The Future of Retinal Disease Treatment

Stem Cell Research, Gene Therapies, and Sophisticated Retinal Imaging Techniques

Susan L. Worley

Degenerative retinal diseases, which damage the cells and intricate structures that comprise the retina (Figure 1), are among the primary causes of irreversible visual loss. It has been estimated that two of the most common of these diseases—age-related macular degeneration (AMD) and diabetic retinopathy—could account for more than 80% of documented cases of bilateral blindness in the world,[1] while a fairly long list of other retinal diseases are relatively rare (Table 1).

The introduction of anti-vascular endothelial growth factor (VEGF) agents near the beginning of this century led to a revolution in the treatment of many of these diseases, including neovascular (wet) AMD, diabetic macular edema, and other conditions in which the retina is damaged by the growth and leakage of abnormal blood vessels.[2] Treatment with anti-VEGF injections has proven to be extraordinarily successful, allowing clinicians to preserve vision in many patients who previously would have gone blind;[3,4] however, drawbacks include lengthy treatment regimens, involving intravitreal injections at roughly one- to two-month intervals, which can be challenging for patients and can in turn interfere with compliance. Under investigation are newer methods of drug delivery and novel drugs,[5–7] which may eventually reduce the treatment burden for patients who currently benefit from anti-VEGF therapy.

Meanwhile, researchers are approaching retinal diseases from a number of other angles. Visual prostheses, for example, which generate artificial vision by the electronic stimulation of remaining healthy cells in the retina, are offering hope to some individuals with severe visual loss who are not candidates for other surgical or medical treatments. Progress in recent years has led to the development of several devices in Europe and in the United States, including the Food and Drug Administration (FDA)-approved Argus II (Second Sight, Inc).[8,9] Although visual prostheses have enabled some patients to detect motion and perform some functional tasks, adverse events such as elevated intraocular pressure and retinal detachment have occurred, and visual acuity with available devices remains poor. At present, researchers are exploring the feasibility of devices that target areas of the visual pathway other than the retina, including the visual cortex.[10]

Perhaps the frontrunners among strategies that have captured the imagination of scientists and lay people alike are biological approaches to retinal disease, which involve the replacement and/or repair of degenerated retinal cells. Stem cell and gene therapy research have gained astonishing momentum, and are not only laying the groundwork for new treatments but also providing new disease models and valuable insights into pathogenesis. Advanced retinal imaging is another area of research that is helping scientists and clinicians better characterize retinal diseases while facilitating the development, use, and monitoring of new treatments. Here, retina specialists discuss highlights of these three areas of research.

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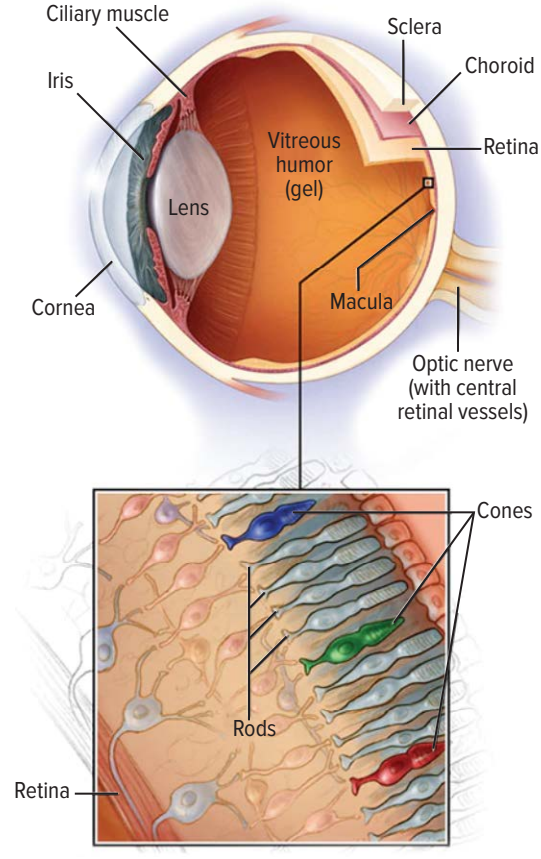
Stem Cell Research

For more than a decade it has been possible to generate large numbers of retinal cells from embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs), two types of precursor cells that have the ability to multiply and remain

Figure 1 Parts of the Eye



Located at the back of your eye in the center of your retina, a healthy macula allows normal central vision acuity. The macula is made up of densely packed light-sensitive cells called cones and rods. Cones are responsible for color vision, and rods enable you to see shades of gray.

Courtesy of Mayo Clinic

Table 1 Selected Retinal Diseases and Disorders: A Glossary

Information courtesy of the American Society of Retina Specialists and the Foundation Fighting Blindness

Retinal Disease/Disorder	Description
Age-related macular degeneration (AMD)	A deterioration of the retina and choroid that leads to a substantial loss in visual acuity (sharpness of vision). AMD is the leading cause of significant visual acuity loss in people aged > 50 years in developed countries. There are two types: wet AMD and dry AMD.
Neovascular (wet) AMD	In wet AMD, there is a sudden or gradual decrease in visual acuity, blind spots in the center of vision, and distortion of straight lines. The hallmark of wet AMD is choroidal neovascularization (CNV).
Dry AMD	In early stages of dry AMD, the hallmark is drusen—pale yellow lesions formed beneath the retina. Drusen are usually harmless, but as they accumulate, dry AMD can progress. Atrophic areas (areas of atrophy or wasting) in the retina also may develop; if the atrophic area is significant and with sharp borders, it is called geographic atrophy (GA).
Choroidal neovascularization (CNV)	Growth of abnormal new blood vessels in the choroid layer of the eye that grow under the retina and macula and disrupt vision.
Diabetic retinopathy	A complication of diabetes that causes damage to the blood vessels of the retina. Tiny blood vessels in the back of the eye can deteriorate and cause fluid to leak into or under the retina, which in turn can cause the retina to swell, leading to blurred or distorted vision. New, abnormal capillaries also may develop and cause additional bleeding that leads to worsened vision.
Diabetic macular edema	The term used for swelling in the macula in eyes with diabetic retinopathy. (The macula is the center part of the retina and is responsible for providing the sharp, straight-ahead vision used for reading and recognizing faces.)
Inherited Retinal Dystrophies (IRDs)	
Choroideremia	An inherited disease that causes progressive loss of vision due to degeneration of cell layers in the retina. The affected layers include the choroid, the retinal pigment epithelium (RPE), and the photoreceptors. The choroid consists of blood vessel layers located between the retina and the sclera. Choroidal vessels provide the RPE and photoreceptors with oxygen and nutrients necessary for normal function.
Leber's congenital amaurosis	An inherited retinal degenerative disease characterized by severe loss of vision at birth. A variety of other eye-related abnormalities including roving eye movements, deep-set eyes, and sensitivity to bright light also occur with this disease. Some patients with the disease also experience central nervous system abnormalities.
Retinitis pigmentosa (RP)	Refers to a group of inherited diseases causing retinal degeneration. The most common feature of RP is a gradual breakdown of rods and cones. Most forms first cause the breakdown of rod cells. These forms of RP, sometimes called rod-cone dystrophy, usually begin with night blindness. A progressive disorder, RP is typically diagnosed in adolescents and young adults. The rate of progression and degree of visual loss varies from person to person. RP can be inherited in a dominant, recessive, or X-linked fashion.
Stargardt disease	The most common form of inherited juvenile macular degeneration. The progressive vision loss associated with Stargardt disease is caused by the death of photoreceptor cells in the central portion of the retina called the macula. Decreased central vision is a hallmark of the disease; side vision is usually preserved. Stargardt disease typically develops during childhood and adolescence.

stem cells (i.e., self-renew), or differentiate into any type of specialized cell in the human body (Figure 2). Research involving retinal cells derived from stem cells aims to halt or reverse visual impairment by replacing or repairing retinal cells damaged by disease. Healthy new retinal cells also can provide support to existing (endogenous) cells in a number of ways, including by secreting growth factors, inhibiting apoptosis, and encouraging new synaptic connections.[11] Although to date there are still no FDA-approved stem cell treatments for retinal disease (the only FDA-approved cell replacement therapy is hematopoietic cell transplantation for the treatment of blood cancers) several developments in recent years point to exciting progress in the field.

In the United States, for example, colleagues at the USC Roski Eye Institute have demonstrated, in a phase 1/2a trial, the feasibility of using an ESC-derived retinal pigment epithelial (RPE) implant designed to replace dead or degenerated RPE cells in patients with dry age-related macular degeneration (AMD).[12] One year after four patients at USC received human ESC-derived RPE cell implants, there was evidence that the implants had integrated with the patients' retinal tissue. The treatment also proved to be well tolerated and three eyes experienced some improvement in visual function. The early results of this research are especially heartening because unlike wet AMD, for which anti-VEGF therapy can improve vision, dry AMD is untreatable. Also working toward a treatment for dry AMD is a team of researchers at the National Institutes of Health (NIH)/National Eye Institute (NEI), led by Kapil Bharti, PhD, who are preparing to launch the first U.S. clinical trial using iPSC-derived RPE cells to treat this

Figure 2 A Stem Cell Glossary

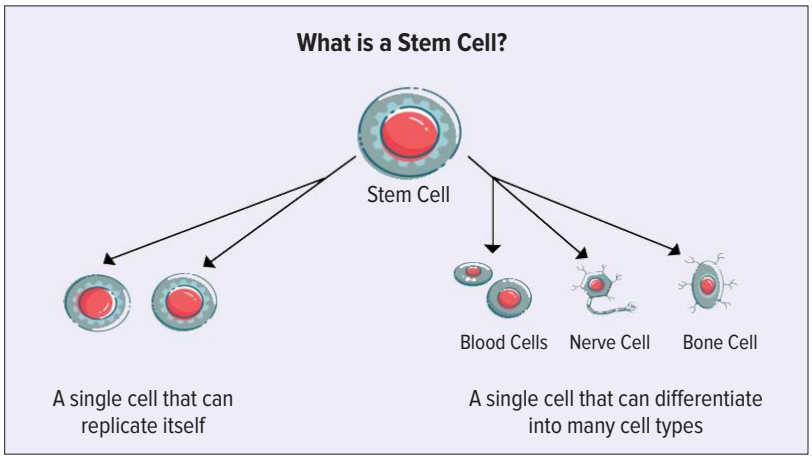


Image courtesy of Diana Molleda/USC

*For more information, see "Stem cells for retinal disease: a perspective on the promise and perils," by Rajesh C. Rao, Vaidehi S. Dedania, and Mark W. Johnson.

Human embryonic stem cells (ESCs)	Undifferentiated cells derived from the inner cell mass of the human blastocyst. These cells can potentially give rise to all cell types in the human body, and can be differentiated to retinal pigment epithelium and photo-receptors for cell therapy in degenerative retinal diseases.
Induced pluripotent stem cells (iPSCs)	ESC-like stem cells that are derived from reprogrammed adult cells, such as blood or skin cells. Like ESCs, these cells can self-renew and are pluripotent (can give rise to all cell types in the body).
Adult or multipotent stem cells/somatic stem cells	Tissue-specific (or adult) stem cells, which can give rise to several different types of specialized cells in specific tissues (e.g., blood stem cells can produce all of the different types of cells that make up the blood; however, they cannot give rise to cells that make up other tissues in the body).
Postnatal/fetal stem cells	Stem cells derived from the developing fetal central nervous system, including the brain, spinal cord and retina.

*Rao RC, Dedania VS, Johnson MW. Stem cells for retinal disease: a perspective on the promise and perils. *Am J Ophthalmol* 2017;179:32–38. doi: 10.1016/j.ajo.2017.04.007

disease. Bharti's team developed a method for reprogramming blood-forming stem cells from patients with AMD into iPSCs and subsequently transforming them into implantable RPE tissue.[13] In the upcoming trial, which is expected to launch in late 2019, RPE cells obtained by this method will be surgically transplanted on biodegradable scaffolds designed to properly orient the stem cells.

Dangers of Unregulated Research

The trials conducted by the USC and NEI teams are well-planned, highly regulated, and conducted with a focus on adhering to rigorous safety measures. However, in recent years, reports of *unregulated* stem cell research and treatments, which in some cases have resulted in severe blinding complications, have caused fear and confusion among the lay public. In 2017, for example, the *New York Times* reported that three patients lost their eyesight after receiving stem cell treatments for macular degeneration at a poorly regulated clinic in Florida.[14,15] The clinic's highly questionable practices included the use of non-ESC or iPSC stem cells that were isolated, by means of a mysterious "proprietary" method, from its clients' own fat tissue.

"Until now, there have been loopholes in the law that have enabled entities to use autologous, non-ocular cells for the treatment of retinal disease, even though there is no compelling research to suggest that it's either safe or effective to do so," says Rajesh C. Rao, MD, professor of ophthalmology and visual sciences at the University of Michigan, and director of the retina service at the VA Ann Arbor Health System. "Traditionally, it hasn't been necessary to follow an FDA-regulated route when transferring fat cells from one part of the body to another. For example, a person undergoing mastectomy may consent to the use of her own fat tissue for breast reconstruction, and such procedures can have very good results. However, in the retina, there is no fat and no known role for fat cells or so-called "stem cells" derived from fat. So loopholes that permit homologous use, or the unregulated transfer of a person's cells from one area of the body to another—regardless of the natural or intended function of the cells—clearly have the potential to be dangerous."

The FDA has begun to crack down on clinics claiming to be exempt from FDA oversight because of rules regarding homologous use or so-called minimal manipulation.[16] In June 2019, for example, a federal judge granted the FDA a permanent injunction against US Stem Cell, forcing the company to stop promoting or using procedures involving the isolation of stem cells from fat tissue.[17] The California Stem Cell Treatment Center Inc. and the Cell Surgical Network Corporation are two other companies against which the FDA has filed a similar suit, whose outcome is still pending. In addition to administering risky, unapproved procedures, stem cell clinics such as these (there are roughly 500 in the country) have misled people into believing that there are approved stem cell treatments for retinal disease, when in fact related research is still very much in the early stages.

Indeed, even after experiencing significant early successes, legitimate stem cell research teams can suddenly face unexpected hurdles. For example, a Japanese team credited with being the first in the world to successfully transplant autologous iPSC-derived RPE cells into a patient's eye for the treatment of a type of wet AMD[18] halted their research after cancer-causing genetic mutations were detected in iPSCs prior to their use in a second patient. In compliance with regenerative medicine

laws in Japan, the team carefully reevaluated its protocols and in the future will be using RPEs from oncogenic mutation-free, allogeneic iPSCs.

Variations in Technique and Stem Cell Type

After a surprisingly small number of stem cell-derived retinal cells are successfully introduced into a suitable area in the eye, they can begin to replace damaged cells or repair them by secreting proteins that might help damaged cells to survive. But researchers still have not determined the optimal method for delivering these stem cells. Subretinal injection of a suspension of cells was the earliest method of delivery[19] and continues to be the most common, while in newer studies, scientists have begun to deliver stem cells on scaffolds.[12,13]

"In the human eye," says Dr. Rao, "retinal pigment epithelium cells are naturally arranged in a series of hexagons that form a honeycomb pattern. It is thought that RPE cells arranged on a scaffolding might help to preserve some of the natural spacing or architecture of this layer of cells, which is thought to be important because the arrangement of these cells can affect their function."

Rajesh C. Rao, MD

When scaffolding is used, notes Dr. Rao, it is necessary to make a much larger incision into the retina, which can pose risks such as excessive bleeding or causing a larger area of damage to existing retinal cells in the macula. In contrast, cells delivered in suspension require a smaller, almost self-sealing incision. And it could turn out that cells introduced in a suspension are capable of attaining somewhat normal spacing and architecture on their own, as a recent update by the Japanese team has suggested.[20]

"Researchers haven't yet conducted enough clinical trials to determine which technique is better," says Dr. Rao, "so it's far too early to draw any conclusions."

Researchers also have yet to determine whether it is more advantageous to use ESCs or iPSCs for retinal stem cell research. Because iPSCs have ESC-like properties and can be created by reprogramming cells taken from a patient's own skin or blood, their use theoretically poses less risk of rejection and should require less immunosuppression. However, the process of reprogramming can stimulate mutations in genes that may increase the risk of tumor formation. And there are other challenges associated with iPSCs.

"If you derive a stem cell line from blood or skin, the resulting iPSCs will share many characteristics of ESCs, which are derived from the embryonic blastocyst, but there are important differences," says Dr. Rao. "iPSCs have a memory of where they come from, an epigenetic memory that is still not well understood. For example, iPSC lines derived from reprogrammed skin cells, in which some skin genes may remain partially turned on, may resist becoming retina cells to some extent. This can affect the efficiency with which a retina cell can be derived from blood or skin, and with differences in efficiency can come differences in safety as well as cost."

According to Dr. Rao, whose own stem cell research is focused on the investigation of genes and proteins that regulate whether cell type-specific genes are turned "on or off" (epigenetic processes that control the fate of pluripotent stem cells), in the future it may be possible to predict and possibly even prevent unwanted epigenetic changes.

"Eventually, we may have technologies that let us know which stem cell lines are the safest and most cost-effective by assessing key epigenetic features that determine the probability, efficiency, and speed by which a given clinical-grade ESC or iPSC line will generate transplantable retinal cells. Such technology should help to provide a form of quality control. We will also likely begin to use next-generation sequencing to help us better understand, and hopefully avoid, pro-tumor genetic and epigenetic changes that arise during the process of reprogramming skin and blood cells to create retina cells for therapy."

As stem cell researchers begin to tackle new retinal diseases[21] (Table 2), they may ultimately discover a way to deliver not only RPE cells but also photoreceptors—the rods and cones that convert light into signals that can be processed by the brain. Clinical trials will likely undergo other changes as well.

"To date, many of the clinical trials have involved the treatment of patients with advanced disease," says Dr. Rao. "But one recent trend has been to treat patients who have more moderate vision loss, with the hope of catching disease at an earlier time, when cell therapies might be more effective. The ultimate goal may be to define a window during which a particular cell therapy will be most effective."

Gene Therapy for Inherited Retinal Dystrophies

In December 2017, the approval of voretigene neparvovec (Luxturna, Spark Therapeutics, Inc.), the first gene therapy for visual loss caused by an inherited retinal disease, was a major medical and scientific milestone.[22] Only the second gene therapy to be approved by the FDA, it was the first to target a retinal disease, and the first to involve the delivery of a gene directly into a person, rather than into a cell in a dish. This astonishing success followed nearly twenty years of research, including early preclinical work on transgenic mouse models (mice created to have specific genetic mutations) as well as naturally occurring animal models, such as dogs with *RPE65* mutations that resulted in blindness.

"The preclinical work that was done in mice and dogs was critical to understanding the way that genes can be delivered to treat inherited retinal diseases," says Jacque Duncan, MD, professor of clinical ophthalmology at UCSF. "The work helped researchers understand what kind of viral vectors can be used, what techniques might be necessary to deliver vectors to the photoreceptors, what kind of side effects to expect, and which cells were the best targets of the treatment."

Inherited retinal dystrophies (IRDs) are rare disorders caused by genetic defects that result in progressive retinal degeneration. Some patients with these disorders experience severe, bilateral, irreversible loss of vision at an early age. By the time gene therapy research in humans began, scientists already had identified genes implicated in many of these disorders, but focused first on mutations in the *RPE65* gene, which

Table 2 Selected Investigational Stem Cell Trials

Manufacturer/Sponsor	Clinical Trial Number	Indications/Comments	Status
University of California, Davis	NCT01736059	Pilot study to determine safety and feasibility of injecting CD34+ stem cells from bone marrow into eye for patients who are irreversibly blind from various retinal conditions.	Phase 1
Pfizer	NCT03102138	Long-term, open-label safety follow-up study of patients with wet AMD and recent rapid vision decline after transplantation of human embryonic stem cell (ESC)-derived retinal pigment epithelium (RPE)	N/A
Southwest Hospital, China	NCT02749734	Clinical study of subretinal transplantation of human ESC-derived RPE in treatment of macular degeneration diseases (including Stargardt disease)	Phase 1/2
Regenerative Patch Technologies, LLC	NCT02590692	Clinical trial to assess feasibility of delivery and safety of human ESC-derived RPE cells on parylene membrane (CPCB-RPE1) in patients with advanced, dry AMD	Phase 1/2a
BioTime, Inc.	NCT02286089	Evalutation of safety and tolerability of OpRegen for treatment of advanced dry-form age-related macular degeneration	Phase 1/2
ReNeuron Limited	NCT02464436	First-in-human, dose escalation study in which participants with retinitis pigmentosa will receive single subretinal injection of human retinal progenitor cells (hRPC) in one eye to evaluate safety and tolerability.	Phase 1/2
jCyte, Inc	NCT03073733	Study to evaluate changes in visual function at 12 months following single injection of hRPC in adult subjects with RP	Phase 2

are known to be associated with Leber's congenital amaurosis (LCA, for which voretigene neparvovec is approved) and early onset retinitis pigmentosa.

"The *RPE65* gene was studied for a number of reasons," says Dr. Duncan. "First, a large animal model of the disease—a naturally occurring dog model—was available. In addition, the gene is expressed in RPE cells and not in the photoreceptors, and for that reason the photoreceptors themselves remain structurally present for a longer time than in many other forms of retinal degeneration caused by mutations in genes that are expressed by the photoreceptor cells. It was believed, and shown to be the case, that by restoring *RPE65* to the RPE cells it would be possible to improve visual function in remaining photoreceptors that had not yet degenerated. By replacing this missing gene, researchers were able to restore more normal cycling of vitamin A, and the photoreceptors were still present to utilize the vitamin A, which caused vision to improve."

Dr. Duncan adds that because *RPE65* is a small gene, it fits easily within the adeno-associated virus (AAV) vector, which is commonly used to deliver genes. This small virus infects humans but is not known to cause disease or side effects other than symptoms of a mild immune response.[23]

A Closer Look at Treatment

Mutations in the *RPE65* gene interfere with the process of making an enzyme that is essential for normal vision, and voretigene neparvovec works by delivering a normal copy of *RPE65* directly to RPE cells. At present, the only method for delivering the gene is by subretinal injection.

"Because of the blood–retina barrier, it's not possible to deliver this gene by giving someone a pill, or by injecting the gene into their bloodstream," says Dr. Duncan. "You have to inject the gene into the eye and inject in a region close to the RPE cells, which are the targets of the treatment. A lot of work has gone into trying to develop treatments that can be injected into the vitreous, which is how we treat patients with AMD who receive anti-VEGF injections. The challenge, though, is that a lot of viral vectors don't make it to the target cells in the outer retina or beneath the retina in the RPE; instead they get stuck on the inner retinal surface and don't effectively reach the cells you are trying to treat, which are the photoreceptors and the RPE cells."

Treatment is delivered underneath the retina by first creating a retinal detachment. Typically, saline is used to detach the retina, so that treatment can be injected right next to cells that must eventually express the new gene.

"In general, retinal detachments are to be avoided, because they can cause vision loss," says Dr. Duncan. "There is probably some damage to the outer segments of the photoreceptors whenever you detach the retina. It is true that the subretinal fluid goes away over the course of the next day, or week at most, and the retina eventually flattens out. But it probably takes some time for cells to fully recover from having been detached, which might reduce the amount of improvement you can expect the patient to experience."

Because retinal specialists are trained to avoid retinal detachments, relatively few specialists are familiar with this gene delivery technique. Currently, this treatment is being delivered primarily by surgeons who were involved in the original clinical trials, or specialists trained by those surgeons.

"These are pretty rare diseases and only a small number of people are receiving treatment at this time," says Dr. Duncan,

"so currently it makes sense to have patients travel to the surgeons who have experience with this kind of treatment. Eventually, if a lot more gene therapies are approved, this type of surgery likely will be more widely taught to vitreoretinal surgeons."

For now, research is focused not only on new delivery techniques, but on identifying novel genes that play a role in these disorders. One goal is to better understand so-called modifier genes, which do not directly cause disease, but can influence disease onset and severity.[24] Scientists also are making use of new tools, including iPSC models of disease, to gain a better understanding of disease pathogenesis and to validate potential new therapeutic strategies.[25] In her own research, Dr. Duncan has been using advanced imaging techniques, such as adaptive optics, to better understand how different mutations affect the arrangement of photoreceptors in patients with IRDs—research that may ultimately enhance drug discovery efforts.[26]

Jacque Duncan, MD

Getting Tested

To date, more than 260 disease genes associated with various IRDs have been identified, and more than 25 gene therapies are currently in development (Table 3). Estimates suggest that it is now possible to identify the genetic causes of disease for about two thirds of all individuals with IRDs, and up to 85% of children who have these rare disorders. Registries for patients with IRDs are providing valuable data for researchers as well as facilitating testing for people who have a family history of disease. My Retina Tracker®, a registry and database created by the Foundation Fighting Blindness, is the largest of these.

"To make their database as informative as possible, the My Retina Tracker organizers have initiated a gene therapy study in which patients can elect to provide a sample of saliva or blood to have genetic testing, through a platform that looks at 266 different genes across IRDs," says Dr. Duncan, who chairs the philanthropic organization's scientific advisory board. "About three months later, individuals who participate will receive a phone call from a genetic counselor, who will explain their results."

Dr. Duncan notes that Spark Therapeutics also recently launched an initiative called *ID Your IRD*, which allows patients to have similar genetic testing at no cost, if their healthcare providers suspect that they have an IRD. The *ID Your IRD* program tests for approximately 250 genes associated with IRDs but omits some genes that are included in the My Retina Tracker panel, such as *RPGR*, the most common cause of X-linked retinal degeneration. Both the Spark Therapeutics and My Retina Tracker genetic testing programs are important resources, because few insurers are currently covering the cost of this type of testing, despite the availability of a new treatment.

"Testing is important because it can help some patients qualify for enrollment in clinical trials, and of course it can make some patients eligible to receive the new FDA-approved treatment for *RPE65*-related retinal degeneration," says Dr. Duncan. "Confirming a diagnosis also helps families with these diseases, in part by helping patients to understand how

Table 3 Selected Investigational Gene Therapy Trials

Manufacturer/Sponsor	Clinical Trial Number	Description	Status
Sanofi	NCT01367444	Study to assess safety and tolerability of ascending doses of SAR422459 in patients with Stargardt disease	Phase 1/2a
Applied Genetic Technologies Corp	NCT02416622	Study to evaluate safety and efficacy of a recombinant adeno-associated virus vector expressing retinoschisin (rAAV2tYF-CB-hRS1) in patients with X-linked retinoschisis	Phase 1/2
Novartis Pharmaceuticals	NCT03374657	Study to determine safety and potential efficacy of CPK850 on improving visual function in patients with decreased visual function from RLBP1 retinitis pigmentosa due to biallelic mutations in RLBP1 gene	Phase 1 /2
Horama S.A.	NCT03328130	Safety and efficacy study in patients with retinitis pigmentosa due to mutations in PDE6B gene	Phase 1/2
Oxford BioMedica	NCT01301443	Study to examine safety of experimental gene transfer agent, RetinoStat, designed to treat neovascular age-related macular degeneration	Phase 1
GenSight Biologics	NCT03293524	Study to assess safety and efficacy of GS010 in improving retina functional and structural outcomes in subjects with LHON due to G11778A ND4 mitochondrial mutation, when vision loss duration is present up to one year	Phase 3
NightstaRx Ltd	NCT03496012	Study to evaluate efficacy and safety of single sub-retinal injection of AAV2-REP1 in subjects with choroideremia	Phase 3
MeiraGTx UK II Ltd	NCT03758404	Clinical trial of AAV - CNGA3 retinal gene therapy for patients with achromatopsia	Phase 1/2

the IRD is inherited in their family, and to focus on research that is relevant to their specific condition."

Advancing Research With New Imaging Technology

Advances in retinal imaging in recent decades have led to better characterization of disease, significant improvements in the diagnosis and monitoring of retinal diseases, and a range of exciting new avenues for research. More than a decade ago, at roughly the same time that anti-VEGF agents began to revolutionize the treatment of many retinal diseases, the growing clinical adoption of optical coherence tomography (OCT) ushered in a new era of retinal imaging.[27] A non-invasive imaging technique, OCT works according to a principal similar to that of ultrasound, except that cross-sectional images of the retina are produced with light rather than sound waves, and at a much higher (micrometer) resolution. OCT permits clinicians to accurately measure the thickness of the retina and obtain detailed information about the anatomic layers that comprise the retina. It also permits real-time visualization of the microstructure of retinal tissues, without the need for a biopsy. In clinical practice, OCT is now an indispensable method of visualizing the pathology of many eye diseases, and a single OCT instrument often is used in lieu of several older diagnostic devices.

Brandon Lujan, MD

Today, a number of newer imaging techniques are building on the OCT platform. OCT angiography (OCTA), for example, is a non-invasive imaging technique that provides both structural and functional information about the retina, including details regarding changes in blood flow and subtle microvascular abnormalities.[28,29]

"OCTA works on the principle that if you image the same part of the retina twice, the parts that are changing significantly represent blood flow," says Brandon Lujan MD, associate professor of ophthalmology at OHSU School of Medicine and the medical director of the Casey Reading Center. "If you acquire multiple images of the same part of the retina, areas that aren't moving are part of the structural tissue of the retina, but in areas where there is change, and therefore blood flow, OCTA can put together a volume of data that ultimately provides a map of the capillaries and all of the flow in the retina, without any kind of dyes or invasive procedures."

Prior to the emergence of OCTA, retina specialists relied exclusively on invasive imaging techniques for such information, including fluorescein angiography (FA) and indocyanine green angiography, both of which require the injection of dye. Both techniques are time consuming and produce limited 2-dimensional images. Although the dyes used are generally considered to be safe, they can cause nausea and rarely significant allergic reactions. In contrast, OCTA quickly and safely delivers far more detailed 3-dimensional images. However, retina specialists will likely continue to use FA for some time because, unlike OCTA, it provides information about the leakage of blood vessels, and also can be conjoined with widefield imaging technology,[30] to permit clinicians to view the periphery of the retina and assess peripheral ischemia.

"OCTA provides a wide range of benefits in research, including the analysis of ischemic areas in diabetic retinopathy and response to treatment in wet age-related macular degeneration," says Dr. Lujan, "and in clinical practice, the images provide critical information about early choroidal neovascularization, or the early detection of new abnormal blood vessels deep in the retina. This information lets clinicians know whether to watch patients more closely and/or potentially begin to treat them earlier."

Another new imaging method that builds on the OCT platform is directional OCT,[31] a technique that Dr. Lujan has pioneered, which allows scientists to assess the integrity and orientation of photoreceptors and other retinal structures.

"Directional OCT obtains images from different pupil positions and uses changes in reflectivity, or the amount of light that "bounces" off the different layers of the retina to provide detailed information about retinal tissue," says Dr. Lujan. "An advantage of this technique is that it allows you to see photoreceptors that are present but are not oriented correctly. This information helps identify photoreceptors that are at risk of dying in certain retinal conditions, and helps to detect photoreceptors that may be at risk, at a much earlier stage of disease."

Although directional OCT is not yet used in clinical practice, it is used in research. For example, studies involving potential treatments for dry AMD have benefitted from this technology, which does a better job than standard OCT when it comes to accurately and precisely measuring the layer of the retina that contains photoreceptor nuclei. Directional OCT also has been used to image a disease called macular telangiectasia (MacTel), which affects the macula and causes loss of central vision.

"In patients with macular telangiectasia," says Dr. Lujan, "there's typically an area of frank photoreceptor loss, but there also appears to be a border zone around that area of photoreceptor loss where the vision cells are still present but misdirected. That means that the supporting structure around them is already damaged or altered. Directional OCT improves visualization of this area, which is important to watch very closely when determining whether a treatment for this disease is having an effect."

Particularly valuable to surgeons providing subretinal delivery of stem cell and/or gene therapies is intraoperative OCT, a new imaging technique that enables real-time visualization of the retina during surgery.

"With intraoperative OCT, which conjoins the OCT system to an operating microscope, you are able to see, on a very fine scale, the amount of fluid that is being put into the retina," says Dr. Lujan. "It's possible to actually see instruments imprinting the retina and to watch the subretinal space expanding with incredibly precise detail. You also can visualize the amount of fluid delivered to that area, and can detect abnormalities that occur, such as reflux."

Adaptive Optics and AI

Adaptive optics (AO) is one of the most exciting new developments in the field of retinal imaging. Used by NASA and

originally developed to improve the resolution of atmospheric telescopes, by correcting for aberrations caused by elements such as wind and precipitation, AO is now helping scientists view individual RPE and photoreceptor cells.[32,33]

"The eye is an imperfect optical system," explains Dr. Lujan, "So the tear film, the cornea, the lens, and all of the media or components inside the eye can cause imperfections in the path that light takes toward the retina. AO corrects for aberrations and distortions that are present in the eye to allow visualization of individual cells in the retina. It's able to measure those imperfections on a moment by moment basis, feed that information into deformable mirrors, and correct for abnormalities repeatedly, in a refreshing state, to deliver incredibly detailed images of individual photoreceptor cells."

Adaptive optics, which can be used in conjunction with several existing imaging systems,[34] is used only for research at present. Research grade AO scanning laser ophthalmoscopy (AOSLO) or AO OCT systems are expensive and relatively difficult to operate, so they have yet to undergo commercial standardization. Yet these systems already provide information that is of value to clinicians.

"In addition to greatly improving disease tracking for clinical trials, AO systems can target and stimulate individual cells," says Dr. Lujan. "You can image a cell, lock onto it, and then shine light onto that cell or on a number of cells around it and determine whether the person you are imaging can see it. So, AO gives you a lot of insight into visual function in addition to retinal disease. You also can measure cells in disease at a much earlier state and, as a clinician, that is my primary interest. In a disease such as macular degeneration, where cell loss occurs very slowly, you can measure disease progression on a very small scale. For clinical trials that take place during a short period of time—one or two years—the value of this technology is more apparent, but for standard clinical practice, AO does not yet offer a clear clinical decision point."

Also used more far more commonly in research than in clinical practice is an extraordinary branch of artificial intelligence (AI) called deep learning, which is beginning to offer scientists not a better way to image the retina, but a better way to interpret images. Deep learning is a type of machine learning that can, among other things, train software to develop algorithms for recognizing patterns in images.[35] Such sophisticated pattern recognition is gradually being embraced by many fields in medicine and health care, including ophthalmology.

"There is still a lot that we are learning about AI," says Dr. Lujan, "But for a number of different conditions, including AMD, retinopathy of prematurity, and particularly diabetic retinopathy, AI's ability to diagnose disease has proven in some cases to approach that of retina specialists."

Last year, results of pioneering collaborative research conducted by colleagues at Moorfields Eye Hospital in London, England, and DeepMind Health showed that an AI system was able to match the referral decisions for more than 50 sight-threatening eye diseases, made by leading global eye experts, with 94% accuracy.[36] This study and others[37] suggest that in the future such programs will eventually help clinicians to make faster and more accurate decisions and in turn improve health outcomes for their patients. However, real world research will be necessary to validate these programs, and experts will have to address a number of problems, including their own reservations about "black box" or unsupervised learning.

"Supervised learning reflects how the human mind works," says Dr. Lujan. "Retinal specialists can program features or variables that we think are important—for example, the size of drusen, the amount of fluid that is present, or the thickness of the retina—and then design a model that tries to use those different features to get to an answer. In contrast, with unsupervised learning, you aren't telling a program what features to pay attention to. Instead, you provide a large data set—for example, images of eyes that have macular degeneration and their vision—and you allow the AI software to decide for itself what the most relevant features of the disease are. Based on those features, the software analyzes photos and draws its own conclusions regarding the presence and extent of disease."

Dr. Lujan adds, "However, with unsupervised learning you never really know exactly what information the software uses to arrive at its conclusions, which, increasingly, are surprisingly accurate."

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