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Future Vision 2020 and Beyond—5 Critical Trends in Eye Research

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Abstract: Ophthalmology has been at the forefront of many innovations in basic science and clinical research. The randomized prospective multicenter clinical trial, comparative clinical trials, the bench to beside development of diagnostic and therapeutic devices, the powerful combination of biostatistics and epidemiology, gene therapy, cell-based therapy, stem cell therapy, regenerative medicine, artificial intelligence, and the development of personalized molecular medicine continue to propel us forward. This article summarizes several critical trends in eye research. Innovative translational research continues to bring new solutions to blinding retinal diseases. The discovery of the genetic code presaged a day when the development of molecular tools and understanding of the basis of disease would lead not only to disease management but potentially lifelong cure. After decades of investigation, gene therapy is now a reality for a single autosomal recessive bi-allelic disease, Lebers Congenital Amaurosis. Its success has paved the way for a myriad of conditions once thought to be untreatable. In parallel, the progress to utilize pluripotential stem cells, immunomodulation, computational biology, and continued investigation into the fundamental mechanisms of cell and molecular biology is breathtaking in its rapidity. The next decade is likely to be the most exciting in the history of medicine. It will be essential that research progresses in a meticulously thoughtful, ethical, and collaborative process that safeguards the trust of our work and that of the society we serve.

Presented as the International Award Lecture, Asia-Pacific Vitreoretinal Society meeting, November 2019, Shanghai China.

Key Words: artificial intelligence, gene therapy, immunomodulation, regenerative medicine, stem cell therapy

(*Asia Pac J Ophthalmol (Phila)* 2020;9:180–185)

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Submitted February 25, 2020; accepted April 1, 2020.

The authors have no conflicts of interest to disclose.

Financial Disclosure: American Academy of Ophthalmology—C; American Society of Retina Specialists—C; Asia Pacific Academy of Ophthalmology—C; Asia Pacific Vitreoretinal Society—C; Diopsys—C, L, S; i2i Innovative Ideas, Inc. —C, O; Lumoptik—C, O; NEHEP/National Eye Institute/NIH—C; RegenXbio—C; Regenerative Patch Technologies—C; Second Sight—C; Spark Therapeutics—C; Volk Optical—C, L; Washington University, St. Louis—C. Category Code Legend: C indicates Consultant/Advisor; E, Employee; L, Lecture Fees; O, Equity Owner; P, Patents / Royalty; S, Grant Support.

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ISSN: 2162-0989

DOI: 10.1097/APO.0000000000000299

GENE THERAPY

Hereditary diseases of the retina represent a group of diseases with several heterogeneous mutations that have the common result of progressive photoreceptor death leading to blindness. Retinal degenerations include multifactorial diseases such as age-related macular degeneration, Lebers congenital amaurosis type 2, Stargardt disease, and retinitis pigmentosa. There is currently no cure for degenerative retinal diseases; ophthalmology has been at the forefront of the development of gene therapy, which offers hope for the treatment of these conditions. Clinical trials for achromatopsia, choroideremia, MERKT, Leber congenital amaurosis 2, Leber hereditary optic neuropathy, neovascular age-related macular degeneration, retinitis pigmentosa, Stargardt disease, Usher syndrome 1B, X-linked retinitis pigmentosa, and X-linked retinoschisis are underway (www.ClinicalTrials.gov)

The first successful gene therapy product for an inherited disease was approved by the US Food and Drug Administration (FDA) in December 2017. Of note, indication statement was not for a specific disease but "for the treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy."[1,2] This seminal observation highlights a key difference between traditional pharmacologic therapy and that of gene therapy where clinical phenotypes may have a common genetic basis. Approval followed 20 years of research to identify the mutation causing Lebers congenital amaurosis type 2 (LCA2), development of an animal model,[3] techniques for viral transfection, and establishment of biomarkers to evaluate efficacy.[2,4–7] The capability to develop animal models for testing of gene therapy is a critical precursor to human clinical trials.

The eye is well-suited for target organ gene therapy. Its uniqueness as an immune-privileged organ that is isolated from much of the systemic host immune response is that retinal cells do not divide after birth, and its small size confers singular advantages over other areas of human gene therapy. These advantages are key factors for gene therapy success, an isolated target organ/tissue with little opportunity for spillover effect, that transfected retinal cells are likely to maintain transgene production for life, and that the volume of distribution is also small. All are critical factors for transfection efficiency and clinical success.

CHALLENGES AND STRATEGIES

Despite the success and approval of gene therapy for biallelic RPRE65 mutation-associated retinal dystrophy, many challenges remain. Identification of a causative gene for retinal and ocular disease is difficult and elusive. Although treatment of single gene mutations is most likely to have positive clinical results, the treatment of common causes of blinding retinal disease [diabetic retinopathy (DR), age-related macular degeneration, and retinopathy of prematurity] are likely to be polygenic in nature. These conditions may require different approaches

targeting neurotrophic factors that preserve function, by blocking apoptotic or degenerative pathway, or through expression of a blocking anti-vascular endothelial growth factor protein.[8]

A second major challenge is the size of genetic payload. Adeno-associated virus (AAV) has emerged has an efficient method of gene delivery with low levels of immunogenicity and side effects as compared with lentivirus, adenovirus, or nonviral transfection systems.[9–11] AAV-2 and AAV-8 are among many possibilities for naturally occurring viral vectors but have an effective payload of only 5 kb of DNA.[12] Most genes encoding for major structural or regulatory functions are significantly larger. Strategies to address this include a double vector approach where each vector carries a unique fragment into the cell. Host mechanisms anneal each transgene via intermolecular recombination to form a product nearly twice the size of a single vector. Protein expression was noted to be lower but was sufficient to improve the retinal phenotype in mouse models of Stargardt disease and Usher IB.[10,11]

Assessment of clinical response and efficiency of transgene expression is a third area of study. The use of promotors and directed evolution of high efficiency mutant AAV has been shown to transfect the outer retina in small animal models but failed to reproduce similar levels of expression in dogs and nonhuman primates.[13,14] It is unknown whether the anatomy of humans, volume of distribution, the level of transgene production needed to ameliorate the gene defect, or other mechanisms contribute to these disappointing results. Measurement of transfection efficiency in human clinical trials is also a challenge. Measurement of aqueous/vitreous transgene protein levels is a useful biomarker for production but does not necessarily reflect whether these are enough to produce similar physiologic changes noted in preclinical studies. Subjective measurement of function including visual acuity, microperimetry, and visual field assessment remains the primary outcome measures in studies of inherited retinal disease but is severely limited in assessing functional changes for the entire retina. Objective testing such as electrophysiology combined with increasingly detailed anatomic assessment holds promise. A unique test of visual function was created to test patients receiving gene therapy for patients with RPE65-mediated Leber congenital amaurosis type 2. A new quantitative for visual function and mobility was created in collaboration of the FDA, the multiluminance mobility test. Twelve different mazes were created for the subject to navigate with arrows to guide the subject around and over a variety of obstacles under light conditions ranging from dim to bright. The subjects were filmed and scored via a masked independent reading center.[2,15] To date, this is the standard for testing visual function in patient with inherited retinal disease who have progressive night blindness. Optimization of delivery systems, viral vectors, and improved functional testing are needed.

To date, immunogenicity of AAV vectors and adverse events are low in early-phase studies for a variety of conditions. On December 16, 2017, the US FDA approved Luxturna (voretigene neparvovec-rzyl) to treat children and adult patients with bi-allelic RPE-65 mutation-associated retinal dystrophy. Luxturna is the first directly administered gene therapy approved in the United States that targets a disease caused by mutations in a specific gene. However, many questions remain unanswered. Will every gene therapy have an acceptable profile of safety and efficacy? What is the durability of effect? Will transgene production remain constant over a lifetime? Will promotors and other enhancements to drive gene production have adverse effects? Could there be unanticipated effects if additional gene therapy is desired in the same eye/fellow eye? What are the financial and economic implications of cure? This milestone is but a first step in the treatment of our most intractable blinding diseases.

SUMMARY

Presaged by the discovery of the genetic code, 25 years of gene therapy research has culminated in the first successful gene therapy for inherited disease in the US. Gene-targeted mutations have determined the functions of genes, their functional domains, and the mechanisms underlying the dysfunction of their disease alleles. Ongoing discovery will provide new molecular targets and will form the basis for clinical trials for many blinding inherited retinal dystrophies. Viral vector-mediated pronuclear transgenesis for retinal disease has promise but polygenic and multifactorial diseases such as age-related macular degeneration (AMD) are likely to require different approaches.[8,16–18] Whole gene editing or gene silencing using CRISPR/Cas9 technology can be used to directly treat target tissues and to develop target-specific strategies using iPSC (induced pluripotential stem cells). Other strategies of inducing mechanisms which promote neuroprotection or retard cell damage are likely pathways to pursue. For eye diseases which have a genetic determinant, we begin a decade where the goal is not just management, but cure.

Immunomodulation

There is increasing recognition of the role of host immunity in disease. Immunotherapy, immunomodulation, tumor targeting, and chimeric antigen receptor T-cell immunosensitization techniques have emerged as another strategy for clinical treatment. Public confusion exists over the term stem cells. Human embryonic stem cells and induced pleuripotential stem cells (iPSCs) can be manipulated in a wide variety of ways in the treatment of retinal disease. These will be discussed in the section on Regenerative Medicine to follow.

Another cell type is the mesenchymal stem cell (MSC). Neither derived from mesenchyme nor a true progenitor cell, the MSC is traditionally defined as a bone marrow stromal cell which may develop into chondrocytes, adipocytes, myocytes, and osteoblasts. They are found in enriched populations in the bone marrow, umbilical cord, adipose tissue, molar teeth, and in amniotic fluid. They do not have the ability to differentiate to produce hematopoietic cells. Long thought to be a structural connective cell, our laboratory in 2012 demonstrated elimination of corneal scarring in a murine model of bacterial keratitis and reduction in the neovascular response in a laser injury model in the rodent retina.[19,20] Numerous other studies have confirmed that MSCs exert strong immunomodulatory effects through a variety of mechanisms including those that "cloak" the MSC surface rendering it invisible to host immune response, that modulates lymphokine and cytokine response, and placing erythropoietin gene modified MSC in the subretinal space for the treatment of retinal degeneration.[21–23] Dubbed "nature's natural healers," these cells have chemotactic attraction to sites of inflammation and exert immunomodulation of scarring in multiple studies of microbial infection.[24] MSCs have been shown to improve corneal regeneration, further supporting the integrated role of limbal and MSC therapy.[25]

In 2011 and 2012, we described a new mechanism for the pathogenesis of DR.[26–28] We found convincing evidence that there was pericyte autoimmunity in the retinal capillaries of deceased patients with DR. An autoimmune mechanism may explain how retinal microvascular disease might progress even with good glycemic control. With time and immune attack on capillary pericytes, endothelial dysfunction leads to loss of endothelial cell integrity, macular edema, and eventually nonperfusion. These findings suggest that therapeutic immunomodulation may be a pathway to retard or ameliorate diabetic microvascular disease throughout the body. Although there is growing recognition for a therapeutic effect of MSCs in ocular degenerative disorders, problems of precision targeting of tissues and survivability remain practical issues of concern.[29,30] The role of systemic or periocular MSC infusion for DR disease requires additional investigation.

Chimeric antigen receptor T-cell therapy is the latest immunomodulatory therapy to use host immune mechanisms to specifically identify target cells. Primarily used in the treatment of cancer, this technique uses T-cells modified by chimeric antigen receptors to selective target tumor surface antigens.[31,32] This approach has not been used in the eye but is an example of a novel approach for targeted immune therapy.

Regenerative Medicine

Strategies for tissue replacement or regenerative therapies have been building momentum as understanding of stem cell biology, substrates, delivery systems, nanomaterials, and 3-D machined structures converge. Investigations using structures which combine cells and/or substrates include preclinical investigations of corneal disease, glaucoma, cataract, age-related macular degeneration, and retinitis pigmentosa. The use of 3-D and microengineering of bionic interfaces is being tried in many areas. Phase I studies include: implantation of ex vivo cultured corneal stroma stem cells to reverse stromal scarring, and replacement of glaucomatous trabecular meshwork with stem cell-derived trabecular meshwork cells for intraocular pressure regulation. Phase II studies include cell suspension and cells on matrix subretinal implantation of stem-cell derived retinal pigment epithelium (RPE), subretinal RPC (retinal progenitor cell) transplant, and trophic support to retard or prevent the progression of retinal cell death.[33]

Retinal stem cell transplantation and tissue engineering have largely focused on replacement or regeneration of photoreceptors (PRs) and retinal pigment epithelial cells (RPEs).[34,35] Retinitis pigmentosa is a heterogeneous genetic mix of diseases which may affect PRs, RPEs, or both. Similarly, age-related macular degeneration is also characterized by PR loss. Whether PR loss is primary or a secondary effect due to RPE dysfunction, the ability to regenerate normal function of the PR and RPE is an attractive therapeutic option. For patients in advanced stage of outer retinal degeneration, gene therapy is unlikely to restore function and, at best, may only preserve the integrity of remaining tissues. Whether tissue replacement techniques ultimately improve vision has yet to be conclusively determined.[35]

Sources for RPE transplantation include human fetal RPE cells, allogenic human cadavers, alternative cell sources, and human pluripotential stem cells (hPSCs) (including human embryonic stem cells, and human iPSCs). Each has demonstrated the ability to assume some of the functions of native tissue without conclusive improvement in vision. The need to provide large numbers of clinical grade replacement cells has tipped the process toward the use of human pluripotential stem cells. hPSCs are induced to undergo embryologic differentiation into immature PR cells or RPE cells. For conditions primarily characterized by PR loss such as some forms of RP, a suspension of immature PR cells may be placed in the subretinal space. For patients with RP or atrophic AMD characterized by absent PR and RPE, both tissues may be required. For RPE dystrophic changes with preservation of PR, an RPE cell suspension alone might be used.[33–35] Successful implantation of a bioengineered RPE cell on a monolayer has been successfully performed and demonstrates proof of principle in patients with advanced, dry age-related macular degeneration, and macular atrophy.[36] Ultimately, success of this technology will be defined by sustained viability of host and transplanted tissue, restoration of physiologic function, and stabilization or improvement in visual function.[33–35]

Rescue strategies that seek to induce positive trophic effects from implanted tissue are being investigated. The subretinal space is believed to have moderate immune privilege. However, inflammation was noted in studies of subretinal injection of transplanted allogenic iPSC-RPE cells in rhesus macaques and minipig models.[37,38] It is becoming apparent that transfer of cytoplasmic materials between transplanted cells and the host cells occurs when enough viable host cells are present but does not occur when disease is advanced, and PRs are in low number.[36] These findings suggest that the timing of tissue transplantation is important to rescue existing PR function and maximize transplantation effect. Numerous Phase 1/2 studies are underway and explore intravitreal cell suspension, subretinal cell suspension, stem cell on scaffold, and suprachoroidal subretinal techniques. Designed primarily as proof of principle studies, these human trials have demonstrated broad tolerability and evidence of cell integration.[35] Whether retinal stem cell transplantation for retinal dystrophy or advanced dry AMD can retard disease progression or to improve retinal function is unknown and awaits further investigation.

Regenerative medicine holds significant promise for those for whom replacement of tissue is the best approach to restore neuroretinal function. An intriguing recent study of in vitro cortical brain organoids demonstrated oscillatory potentials at a level equal to the visual potentials noted in a 12-week-old premature infant. This extraordinary finding demonstrates the potential of regenerative medicine strategies to repair or replace neural tissue. Because the eye is an extension of the central nervous system, guiding eye precursor cells to full development may be a future approach to inherited and degenerative eye disease.[39]

Artificial Intelligence

Perhaps no technological advance is as widely anticipated as the introduction of artificial intelligence (AI) into the medical biome. Alternately feared and hailed as a paradigm shift in clinical care, AI engenders deep feelings that accompany transition into civilization's fourth industrial revolution.[40] Each medical specialty will have areas where AI will help analyze massive data sets, identify clinically relevant patterns of disease, anticipate disease progression, guide clinical care, and optimize operations and resources. Electrocardiography, dermatology, radiology, and breast cancer screening all utilize image recognition.[40]

In ophthalmology, the data generated from increasingly sophisticated imaging modalities are surpassing the ability to synthesize the clinical dataset. The rapidly expanding number of tests and the amount of data gathered are overwhelming the clinician's capacity to perform detailed analysis. AI will have the ability to identify patterns, progression, and most importantly, new markers that guide management.[41–43] It is worthwhile goal of AI programs to enhance the clinician's ability to practice more efficiently, more effectively, and to intervene before function is lost.

DEFINING AI

AI broadly refers to computer programs with the ability to learn and reason like humans. After training extensively on fundus images of patients with known grades and severity of DR, AI independently provides diagnostic recommendation. In the example of DR, AI might perform screening of patients with known diabetes and provide a simple output: DR present, DR absent, or needs triage for additional testing.

Machine Learning algorithms feature programing that instills the ability to learn without being explicitly programed. Machine learning is a subset of AI that permits independent review of datasets with previously known DR to "learn" what DR looks like, identify key features, and assign a diagnosis and stage. Much more sophisticated than "simple" AI, most current AI is at or near this level of sophistication. Ten thousand known imaging data sets are needed to begin to acquire this more sophisticated level of analysis.

Deep Learning subset of machine learning is the most powerful of the 3 modalities. Deep learning utilizes artificial neural networks that can learn and adapt from vast amount of data. Deep learning requires large curated data sets. One hundred thousand image sets were used in the National Institutes of Health chest x-ray studies and for dermatology deep learning projects.[44,45] Deep learning for DR would be able to identify and assign classification of DR from unknown retinal images. Over time, continuous learning on millions of images would permit searches for new patterns, biomarkers, and prognostic factors for disease progression. The ability to predict progression and the optimal time for intervention would forever change the paradigm for management, selection of subjects for clinical trials, and the efficiency of clinical care.

It is estimated that Deep Learning on 3D reconstructed images combining multimodal imaging would require training on 500,000 stereo paired images. Image sharpening using adaptive optics and image synthesis that sharpen weak imaging will be critical to avoid imprecise interpretation to default to human imaging.[46,47,42] Other challenges include the identification of training data sets, validation testing of data sets, sensitivity testing for heterogeneous populations, generalizability, display of analyzed data, algorithms for clinical care, deployment of systems, and financial impact.[41]

Opportunities for AI in Medicine

Diagnostics

Reading imaging scans such as x-Rays, magnetic resonance imagings, ultrasounds, and so on, with increased accuracy

Conduct virtual biopsies and assist in screening for cancer

Administrative

Automate routine tasks for hospital staff

Monitor member interactions with call center staff to ensure that calls are compliant

Utilize Electronic Health Records as a predictor of patient risk

Patient Monitoring

Integrate with smart devices to alert and to aid in the prevention of complications such as sepsis

Leverage data provided by patient devices such as smart watches and mobile pictures to scan for risk factors while the patient is not at the site of care

Clinical Research

Collapse data set to eliminate patients who are unlikely to progress or to respond to treatment

Will help direct patient management by making recommendations on follow-up

This daunting challenge would seem to be on a distant horizon but consider the story of Stockfish and AlphaZero.[48] From its earliest inception, computer programming had its roots in game theory. The race to surpass the human mind in games of multidimensional thinking like Go, Shogi, and Chess were felt to be forever beyond the grasp of nascent computer programming. In 2018, Stockfish version 9 was the undisputed king of the chessboard. With every move of every game in recorded history in its database and computational speed of 60 million moves per second, Stockfish was lightyears ahead of the programs than first conquered the best human players in the world. Pitted against an upstart program in a 1000 match challenge was AlphaZero. The program could calculate a mere 60,000 moves per second and had not one game in its database. With no match experience and armed only with the instructions of play, AlphaZero was armed only with Deep Learning and a novel neural network. The rest is history. After 3 hours of self-study, AlphaZero registers its first win against the most powerful chess program in history. After 9 hours of play at a limit of 15 minutes a move (a lifetime in computer chess), AlphaZero never lost again.

The power and speed of deep learning programs will improve our ability to care for patients and accelerate every aspect of our journey to molecular medicine and individualized care. The future is now.

CONCLUSIONS

This review of critical trends in retinal research has touched on some of the vast efforts of clinician scientists around the world. The last critical trend is the need for collaboration. The randomized clinical trial, the Diabetic Retinopathy Clinical Research network, the Human Genome Project, and virtually every area and strata of civilization will depend on the ability of people to work together toward a common goal. Opportunities for misconduct, misuse, or worse may intensify. The ability to alter the human genome through gene editing and somatic gene rearrangement provides limitless chances for unintended consequences.

And yet, we are living in the most hopeful time in our history. We have limitless chances to improve the human condition and the opportunity to do so together. "If you want to go fast, go alone. If you want to go far, go together."

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