the retina. To date, autologous stem cell clinical studies confirm the presumed advantages of limited immune rejection and decreased need for postoperative immunosuppression. Further studies to validate the safety of therapies prior to efficacy trials will only be achieved through careful planning and regulated clinical trials. Initial data is promising and gives a hope for sight restoration through cellular transplantation.

Executive summary

Background

- To reverse disease process in inherited or acquired retinal disease with cell loss, regenerative medicine is likely needed over other biologic strategies.

At the bench

- *In vitro* and *in vivo* studies suggest future clinical translatability.

Integration into neural circuitry

- Delivery and integration of administered cells into the host retina remains a challenge for all cell-based therapies.

At the bedside

- Human clinical trials are ongoing for various types of stem cell therapies and conditions of the retina.

Authors' contributions

MBL Apatoff, JD Sengillo, EC White and MF Bakhom performed literature searches and wrote the manuscript. AG Bassuk and VB Mahajan assisted in manuscript composition. SH Tsang oversaw all aspects of manuscript preparation and holds final responsibility for contained information and the decision to submit for publication.

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References

Papers of special note have been highlighted as: ● of interest; ●● of considerable interest

- Jorkasky JF. Attitudinal survey of minority populations on eye and vision health and research. *Research America, EyeResearch.org* www.eyersearch.org/pdf/AEVRslides91714.pdf (2014).
- Giacalone JC, Wiley LA, Burnight ER *et al.* Concise review: patient-specific stem cells to interrogate inherited eye disease. *Stem Cells Transl. Med.* 5(2), 132–140 (2016).
- Sengillo JD, Justus S, Tsai YT, Cabral T, Tsang SH. Gene and cell-based therapies for inherited retinal disorders: an update. *Am. J. Med. Genet. C Semin. Med. Genet.* 172(4), 349–366 (2016).
- Wiley LA, Burnight ER, Mullins RF, Stone EM, Tucker BA. Stem cells as tools for studying the genetics of inherited retinal degenerations. *Cold Spring Harb. Perspect. Med.* 5(5), a017160 (2014).
- Hamel C. Retinitis pigmentosa. *Orphanet J. Rare Dis.* 1, 40 (2006).
- **Provides an excellent and highly cited overview of retinitis pigmentosa. Highlights the genetic heterogeneity of the condition and challenges in diagnosis and management.**
- Garg A, Yang J, Lee W, Tsang SH. Stem cell therapies in retinal disorders. *Cells* 6(1), 4 (2017).
- Fu X. The immunogenicity of cells derived from induced pluripotent stem cells. *Cell. Mol. Immunol.* 11(1), 14–16 (2014).
- Li Y, Tsai YT, Hsu CW *et al.* Long-term safety and efficacy of human-induced pluripotent stem cell (iPS) grafts in a preclinical model of retinitis pigmentosa. *Mol. Med.* 18, 1312–1319 (2012).
- Okano H, Nakamura M, Yoshida K *et al.* Steps toward safe cell therapy using induced pluripotent stem cells. *Circ. Res.* 112(3), 523–533 (2013).
- Sugita S. Role of ocular pigment epithelial cells in immune privilege. *Arch. Immunol. Ther. Exp. (Warsz.)* 57(4), 263–268 (2009).
- Sugita S, Kamao H, Iwasaki Y *et al.* Inhibition of T-cell activation by retinal pigment epithelial cells derived from induced pluripotent stem cells. *Invest. Ophthalmol. Vis. Sci.* 56(2), 1051–1062 (2015).
- **Explores the mechanism behind which induced pluripotent stem cells retinal pigment epithelium may contribute to the immune privileged status of the retina. Thorough description of signaling involved in evading immune response.**
- Zamiri P, Sugita S, Streilein JW. Immunosuppressive properties of the pigmented epithelial cells and the subretinal space. *Chem. Immunol. Allergy* 92, 86–93 (2007).
- Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. *Nature* 474(7350), 212–215 (2011).
- Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126(4), 663–676 (2006).
- **Represents one of the major milestones in regenerative medicine. Describes the induction of pluripotent stem cells from adult fibroblasts using factors: Oct3/4, Sox2, c-Myc and Klf4.**
- Alvarez Palomo AB, Mclenachan S, Chen FK *et al.* Prospects for clinical use of reprogrammed cells for autologous treatment of macular degeneration. *Fibrogenesis Tissue Repair* 8, 9 (2015).
- Takahashi K, Tanabe K, Ohnuki M *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131(5), 861–872 (2007).

17. Sommer CA, Sommer AG, Longmire TA *et al.* Excision of reprogramming transgenes improves the differentiation potential of iPS cells generated with a single excisable vector. *Stem Cells* 28(1), 64–74 (2010).
18. Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* 458(7239), 771–775 (2009).
19. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature* 448(7151), 313–317 (2007).
20. Warren L, Manos PD, Ahfeldt T *et al.* Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 7(5), 618–630 (2010).
21. Zhou H, Wu S, Joo JY *et al.* Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* 4(5), 381–384 (2009).
22. Hou P, Li Y, Zhang X *et al.* Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. *Science* 341(6146), 651–654 (2013).
23. Edel MJ, Menchon C, Menendez S, Consiglio A, Raya A, Izpisua Belmonte JC. Rem2 GTPase maintains survival of human embryonic stem cells as well as enhancing reprogramming by regulating p53 and cyclin D1. *Genes Dev.* 24(6), 561–573 (2010).
24. Mclenachan S, Menchon C, Raya A, Consiglio A, Edel MJ. Cyclin A1 is essential for setting the pluripotent state and reducing tumorigenicity of induced pluripotent stem cells. *Stem Cells Develop.* 21(15), 2891–2899 (2012).
25. Eiraku M, Sasai Y. Mouse embryonic stem cell culture for generation of three-dimensional retinal and cortical tissues. *Nat. Protoc.* 7(1), 69–79 (2011).
26. Tucker BA, Anfinson KR, Mullins RF, Stone EM, Young MJ. Use of a synthetic xeno-free culture substrate for induced pluripotent stem cell induction and retinal differentiation. *Stem Cells Transl. Med.* 2(1), 16–24 (2013).
27. Tucker BA, Park IH, Qi SD *et al.* Transplantation of adult mouse iPS cell-derived photoreceptor precursors restores retinal structure and function in degenerative mice. *PLoS ONE* 6(4), e18992 (2011).
28. Zhong X, Gutierrez C, Xue T *et al.* Generation of three-dimensional retinal tissue with functional photoreceptors from human iPSCs. *Nat. Commun.* 5, 4047 (2014).
29. Assawachananont J, Mandai M, Okamoto S *et al.* Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice. *Stem Cell Rep.* 2(5), 662–674 (2014).
30. Hiram Y, Osakada F, Takahashi K *et al.* Generation of retinal cells from mouse and human induced pluripotent stem cells. *Neurosci. Lett.* 458(3), 126–131 (2009).
31. Kamao H, Mandai M, Okamoto S *et al.* Characterization of human induced pluripotent stem cell-derived retinal pigment epithelium cell sheets aiming for clinical application. *Stem Cell Rep.* 2(2), 205–218 (2014).
32. Sonoda S, Spee C, Barron E, Ryan SJ, Kannan R, Hinton DR. A protocol for the culture and differentiation of highly polarized human retinal pigment epithelial cells. *Nat. Protoc.* 4(5), 662–673 (2009).
33. Carr AJ, Vugler AA, Hikita ST *et al.* Protective effects of human iPS-derived retinal pigment epithelium cell transplantation in the retinal dystrophic rat. *PLoS ONE* 4(12), e8152 (2009).
34. Sengillo JD, Justus S, Cabral T, Tsang SH. Correction of monogenic and common retinal disorders with gene therapy. *Genes* 8(2), pii: E53 (2017).
- **Provides an update of current gene therapy clinical trials for inherited retinal disease. Thorough overview of delivery strategies and technical considerations in the personalized medicine approach to inherited dystrophies.**
35. Li Y, Wu WH, Hsu CW *et al.* Gene therapy in patient-specific stem cell lines and a preclinical model of retinitis pigmentosa with membrane frizzled-related protein defects. *Mol. Ther.* 22(9), 1688–1697 (2014).
36. Casaroli-Marano RP, Zarbin MA. Cell-based therapy for retinal degenerative disease. Preface. *Dev. Ophthalmol.* 53, XI–XII (2014).
37. Chen X, Chen Z, Li Z *et al.* Grafted c-kit+/SSEA1– eye-wall progenitor cells delay retinal degeneration in mice by regulating neural plasticity and forming new graft-to-host synapses. *Stem Cell. Res. Ther.* 7(1), 191 (2016).
38. Luo J, Baranov P, Patel S *et al.* Human retinal progenitor cell transplantation preserves vision. *J. Biol. Chem.* 289(10), 6362–6371 (2014).
39. Pearson RA, Barber AC, Rizzi M *et al.* Restoration of vision after transplantation of photoreceptors. *Nature* 485(7396), 99–103 (2012).
40. Santos-Ferreira T, Volkner M, Borsch O *et al.* Stem cell-derived photoreceptor transplants differentially integrate into mouse models of cone-rod dystrophy. *Invest. Ophthalmol. Vis. Sci.* 57(7), 3509–3520 (2016).
41. Warre-Cornish K, Barber AC, Sowden JC, Ali RR, Pearson RA. Migration, integration and maturation of photoreceptor precursors following transplantation in the mouse retina. *Stem Cells Develop.* 23(9), 941–954 (2014).
42. West EL, Pearson RA, Barker SE *et al.* Long-term survival of photoreceptors transplanted into the adult murine neural retina requires immune modulation. *Stem Cells* 28(11), 1997–2007 (2010).
43. Pearson RA, Gonzalez-Cordero A, West EL *et al.* Donor and host photoreceptors engage in material transfer following transplantation of post-mitotic photoreceptor precursors. *Nat. Commun.* 7, 13029 (2016).

44. Lamba DA, Mcusic A, Hirata RK, Wang PR, Russell D, Reh TA. Generation, purification and transplantation of photoreceptors derived from human induced pluripotent stem cells. *PLoS ONE* 5(1), e8763 (2010).
45. Barnea-Cramer AO, Wang W, Lu SJ *et al.* Function of human pluripotent stem cell-derived photoreceptor progenitors in blind mice. *Sci. Rep.* 6, 29784 (2016).
46. Wiley LA, Burnight ER, Deluca AP *et al.* cGMP production of patient-specific iPSCs and photoreceptor precursor cells to treat retinal degenerative blindness. *Sci. Rep.* 6, 30742 (2016).
47. Gill KP, Hewitt AW, Davidson KC, Pebay A, Wong RC. Methods of retinal ganglion cell differentiation from pluripotent stem cells. *Transl. Vis. Sci. Technol.* 3(4), 7 (2014).
48. Parameswaran S, Balasubramanian S, Babai N *et al.* Induced pluripotent stem cells generate both retinal ganglion cells and photoreceptors: therapeutic implications in degenerative changes in glaucoma and age-related macular degeneration. *Stem Cells* 28(4), 695–703 (2010).
49. Tanaka T, Yokoi T, Tamalu F, Watanabe S, Nishina S, Azuma N. Generation of retinal ganglion cells with functional axons from mouse embryonic stem cells and induced pluripotent stem cells. *Invest. Ophthalmol. Vis. Sci.* 57(7), 3348–3359 (2016).
50. Klassen H, Kiilgaard JF, Zahir T *et al.* Progenitor cells from the porcine neural retina express photoreceptor markers after transplantation to the subretinal space of allograft recipients. *Stem Cells* 25(5), 1222–1230 (2007).
51. Shirai H, Mandai M, Matsushita K *et al.* Transplantation of human embryonic stem cell-derived retinal tissue in two primate models of retinal degeneration. *Proc. Natl Acad. Sci. USA* 113(1), E81–E90 (2016).
52. Ballios BG, Cooke MJ, Donaldson L *et al.* A hyaluronan-based injectable hydrogel improves the survival and integration of stem cell progeny following transplantation. *Stem Cell Rep.* 4(6), 1031–1045 (2015).
53. Nadri S, Kazemi B, Eslaminejad MB, Yazdani S, Soleimani M. High yield of cells committed to the photoreceptor-like cells from conjunctiva mesenchymal stem cells on nanofibrous scaffolds. *Mol. Biol. Rep.* 40(6), 3883–3890 (2013).
54. Yao J, Tucker BA, Zhang X, Checa-Casalengua P, Herrero-Vanrell R, Young MJ. Robust cell integration from co-transplantation of biodegradable MMP2-PLGA microspheres with retinal progenitor cells. *Biomaterials* 32(4), 1041–1050 (2011).
55. Ramsden CM, Powner MB, Carr AJ, Smart MJ, Da Cruz L, Coffey PJ. Stem cells in retinal regeneration: past, present and future. *Development* 140(12), 2576–2585 (2013).
56. Algvere PV, Gouras P, Dalfgard Kopp E. Long-term outcome of RPE allografts in non-immunosuppressed patients with AMD. *Eur. J. Ophthalmol.* 9(3), 217–230 (1999).
57. Peyman GA, Blinder KJ, Paris CL, Alturki W, Nelson NC Jr, Desai U. A technique for retinal pigment epithelium transplantation for age-related macular degeneration secondary to extensive subfoveal scarring. *Ophthalmic Surg.* 22(2), 102–108 (1991).
58. Binder S, Krebs I, Hilgers RD *et al.* Outcome of transplantation of autologous retinal pigment epithelium in age-related macular degeneration: a prospective trial. *Invest. Ophthalmol. Vis. Sci.* 45(11), 4151–4160 (2004).
59. Van Meurs JC, Ter Averst E, Hofland LJ *et al.* Autologous peripheral retinal pigment epithelium translocation in patients with subfoveal neovascular membranes. *Br. J. Ophthalmol.* 88(1), 110–113 (2004).
60. Mandai M, Watanabe A, Kurimoto Y *et al.* Autologous induced stem-cell-derived retinal cells for macular degeneration. *N. Engl. J. Med.* 376(11), 1038–1046 (2017).
61. Boudreaux K, Justus S, Lee W, Mahajan VB, Tsang SH. Complication of autologous stem cell transplantation in retinitis pigmentosa. *JAMA Ophthalmol.* 134(6), 711–712 (2016).
- **Important report documenting a complication of autologous stem cell transplantation in a retinitis pigmentosa patient. Discusses possible mechanisms by which the complication occurred.**
62. Kuriyan AE, Albin TA, Townsend JH *et al.* Vision loss after intravitreal injection of autologous 'stem cells' for AMD. *N. Engl. J. Med.* 376(11), 1047–1053 (2017).
- **Documents complications that arose following autologous adipose tissue-derived stem cell administration at a stem cell clinic. Emphasizes the oversight and patient education required for stem cell clinics that do not fall under US FDA regulations.**