Drug and Gene Delivery to the Back of the Eye: From Bench to Bedside

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The ARVO 2012 Summer Eye Research Conference (SERC 2012) on “Drug and Gene Delivery to the Back of the Eye: From Bench to Bedside” was held June 15 and 16, 2012, at the University of Colorado Anschutz Medical Campus in Aurora, Colorado. The SERC provided a diverse group of approximately 150 scientists and physicians representing industry and academia from 14 countries with a unique opportunity to explore the latest approaches to drug and gene delivery to the posterior segment of the eye. Unlike the 2009 SERC meeting, which focused on novel drug delivery platforms while elucidating the anatomic barriers to reach the posterior segment,1 the most recent meeting explored strategies for bypassing ocular barriers using novel materials, nanoparticle delivery systems, and gene therapy. It brought together experts in both ophthalmology and tangentially related areas to discuss the application and inherent technical challenges for translating experimental results from the laboratory bench to dependable medical therapies at the bedside and, where possible, it exemplified findings in ocular models with methods and results gleaned from disciplines outside of ophthalmology. The present review of the SERC provides investigators with tools to navigate these nascent approaches by exploring strategies from key laboratory investigations, drug development specialists, and clinical trials.

The 2-day conference comprised the following six sessions: (1) barriers to drug delivery and transporter-guided drug design; (2) drug/gene delivery systems and cell therapies for the eye; (3) pharmacokinetics (PK), pharmacodynamics, and alternative routes of drug delivery; (4) nanotechnology for diagnosis and treatment of posterior eye disease; (5) translation of gene delivery for posterior eye disease; and (6) clinical trials.

Rather than being a deliberate summary of each presentation, this review describes the common themes expressed during the six sessions.

OVERVIEW: URGENT NEED FOR MORE EFFECTIVE, COST-EFFICIENT TREATMENTS

According to the Centers for Disease Control, 21 million Americans report functional vision problems or eye conditions that may compromise vision. Age-related vision disorders, including macular degeneration, diabetic retinopathy, cataract, and glaucoma, are the leading causes of blindness in the world. Estimates suggest that the world population will grow to approximately 7.9 billion by 2020 and 76 million people will suffer from blindness.2 The World Health Organization estimated that diabetic retinopathy accounted for approximately 5% of all blindness out of 5 million cases of blindness in the world in 2002 and forecasts an even greater impact in 2020 as the epidemic of diabetes increases worldwide. Globally, total healthcare expenditures associated with vision disorders are expected to reach $2.7 billion by 2020 as the aging population grows.2 Thus, in addition to novel therapies, the current drug development landscape leads to increases in treatment costs, treatment efficiency (personalized medicine, early diagnosis, etc.), and time to market.

The keynote lecture by Henry Edelhauser (Emory Eye Center, Atlanta, GA, USA) provided a holistic view of the current state of drug and gene delivery to the back of the eye, describing the origins of the field, its current status, and its direction. Although many delivery methods have been devised...
over the past few years, intravitreal injection remains the most common method to deliver drugs to the back of the eye, likely due to its relatively low cost, level of familiarity to retinal physicians, and ability to allow practitioners to control the timing of treatment, which removes the burden of compliance from the patient. However, this delivery route poses several distinct disadvantages, including patient discomfort, and the need for repeat injections, which increases the burden of treatment on the patient and physician. Furthermore, age-related changes in vitreal structure and composition, such as liquefaction (synchisis) and collapse (syneresis), can potentially result in differences in drug delivery.

While alternative routes of administration have been investigated, each is met with challenges from both dynamic and static anatomical barriers that contribute to inefficiencies and significant hurdles to drug and gene delivery, as summarized in Table 1. Topical delivery is the safest and least invasive route of administration for ocular drugs. Nevertheless, successful topical delivery to the back of the eye is impeded by static barriers to penetration including conjunctiva, sclera, choroid, retinal pigment epithelium, and the cornea. Dynamic barriers include tear flow and nasolacrimal drainage, subconjunctival-episcleral lymph and blood vessels, choroidal circulation, retinal circulation, and the anterior-directed bulk flow of aqueous humor.4 Although pioneering work by Anders Bill demonstrated that 85% of labeled albumin that was injected into the suprachoroidal space in rabbits crosses the sclera,5 in vivo barriers to back of the eye delivery following topical dosing include static conjunctival barrier as well as dynamic barriers including conjunctival and episcleral lymphatic and blood flows. Alternative periorcular or transscleral drug delivery routes, such as sub-Tenon’s or subconjunctival injection, avoid the physical barriers posed by the conjunctiva, but they still require drugs to traverse several layers of ocular tissues (episclera, sclera, choroid, Bruch’s membrane, and RPE) before entering the posterior segment. Edelhauser noted that the suprachoroidal space is an alternative for topical and transscleral routes of drug delivery and overcomes the conjunctival and scleral static and dynamic barriers.7 Particles as large as 10 μm in diameter have been efficiently introduced into the suprachoroidal space using hollow microneedles penetrating across the sclera.8 Unlike intravitreal injections that penetrate the globe, microneedles only breach the sclera, depositing drug between the choroid and the sclera. Drugs are delivered convectively or via passive diffusion into the chorioretinal tissues within seconds, and large particles can persist there for up to 2 months.9

### Hurdles to the Development of Drug/Gene Ocular Therapeutics

Although the individual steps in research and clinical development for bringing a drug to market are known, the lances for navigating this pathway for ocular drug and gene delivery are not clearly delineated. While most sponsors of clinical trials and their investigators rely on guidance from the Food and Drug Administration (FDA), this guidance has not kept pace with rapid innovations in ocular drug and gene delivery. The FDA recommends that developers meet with the agency early in the clinical research process to clarify assumptions and procedures before committing to expensive clinical trials. The development of new drug delivery systems varies with the drug and its indication. Maturation of a concept in drug development from bench to bedside requires that parallel research programs be conducted that demonstrate feasibility, safety, and efficacy via clinical trials, which can cost several millions of dollars over the course of the development cycle (Table 2).

Vincent H. L. Lee (School of Pharmacy, The Chinese University of Hong Kong) reviewed the common hurdles that innovators face when developing novel drug delivery systems. He explained that in order to promote a concept or platform to the investigational level, innovators must first identify commercial sponsors, secure funding, and understand the regulatory pathways and potential market for the new drug or delivery system. The design of clinical trials and the selection of appropriate patient populations for early-stage safety studies are prerequisites for attracting sponsors and funding for expensive proof-of-concept studies. Feasibility analyses of both the science and the market should be conducted to determine which delivery system and disease to evaluate in clinical trials. Strategies to gain approval for a clinical intervention may need to be developed (Table 2).
Critical questions need to be addressed early in the product development process; some of these include: Are preclinical models that are predictive of efficacy in humans available to generate preliminary evidence of efficacy? Are the clinical and regulatory pathways for approval known? Is there an orphan or reasonably sized market for the selected indication? What is the unmet medical need? How will patients gain access to the new therapy? What are the landscapes of the competitive market and pipeline for the selected indication? What are the costs, timeline, and likelihood of success for the intervention under study?

Regulatory Guidance

The agency vested with the authority to approve therapeutics for patient use in the United States is the FDA. Other countries have similar licensing agencies, such as the European Medicines Agency and the Japanese Ministry of Health and Welfare. Although the approach to drug development differs slightly for each agency, Lee outlined some good development practices for sponsors and investigators to follow. The FDA recommends that sponsors submit target product profiles (TPP) aimed at summarizing the intended labeling and product attributes of a drug candidate or delivery platform. The TPP is a practical tool for outlining the indication, usage, dose (including administration, dose form, and strength), contraindications, warnings/precautions, adverse reactions, drug interactions, target populations, potential for abuse, pharmacology, toxicology, and clinical studies for a novel drug or therapeutic method. In the early phases of research, there may not be sufficient information to provide a complete statement for each attribute in a TPP. However, development of the document is an exercise that helps the sponsor anticipate future studies that are required to support a new drug application (NDA).

Several years before an NDA is submitted, and well before the first clinical trial is designed, innovators should explore the feasibility of a concept using experimental models of the disease. These preclinical trials can include in vitro assays; pharmacokinetic and release kinetic studies; animal safety and toxicologic evaluations; animal models of disease or non-ophthalmic models that have pathologic resemblance to human disease; biomarker and cell fate evaluations; and ADME (absorption, distribution, metabolism, and excretion) studies. The results of these studies can be discussed with the agency at a pre-investigational new drug (IND) meeting. In the United States, pre-IND meetings for biologics and drugs intended for ophthalmic use should be discussed respectively with the Center for Biologics Evaluation and Research (CBER) or the Center for Drug Evaluation and Research (CDER).

Preclinical Studies

Preclinical safety and efficacy testing in animal models is recommended before embarking on clinical studies conducted in humans, and the results of these preclinical studies comprise the initial application for an IND. These applications may come from commercial sponsors or physician-investigators. If a new molecule has never been screened before in humans, then the document will contain the results of safety and toxicology studies that were conducted, preferably in at least two animal species for durations of up to 6 months, to ensure that the drug or delivery system does not adversely affect the anatomy or function of important body systems such as the cardiovascular, respiratory, or central nervous systems (CNS). Additional studies are conducted to define the metabolism and route of elimination. The results of these studies are used to guide clinical trial design and to inform investigators whether studies in special populations (i.e., patients with impaired clearance or
metabolic variances) are needed. Together, results of preclinical studies provide the foundation for selecting the appropriate lower and upper threshold for doses of drugs used in early dose-ranging studies in humans. The FDA provides guidance on the factors to be considered, such as body weight and surface area, when scaling doses between various preclinical toxicology models and humans.9

When estimating the maximum safe dose for ocular drugs, the potential for systemic and/or local ocular toxicity is also taken into consideration. Initial determination of a safe dose in humans is based on careful titration of drug content in samples of animal blood and urine. When the target is in the back of the eye, these bioavailability studies include additional sampling of the aqueous, vitreous, and retina, and histopathological analysis of animal ocular tissues. Other preclinical considerations for devices that deliver drugs to the back of the eye include photosensitization to the drug and/or its carrier, axonal transport of drug to the CNS, and toxicity to the retina/RPE induced by the carrier or by a burst effect. Samples of human blood and urine are also tested during early clinical trials to confirm that the safety thresholds established from animal models are predictive of safety in humans. For new chemical entities, the following additional tests for toxicity are also required: systemic toxicology testing in two species, in vitro and in vivo tests of mutagenic potential, and in vitro evaluations of melanin binding. Gene products/drugs that are delivered to the eye using viral vectors also require long-term monitoring of animal models to predict the possibility for integration of the viral vector into the host genome.

Animal Models

Kay Rittenhouse (Pfizer, San Diego, CA, USA) explained that the selection of an appropriate animal model for efficacy studies should take both the disease and the anatomical similarity to humans into consideration. Incomplete or suboptimal animal studies are commonplace and can hinder the predictive capacity of safety and efficacy studies for humans. Issues that may confound analysis in animal studies include cross-contamination of ocular tissues after euthanasia, variability in time to analysis of tissues, influence of administration route on ocular and systemic pharmacokinetics, and disparities in drug distribution in tissues among dead, anesthetized, and awake animals. As an example of the latter, Rittenhouse et al. demonstrated that after topical application of propranolol, there was nearly a 10-fold higher drug concentration in the aqueous humor of anesthetized rabbits compared to awake rabbits, possibly because anesthesia results in reduction in aqueous humor turnover.10

The use of animal disease models to demonstrate proof of concept for drug delivery systems often yields misleading results because of anatomical differences between species. For example, although rats are routinely used in efficacy models (e.g., laser-induced choroidal neovascularization, CNV), their vitreous volume is approximately 100-fold lower than that of humans. Thus, rats and rodents are not optimal models for scaling exposure or response relationships to humans. Instead, stepwise scaling or examination of ocular pharmacokinetics in larger mammalian species is more desirable.

Rittenhouse also discussed the work of Short, who described the differences in ocular anatomy between nonhuman primates (Cynomolgus monkey) and rabbits.11 In brief, the anterior chamber and lens are comparatively larger in the rabbit, but the vitreous volume is larger in the monkey (3.2 mL). In humans, the vitreous volume is 4 to 5 mL and, depending on age, its composition is 40% to 80% gel. Thus, animal models usually have a lower vitreous volume than humans, but a higher vitreous gel content, as the gel content in rhesus monkeys is 60% and in rabbits, it is nearly 100%. These differences can contribute significantly to the outcomes of intravitreal drug delivery. Although the monkey model bears the greatest similarity to human vitreous, the protein content in the vitreous is significantly lower than that of human vitreous (80–140 µg/mL), and the colloidal levels in human vitreous are ~3 to ~13-fold higher than monkey levels.12 However, the human vitreous protein content is relatively low (~1%–2% of plasma protein levels) and thus, it is likely that these differences would not be important for most drugs. Other inter-species differences also abound, particularly age-related changes in the hyaluronic acid and collagen networks that affect vitreous liquefaction. Liquid content of the vitreous increases with age in humans from 20% at age 14 to 18 years to 50% at age 80 years.13 These biophysical differences can lead to significant disparities between animal models and humans in pharmacokinetics and diffusion of drugs that are targeted for intraocular delivery. These differences must be considered when choosing an appropriate animal model.

While the monkey model may provide the best predictive value for humans, other mammalian models may be selected on the basis of ethical concerns and cost. When this is the case, mathematical modeling or simulation can be used to predict human physiological responses using data collected from several different animal species. For example, Kompella and colleagues calculated the apparent permeability coefficients for eight different beta-blockers across the sclera-choroid-RPE (SCRPE).14 Estimates of drug permeability were calculated across pigmented and albino scleras of different thicknesses from several species. Melanin binding in the choroid-RPE is a key static barrier for drugs that target retina. Melanin is a polyamionic macromolecule that binds lipophilic and cationic drugs through hydrophobic and electrostatic interactions. A comparison of drug permeation between tissues from several animal models and human tissue indicated that together, the animal models could predict the extent of delivery of the beta blockers in human ocular tissue.14

Another important consideration when selecting which animal best models human physiological response is its similarity with respect to local (ocular) and systemic pharmacokinetics. Rittenhouse presented an analysis of two independent studies that compared the pharmacokinetics of bevacizumab (Avastin; Genentech, San Francisco, CA, USA) after intravitreal injection in humans (1.5 mg bevacizumab) with that in rabbits (1.25 mg). Although the aqueous volume in humans and rabbits is similar (~250 µL), the concentration of bevacizumab in human aqueous humor was 35- to 75-fold higher than in rabbit.15,16 The concentration differential was not attributed to the difference in clearance or turnover mechanisms between species since the rate of aqueous turnover in humans, as measured with fluorophotometry, was 2.41 µL/minute and was not significantly different from that of 2.31 µL/minute measured in rabbits. These observations may support the idea that the ~2- to 3-fold difference between human versus rabbit vitreous volume was not a major contributor to the observed ~35- to 75-fold difference in their aqueous humor concentrations of bevacizumab. On the contrary, we would anticipate a dilution of aqueous humor concentration in humans rather than increased aqueous humor levels compared with rabbits (human vitreous ~4 mL versus rabbit ~1.5 mL). Other factors that may cause such differences are possible, including species-related differences in active transport mechanisms; antibody affinity/avidity; and in intraocular neonatal Fc receptor-mediated clearance mechanisms that may modulate intraocular pharmacokinetics and pharmacodynamics. Craig Struble (Covance Laboratories, Madison, WI, USA) described the usual collection of tissues that support ocular ADME and pharmacokinetic studies. Struble explained
that radiolabeled or nonradio-labeled test articles can be used to determine the disposition of drugs in the anterior segment (i.e., aqueous humor, conjunctiva, cornea, iris, ciliary body, lens, tears, and trabecular meshwork/outflow apparatus) and posterior segment (i.e., choroid, optic nerve, optic nerve head, retina, RPE, sclera, and vitreous humor). Aqueous humor, which can be tapped from humans undergoing cataract surgery, represents a surrogate sample for evaluating drug concentration and clearance in the vitreous humor after intravitreal injection. Although the physiologic status of the aqueous-vitreous interface varies with age and disease, there is usually a positive bulk flow from the vitreous humor to the aqueous humor. Rabbits, dogs, and monkeys are appropriate animal models for using the aqueous humor as a surrogate sample for vitreous humor. Sampling of the aqueous humor is performed when the animal is either under general or local anesthesia, and the fluid is tapped using a 29- or 30-gauge needle to remove a sample of the aqueous (25–50 μL volume). In skilled hands, this technique does not cause lens damage, hemorrhage, or endophthalmitis, although transient inflammation is a common consequence. Repeated sampling should be limited to 14- to 30-day intervals. Another sampling technique, microdialysis of the aqueous and vitreous humor, is used less often due to the required complexity of the procedure.

Struble emphasized that careful planning of ocular pharmacokinetic and ADME studies are requisite for successful drug development. In addition to meticulous sample collection—to avoid cross-contamination—other factors that influence the quality of the results should be considered, including: use of an adequate number of animals; anticipation of an appropriate time schedule for sample collection that accurately captures peak and trough levels of the drug; choice of physiologic route of administration; use of bioactive formulations of the test article; and use of robust analytical techniques.

**Strategies for Overcoming Barriers to Drug/ Gene Delivery to the Back of the Eye**

Delivery of drug and gene therapy to the back of the eye is a challenging task due to static, dynamic, and metabolic barriers. The development of predictive models that recreate these barriers remains a challenge in the preclinical setting. Since the main focus of SERC 2012 was translation of novel drug delivery methods, many presenters described research on innovative strategies for circumventing the barriers to drug delivery, such as nanoparticles, polyethylene glycol conjugated (PEGylated) macromolecules, and erodible implants (Table 1).

**Overcoming Barriers to Topical Drug Delivery**

Topical instillation of ophthalmic drops is the most common method of administering drugs to treat ocular disease; 90% of ophthalmic drug formulations are for topical use. The cornea and the anterior-directed aqueous humor bulk flow are key static and dynamic barriers to the absorption of topical drugs, respectively. Prolonged contact time with the cornea has been successfully exploited to achieve high intraocular levels of topically applied drugs by using ointments and drug-eluting contact lenses, which in turn increase the potential for topical formulations to penetrate to the aqueous and access the posterior segment via trans-scleral routes following prolonged contact with the conjunctiva, which is more permeable than the cornea. Still, penetration of the vitreous and retina have only been marginally successful with these approaches.

A number of experimental topical drug therapies for treating posterior segment diseases have been evaluated in the 2 years since the last SERC on drug delivery. Clinical trials for nepafenac (ocular inflammation); TG100801 (choroidal neovascularization); mecamylamine (diabetic macular edema); and pazopanib (choroidal neovascularization) suggest that new topical formulations are potentially capable of penetrating the barriers and accessing the back of the eye, possibly via the transconjunctival-trans-scleral pathway. Still, bioavailability needs to be improved.

**Polyguanidylated Translocators for Topical Delivery**

Antibiotics are commonly applied topically multiple times each day to maintain effective drug levels in tissues of the ocular surface and aqueous humor. Furthermore, antibiotic eye drops do not deliver adequate drug levels to the back of the eye for treating complications such as endophthalmitis. Key limitations to delivery of any drug from an eye drop to the back of the eye include: low solubility, which limits dose strength; poor permeation of ocular surface tissues, especially conjunctiva, since topical drugs are primarily made for corneal entry; and short retention in the precorneal area and the ocular surface tissues. To overcome these limitations, Uday Kompella (University of Colorado Denver, Aurora, CO, USA) and team developed dendrimeric polyguanidylated translocator (DPT)-based, nano-sized drug complexes for enhanced drug solubility, cell entry, and sustained delivery (Fig. 1). In their study, g6 DPT (~1.7 kDa) possessing six cell-penetrating guanidine surface groups with flexible amino-triol branches and a functionalized core available for conjugation, was used. Dendrimers are organic chemical-branched, tree-like structures that offer several advantages over structures used in other types of nanoparticles, particularly their smaller size (<20 nm compared with >80 nm for polymeric and liposomal nanoparticles). The solubility of gatifloxacin could be increased by several-fold in a dose-proportionate manner with an increase in the concentration of g6 DPT in the formulation. This allowed the development of eye drop solution formulations with 5-fold greater strength than those available in the market at the time of this research. In additional experiments, a g6 DPT formulation of the fluoroquinolone antibiotic gatifloxacin increased the transport of gatifloxacin by 40% across bovine SCRPE over 6 hours. In cell cultures, the dendrimeric formulation gained entry into human corneal epithelial cells within 5 minutes of exposure. However, it should be noted that cultured cells may not fully mimic the biologic and barrier properties of intact cornea. Studies in New Zealand white rabbits demonstrated that aqueous humor levels of the modified drug were maintained for at least 24 hours after topical instillation of two drops.

**Hydrogels for Topical Delivery**

Kompella also described the reformulation of two antiglaucoma drugs, brimonidine (M₁ 442.24) and timolol (M₁ 432.50), in a hybrid dendrimer hydrogel/nanoparticle platform (HDNP) designed to deliver sustained therapeutic levels of both drugs following a single topical administration. Commercial formulations of both drugs require dosing of at least twice a day. A hybrid dendrimer nanoparticle formulation of brimonidine and timolol was designed to both sustain the release of brimonidine and timolol and increase the ocular surface residence time. The formulation consisted of biodegradable nanoparticles loaded with brimonidine and/or timolol maleate and cross-linked with a polyamidoamine dendrimer hydrogel. HDNP uptake by human corneal cell epithelium was significantly higher (~3-fold) than that of plain nanoparticles at all time points tested (5 min, 1 hour, and 3 hours) and was not cytotoxic after 24 hours of exposure to human corneal...
epithelial cells. The HDNP formulations were adhesive to mucin as determined by a mucin adhesive assay. Initially, mucin particles were isolated from gastric porcine mucin by mechanically disrupting the gastric solution. The zeta potential of mucin particles increased from $7.13\text{ mV}$ to $3.6\text{ mV}$ after incubation with dendrimer hydrogels. The increase in zeta potential is due to the adsorption of the hydrogel to the mucin particles. In human corneal epithelial cell cultures, the uptake of the HDNP formulation was higher than the uptake of plain nanoparticles in phosphate-buffered saline.\(^{19}\)

The in vitro release of both drugs from HDNP was sustained for 28 days, which compared very favorably to dendrimer hydrogel formulations that sustained release for less than 1 day, and to unprocessed drugs that sustained release for less than 12 hours. Seven days after a single eye drop containing brimonidine and timolol was administered to Dutch-belted male rabbits, levels of each drug in the aqueous humor were higher for the HDNP formulation compared to the dendrimer only formulation, which in turn was higher than the unprocessed drugs.\(^{19}\) Reduction in intraocular pressure (IOP) could be measured in as little as 30 minutes after administration of the HDNP formulation and the peak effect of 37.6\% reduction from a baseline of 22 mm Hg was observed at 6 hours after dose. By comparison, saline formulations of brimonidine/timolol produced IOP lowering after 1.5 hours with a peak effect of 18\% at 3 hours. The pressures in HDNP-treated animals continued to be lower than those treated with saline and dendrimer control formulations at 7 days after treatment, with the efficacy being statistically significant up to day 4.\(^{19}\) Thus, animal studies have shown that a hydrogel and dendrimer formulation of two topical antiglaucoma drugs extended the duration of IOP lowering for 4 days in rabbits.

**Overcoming Barriers to Intraocular Drug Delivery**

One approach to retinal drug delivery is the introduction of a needle that penetrates the globe, thereby permitting the placement of a therapeutic substance in the vitreous (intravitreal injection) or between the retina and the RPE (subretinal injection). Though invasive, these routes of drug delivery achieve the highest intraocular bioavailability by bypassing...
several anatomic and dynamic barriers of the posterior segment. Most intravitreally administered drugs are of short- to medium duration because dynamic clearance mechanisms, such as anterior bulk aqueous flow or posterior vitreoretinal-choroidal flow, eliminate them from the site of deposition. As a result, it is necessary to administer the drug frequently to maintain adequate intraocular concentrations. In chronic ocular disease, this poses a burden for both caregiver and patient. Frequent injections increase the risk of injection-related adverse events, including endophthalmitis, hemorrhage, retinal detachment, and transient glaucoma. One strategy for mitigating the challenge of frequent injections is to change the drug’s formulation or modify specific properties of the drug, such as size, charge, and lipophilicity.

Hyuncheol Kim (Sogang University, Republic of Korea) shared his understanding of the biophysical properties of the vitreous to enhance the effectiveness of drugs after intravitreal injection. Intravitreal nanoparticles rely on the charged surface of the nanoparticle to permit penetration of the retina and diffusion in the vitreous. Kim found that cationic nanoparticles of human serum albumin (HSA) interacted with the negatively charged glycosaminoglycans in the vitreous, consequently impeding their diffusion in the vitreous and penetration of the retina. Conversely, anionic nanoparticles of non-viral DNA nanoparticles carrying the mouse opsin promoter and wild type mouse Rds gene has shown promise in rescuing a mouse model of retinitis pigmentosa.
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HSA diffused readily in the vitreous before penetrating the retina.25 Kim emphasized that the vitreous acts as a static barrier to drug delivery to the posterior segment, and illustrated the role of nanoparticle surface charge as instrumental in hindering or facilitating diffusion across the vitreous and entry into the retina with several examples of engineered nanoparticles. Assessment of the transit of four different types of spherical nanoparticles, which were similar in size (230–300 nm) but differing in surface properties, was tracked with fluorescent dyes for their delivery from the vitreous to the retina after intravitreal injection.22,23 Polyethyleneimine (PEI) nanoparticles were fully aggregated before they reached the retina due to their strong positively charged surfaces (33.4 ± 5.1 mV). Glycol chitosan (GC) nanoparticles, also positively charged but to a lesser degree than PEI nanoparticles (16.4 ± 3.2 mV), diffused across the vitreous and reached the inner limiting membrane (ILM) of the retina, but were unable to penetrate deeper retinal structures. Hyaluronic acid (HA) nanoparticles did not aggregate in the vitreous due to their strong negatively charged surfaces (~26.2 ± 4 mV), and most of these nanoparticles penetrated the retina and entered the RPE cell layer. HSA nanoparticles, whose sizes ranged from 200 to 300 nm, rapidly penetrated the ILM via clathrin-mediated endocytosis to the subretinal space, due to their negatively charged surfaces (~20.9 ± 2 mV), and likewise penetrated the Müller cell layer through endocytosis. Hybrid combinations of nanoparticles exhibited surface properties that reflected their constituents. For example, PEI/GC hybrid nanoparticles (20 ± 3.2 mV) were also able to reach the ILM due to the reduced positive charge conferred by the PEI. Conversely, HSA/GC hybrid nanoparticles (~1.9 ± 4.1 mV) accumulated in the ILM but could not penetrate it to reach deeper retinal structures, unlike HSA nanoparticles. HSA/HA hybrid nanoparticles (~23.5 ± 4.4 mV) were able to penetrate all retinal layers and easily reached outer retinal structures, including the photoreceptor layer and RPE.

PEGylation for Intraocular Delivery

One novel approach to overcoming the burden of frequent injections often required for intraocular drug delivery is to design sustained or continuous delivery platforms, such as implants, nano/microparticles, or cell encapsulating technologies. At the previous 2010 SERC on retinal drug delivery, Mark Kester theorized that nanoparticles could be PEGylated on their exterior surface to both evade the immune system and target specific tissues. At the 2012 meeting, Steve Brocchini (UCL Institute of Ophthalmology, London, UK) expounded on this approach by investigating the effects of PEGylation on the dissociation kinetics of various versions of bevacizumab and ranibizumab for intravitreal delivery. Attachment of PEG molecules to proteins has been widely used in the biotechnology industry to increase the vascular half-life of a protein while maintaining the therapeutic activity of the molecule. The treatment of hepatitis C with interferon α-2 is an example where the attachment of PEG polymers has led to sustained vascular levels of these proteins for 7 days. Brocchini described methods to create PEGylated versions of the anti-VEGF proteins bevacizumab and ranibizumab,24 two therapeutic antibodies that are cleared rapidly from the vitreous.

At this conference, Brocchini presented preliminary binding results for 2 PEG constructs: PEG-Fab and Fab-PEG-Fab, where “Fab” was derived from bevacizumab (Fig. 2). Comparative binding studies were conducted by either enzyme-linked immunosorbent assay or surface plasmon resonance using Biacore (GE Healthcare Biosciences, Pittsburgh, PA) with low ligand immobilization density and VEGF(165) for the bevacizumab-derived products.25 Binding constants (Kd and Kd) were calculated for Fab-PEG-Fab, and the equilibrium constants Kd (Kd/Kd) were compared and shown to be similar to un-PEGylated bevacizumab (Kd = 1.27–1.54 nM versus Kd = 1.33 nM), which was lower than PEG-Fab (Kd > 10 nM) according to the Biacore results.24 In a cell culture-based functional assay, anti-VEGF Fab products with longer PEGylation sequences (e.g., Fab-PEG[20 kDa]-Fab) reduced both tight junctions and tube formation to a greater extent than those with shorter PEGylation sequences (e.g., Fab-PEG[6 kDa]-Fab).24 Future work will focus on demonstrating that these PEGylated proteins can find their targets in the diseased eye (Fig. 2).

Overcoming Barriers to Periocular Drug Delivery

Periocular drug delivery methods, such as sub-Tenon’s and subconjunctival injections, can utilize the trans-scleral pathway to effectively deliver drugs next to the choroid. Since the drug has to pass through several layers—including the episclera, sclera, choroid, Bruch’s membrane, and RPE—while overcoming choroidal circulatory clearance, delivery is not as effective as intraocular injections in targeting the retina.

Edelhauser reported that literature describing the dynamic clearance mechanisms and metabolic impediments to periocular drug delivery is lacking.1 Therefore, this type of drug delivery is the subject of intense research. Improvements to formulations that either increase residence time in the middle coat or promote diffusion from the middle coat may be effective ways to overcome the barriers to periocular drug delivery.

Hydrogels for Periocular Delivery

Hydrogels are cross-linked (chemically and physically) three-dimensional structures that can control drug release in response to a stimulus. The gels are mostly liquid at room temperature and solid at body temperature. When implanted in the subconjunctival space, hydrogels can also sustain the release of drugs for delivery to the back of the eye (Fig. 3). Tao Lowe (University of Tennessee Health Science Center, Memphis, TN, USA), discussed how subconjunctival placement of insulin-impregnated hydrogels could potentially be used to treat diabetic retinopathy. Subconjunctival placement of a hydrogel is a minimally invasive procedure that has advantages over both topical and intravitreal injections. According to Lowe, certain hydrogels are thermo responsive and hydrolytically degradable polymers that can be cured with ultraviolet light.25 At temperatures below the lower critical solution temperature (LCST), the hydrogel expands and swells, whereas at temperatures above the LCST, the hydrogel shrinks and collapses. Biocompatible and thin (1.6-mm thick) hydrogels were fabricated by copolymerizing N-isopropylacrylamide monomer (NIPAAm) with a dextran macromere containing multiple hydrolytically degradable oligolactate-2-hydroxyethyl methacrylate units (Dex-lactateHEMA).

When gels were loaded with FITC-labeled insulin and placed in the superobulbar-subconjunctival space of Sprague Dawley rats, the release of drug could be sustained for at least 18 days.25 When a hydrogel was constructed with a LCST of 32°C, its properties were such that at physiological temperature (37°C) it shrank and collapsed, allowing labeled insulin to be released slowly. The rate of drug release could be altered by changing the ratio of NIPAAm to DEX-lactate-HEMA, where the highest ratio of 8:1 resulted in the longest duration of sustained release (18 days). The size of the hydrogel also influenced the release profile, wherein larger diameter hydrogels (4.5 mm diameter and 1.6 mm thick) exhibited a zero-order release.
profile and smaller diameter hydrogels (2 mm diameter and 1.6-mm thick) exhibited diffusion-controlled release. The insulin loading efficiency in these hydrogels was very high, at 98% for 15% wt/vol insulin. The insulin released from the hydrogel exhibited similar activity to that of control insulin, as determined by a Western blot assay.

In the Sprague-Dawley rat, there was no acute or delayed retinotoxicity after implantation of the hydrogel, as measured by histology studies. The hydrogel seemed well-tolerated, based on signs of ocular irritation and visual performance as evaluated by full field electroretinograms.

Although the proof of concept for hydrogel-based delivery of insulin is robust, the usefulness and generalizability of this drug delivery system in an animal model of ocular disease remains to be proven.

**Microneedles for Suprachoroidal Delivery**

Hollow microneedles are an experimental device used for intrascleral and suprachoroidal delivery of drugs.8 The choroid is part of the uveal tract, which is attached to the overlying sclera at three sites: the sclera spur, exit channels for vortex veins, and the optic nerve. The virtual space between the sclera and choroid is called the suprachoroidal space, and exploration of the capacity and access to this space for drugs has been an exciting area of development (Fig. 4). Microneedle-facilitated drug delivery uses a trans-scleral approach to the suprachoroidal space, which may avoid most of the safety shortcomings of repeated intravitreal injections as well as the bioavailability shortcomings of periocular injection.

In rabbits, the volume of this space is approximately 20 μL and molecules are cleared from it within 1 day. In a rabbit model, direct injection of India ink into the suprachoroidal space revealed the tracer’s location in this space and its swift diffusion around the entire globe within seconds.8 This method is capable of being used with particles ranging in size from 20 nm to 10 μm in diameter. For retinal drug delivery, the barriers for drugs that are injected into this space include choroid and RPE. Alternatively, if the choroid vasculature is the target of drug therapy, this approach might be ideal (Fig. 4).

Microneedles offer a minimally invasive approach for delivering drug to this space and Clearside Biomedical, Inc.
Alpharetta, GA, USA) is currently developing platforms for microneedle-assisted delivery of different types of drugs to the back of the eye. In animals that had been injected with microneedles, the insertion site was no longer visible 1 hour after injection and the eye appeared indistinguishable from a naïve eye upon histologic inspection.8 In the clinical setting, microneedle injection in the suprachoroidal space would be an outpatient surgery procedure, similar to intravitreal injection but much less invasive.

Overcoming Barriers to Systemic Delivery

The penetration of almost all drugs from the systemic circulation is restricted by the blood-retinal-barrier (BRB). The vascular and epithelial components of the BRB maintain the specialized environment of the neural retina.26 The inner BRB includes all blood vessels in the retina with tight intercellular junctions between the retinal vascular endothelial cells. The outer BRB is constituted by the RPE, which also possesses well-developed tight junctions that confer a high degree of control over solute and fluid permeability.

Large molecules and/or hydrophilic drugs, such as fluorescein, are able to penetrate the choroid from the systemic circulation, but are unable to cross the inner BRB into the retina. Thus, in order to breach these static barriers to the retina from the systemic circulation, drugs must exit the choroidal circulation and permeate the outer BRB. With some drugs this can be accomplished with large systemic doses, but the dilution effect restricts the concentration and efficacy of drugs at the target.1 However, these and other vascular barriers, including choroid, may be compromised in disease states, resulting in enhanced drug delivery. Kompella presented a scenario in which systemic delivery of surface-functionalized nanoparticles could enable treatment of choroidal neovascularization (CNV) in an animal model. A single intravenous dose of anti-VEGF intracavity plasmid-loaded nanoparticles that were decorated with RGD peptide led to a statistically significant reduction in retinal and choroid-RPE expression of VEGF in Brown-Norway rats that had laser-induced CNV27 (Fig. 5). Treatment was administered intravenously 14 days after laser injury; delivery was assessed by flatmounts; VEGF expression was characterized by ELISA; and tissue was characterized via histopathology.
Modification of the nanoparticle surface with RGD, transferrin, or both markers resulted in enhanced delivery of nanoparticles to the back of the eye in the CNV model. In normal eyes with no laser injury or CNV induction, nanoparticle extravasation at 24-hours after dosing was not evident. Fluorescence imaging of retina-choroid-sclera flatmounts demonstrated the presence of nanoparticles in the laser-treated eye only, due to vascular leakage of the damaged tissue. Delivery to the posterior segment of the eye was more robust with RGD-functionalized nanoparticles, possibly due to the abundant expression of integrin receptors on neovascular endothelial cells. No nanoparticles were detected in the brain of any animal. All functionalized nanoparticles (RGD, transferrin, or RGD plus transferrin) traversed the RPE cell layer in vivo, thereby reducing retinal VEGF levels more effectively than nonfunctionalized or naked plasmid. Additional studies showed that intravenously delivered, RGD-functionalized, VEGF intracorder gene-loaded nanoparticles are effective in mouse and monkey models of choroidal neovascularization. If future testing of these functionalized nanoparticle constructs reveals them to be relatively safe, it may ultimately enable systemic delivery of gene therapy while avoiding more invasive methods such as subretinal injections (Fig. 5).

**OVERCOMING BARRIERS TO THE DELIVERY OF SMALL MOLECULES, PROTEINS AND GENES**

One of the major barriers to the development of drugs made from small molecules, proteins, and genes is chemical and physical instability. Such instability can lead to loss of activity and immunogenicity, when the drugs aggregate in tissues, are further destabilized by nucleases, or are incompletely released from the delivery vehicle. Formulation excipients such as sugars, surfactants, and basic salts are used routinely to help stabilize proteins and small molecules intraocular drug solutions. This section describes some of the novel approaches that have been used to overcome barriers to the delivery of small molecules, proteins, and genes.
Small Molecules

An innovative approach to improve the permeability and delivery of small molecules targeted for the posterior segment is to use biological transporters that can chauffeur the therapeutic protein across the barrier to its intended target. The implications of using such solute transporters for ocular drug delivery were discussed by Vadivel Ganapathy (Georgia Health Sciences University, Augusta, GA, USA), who previously described the presence of two different endogenous Na+-coupled oligopeptide transporters in cells cultured from the gut. Similar to the gut, RPE and corneal cells both possess the same transport systems, sodium-coupled oligopeptide transporters (SOPT)-1 and 2, which recognize synthetic peptides containing five or more amino acids. These two endogenous transporter systems, SOPT1 and SOPT2, can be experimentally distinguished by their differential inhibition by dipeptides and tripeptides. When the intended ocular target is the retina, therapeutic agents can be coupled to peptide substrates which are preferentially transported by SOPT 1 and SOPT 2 and used as prodrugs designed for retinal delivery.

Ashim Mitra (University of Missouri-Kansas City, Kansas City, MO, USA) provided an update on the potential value of using transporters to deliver drugs across the corneal epithelium, the primary barrier to topical drug delivery. As a case study, Mitra discussed the use of acyclovir for the treatment of keratitis, a drug that presents delivery challenges due to its hydrophilicity and inability to permeate the cornea. Acyclovir (M, 225) is a guanosine-analogue antiviral. Pharmacology studies indicated that when the lipophilicity of acyclovir was increased to improve permeability in the cornea, its solubility and bioactivity in the aqueous humor was consequently reduced. To circumvent these obstacles, a transporter was constructed that produced a permeable prodrug, dipeptide-acyclovir, capable of being hydrolyzed to its active form within the deeper layers of the cornea without sacrificing its solubility in the aqueous humor.

Dipeptide-acyclovir (L-Val-L-Val-acyclovir) was a very effective prodrug that could be taken up by dipeptide transporters in the cornea. Mitra explained that the stereoisomer of the dipeptide was recently shown to more specifically affect transport, such that its L-isomer is transported more efficiently than its D-isomer, but hydrolysis of the D-isomer was discovered to be slower than that of the L-isomer. The researchers discovered that the dipeptide containing the sequence L-Val followed by D-Val was delivered to the cornea more effectively after oral administration than the original L-isomer (Val-Val) dipeptide. Thus, as illustrated by this example, dipeptide prodrugs can be utilized to improve ocular permeability following topical or oral administration.

Mitra also demonstrated that cellular entry of acyclovir is enhanced by placing a lipophilic linker between the drug and its transporter moiety. Transport of acyclovir across the membranes of MDCK and Caco-2 cells was significantly higher when acyclovir was linked to a biotin transporter via a ricinoleic acid molecule (an unsaturated omega-9 fatty acid) than either acyclovir that was conjugated directly to biotin or acyclovir alone. This represents a novel approach to targeting lipid prodrugs that also improves absorption of the parent drug.

Rocio Herrero-Vanrell (Universidad Complutense de Madrid, Madrid, Spain) described her novel microencapsulation technique for prolonging the release of acyclovir from biodegradable microspheres for use in intraocular administration. Using additives, such as vitamin A in poly-lactic-co-glycolic acid (PLGA) microspheres, changes the release profile of acyclovir from bimodal to zero-order, although the overall length of sustained release (40 days) remains unchanged. The antioxidant properties of vitamin A provided additional protection for the drug. Herrero-Vanrell demonstrated the additional applicability of this method for prolonging the release for up to 11 weeks of glial cell line-derived neurotrophic factor (GDNF) from GDNF/Vitamin E PLGA microspheres, which could be sustained. This application may prove clinically useful as a neuroprotective tool to preserve retinal ganglion cell survival.

Patrick Hughes (Allergan, Irvine, CA, USA) discussed bioerodable and nonerodable implants, which have been described extensively in the delivery of medical therapy to the vitreous. Consequently, a detailed discussion will not be presented here except to describe novel ways of regulating small molecule release from microsphere-based PLGA erodible implants. The Ozurdex implant (Allergan), a PLGA copolymer matrix that contains 0.7 mg dexamethasone, has been approved by the FDA most recently (in 2012) for clinical use in branch retinal vein occlusion.

Bioerodable implants like Ozurdex offer several advantages over nonerodable implants including the ability of the polymer matrix to degrade into nontoxic metabolites, and the lack of a need for implant removal at the end of drug delivery.

Proteins

Stabilization of a protein-based drug product can be challenging and emerging methods to enhance stability by producing the protein within the body or using engineered cells or genes to deliver the drug product in the eye have been examined in clinical trials. Alternatively, if the final protein product is produced ex vivo and delivered in its final form, it can be engineered with nanoparticles to achieve enhanced stability and reduced immunogenicity.

 Nanoparticles can also be used to stabilize microparticles that deliver therapeutic proteins (e.g., bevacizumab) to the eye. This method, in which microspheres are loaded with specific nanoparticles, may also increase the residence time of a drug, such as anti-VEGF, obviating the need for frequent injections. Kompella reported that release of the protein therapeutic bevacizumab from microspheres can be modulated by varying the percentage of nanoparticles that are encapsulated in the microparticles. For example, loading of microspheres with a relatively smaller percentage (5%) of nanoparticles results in an initial burst release of approximately 25% followed by a zero-order release for up to 120 days. In contrast, loading a larger percentage of nanoparticles (25%) results in a much larger burst effect of approximately 50% and followed by slow release for up to 90 days. The stability of bevacizumab after its release from microspheres was similar to that of the native protein for up to a few months, as determined by size exclusion chromatography and circular dichroism studies.

Gene Therapy

Gene therapy is a uniquely suited treatment modality for retinal diseases due to direct access by intravitreal or subretinal injection and the immunologically privileged status of the eye. Many gene mutations contribute to the pathogenesis of ocular diseases including glaucoma and retinitis pigmentosa (RP), and several treatment strategies for overcoming these genetic defects have been attempted and proven in tissue culture and animal models. Gene therapy is also an attractive approach for the treatment of diseases that require gene replacement, including Leber congenital amaurosis (LCA), X-linked retinoschisis, and Stargardt disease, as well as in other ocular diseases: neovascular diseases, ischemic retinopathies with macular edema, retinal degenerations, and hereditary
Drug delivery trials

Gene delivery trials

- LCA2
  - NCT00821540: rAAV2.hrPE65
  - NCT01496040: rAAV2/4.hrPE65
  - NCT00481546: rAAV2-CBSB-hRPE65
  - NCT00749957: rAAV2-CBHRPE65

- Choroideremia
  - NCT01461213: rAAV2.REP1

- Nonarteritic anterior ischemic optic neuropathy (NAION)
  - NCT01064450: QPI-1007; siRNA inhibitor targeting caspase-2

- AMD
  - NCT00109499: AdGVPDE11D

- Stargardt disease macular degeneration
  - NCT01367444: StarGen equine infectious anemia virus (EIAV) lentiviral vector that expresses ABCA4
  - NCT01736592: RetinoStat EIAV lentiviral vector that expresses endostatin and angiostatin

- RP associated with Usher syndrome
  - NCT01590621: (U)Stat EIAV lentiviral vector that expresses MYO7A

- Retinal disease
  - NCT01024998: AAV2-sFLT1
  - NCT01482195: rAAV2.VMD2-hMERTK

Drug delivery trials

- Metastatic melanoma of the eye
  - NCT00738861: Albumin nanoparticles

- Diabetic macular edema
  - NCT01523514: Cyclodextrin microparticles

Table 3. Clinical Trials in Ocular Gene and Drug Delivery

<table>
<thead>
<tr>
<th>Indication</th>
<th>ClinicalTrials.gov Identifier</th>
<th>Vector/Delivery System</th>
<th>Route of Delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gene delivery trials</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCA2</td>
<td>NCT00821540</td>
<td>rAAV2.hrPE65</td>
<td>Intraocular</td>
</tr>
<tr>
<td>Choroideremia</td>
<td>NCT01461213</td>
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<td>Diabetic macular edema</td>
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<td>Topical</td>
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optic neopathies. Gene delivery to the back of the eye tissues is fraught with several problems including poor cell permeability and degradation by ubiquitous nucleases. Both nanoparticles and virus delivery systems have demonstrated potential in achieving therapeutically relevant gene therapy formulations.

Self-Assembling DNA Nanoparticles for Gene Therapy

The delivery and proof-of-concept for self-assembling nonviral DNA nanoparticles via different routes of injection for the treatment of RP was described by Muna Naash (University of Oklahoma, Oklahoma City, OK, USA). DNA nanoparticles were fabricated by mixing plasmid DNA with a 30-mer polylysine peptide containing an N-terminal cysteine conjugated via a maleimide linkage to a 10-kDa PEG linker. The cationic nature of polylysine interacts electrostatically with negatively charged tissues is fraught with several problems including poor cell permeability and degradation by ubiquitous nucleases. Both nanoparticles and virus delivery systems have demonstrated potential in achieving therapeutically relevant gene therapy formulations.

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Nanoparticle strategies to deliver therapeutic genes have been evaluated in clinical trials in fields other than ophthalmology. In 2004, compacted DNA nanoparticles carrying the cystic fibrosis transmembrane regulator-encoding gene were administered to the nasal mucosa of subjects with cystic fibrosis in an effort to evaluate safety and tolerability in a phase 1 clinical trial. The nanoparticles were well-tolerated, a result which could be translated into drug delivery to the posterior segment. Naash and colleagues have found that nanoparticles can efficiently transfect RPE cells as early as 2 days after treatment and can persist for up to 30 days.

Adeno-Associated Virus for Gene Therapy

Recombinant viruses provide an effective method for gene delivery to the retina, and AAV has taken the lead in the development of gene therapies for inherited retinal degenerations. While lentiviral vectors are in development, they transduce photoreceptors poorly. In contrast, infects both photoreceptor and RPE following subretinal injection. Alfred Lewin (University of Florida, Gainesville, FL, USA) described how the efficiency of AAV transduction of retinal neurons has been increased by modification of the viral capsid proteins: replacing surface tyrosine residues with phenylalanine permits infection of retinal ganglion cells, bipolar cells, photoreceptors and RPE following delivery of the virus to the vitreous. Nevertheless, cell specificity can be achieved using cell type-specific promoters: the VMD2 promoter for RPE and the ribodofin promoter for rod cells. A major limitation of AAV is its small carrying capacity: 5 kb of DNA. This impediment is being addressed by co-injection of two viruses constructed such that recombination reconstructs the reading frame for a large gene. As shown in Table 3, AAV delivery has...
proved safe for gene therapy in several clinical trials for Leber congenital amaurosis type 2 (LCA-2). Expression was stable for over 3 years.54 All patients gained vision benefits except those experiencing a foveal detachment during delivery. With the advent of AAV that can be delivered from the vitreous, this danger should be minimized.

Recent gene therapy efforts have shown great clinical success in treating a relatively rare form of RP (i.e., an RPE65 mutation) in patients.55–59 However, the use of gene therapy for vision loss is complicated by the extraordinary genetic heterogeneity of retinal degeneration (over 180 different genes are associated with RP)60 and the loss of photoreceptors.61 Alan Horsager (University Southern California Institute For Genetic Medicine, Los Angeles, CA, USA) postulated that an ideal therapy would be able to restore vision in blind patients, independent of the specific gene mutation and subsequent loss of photoreceptors, by targeting a downstream point in the visual pathway. Indeed, the use of microelectronic retinal prostheses has shown fundamental success in generating visual perception in blind subjects by electrically stimulating spared neurons of the inner retina.62–64 Still, electrically activating these neurons requires large disc electrodes (at least 25 times the diameter of a retinal ganglion cell), leading to broad and indiscriminate activation of multiple cell types,65 greatly complicating the normal spatial and temporal process of the retina.66 Thus, there is great need for a therapy capable of treating photoreceptor degeneration independent of the mutation or cause, and accurately driving specific retinal cell subpopulations so as to mediate naturalistic neural responses and high-resolution visual perception.

Recently, several groups have separately demonstrated the building blocks for a successful therapy in which spared retinal neurons are sensitized to light, although they have not assembled these ideas into a coherent translational technology which is capable of treating blindness. One group used AAV to safely and permanently label retinal ganglion cells in mice that were homozygous for the rd1 mutation (rd1; Pde6brd1), with 20% expression efficiency of Channel rhodopsin-2 (ChR2),67 rendering ganglion cells light-sensitive. However, targeting ganglion cells bypasses substantial inner retinal circuitry, much of which is involved with spatial and temporal processing of visual input.65,68 Another group targeted ChR2 to the ON bipolar cells of rd1 mouse pups using electroporation and the GRM6 promoter, leading to improved physiological and behavioral responses to light stimuli.69 However, ChR2 expression levels were low (~7%) and delivery was conducted via electroporation, a technique that is not clinically viable.

Horsager and colleagues demonstrated a potential therapy for late stage retinal degeneration using ChR2, a light-sensitive cation channel derived from algae. Using an AAV vector, they have expressed ChR260 in the bipolar cells of the inner retinae of blind mice with approximately 20% efficiency using the metabotropic glutamate receptor 6 (GRM6) promoter sequence. Expression of ChR2 has restored visual light sensitivity in these mice, as detected by both behavioral and physiological measures. Targeting retinal bipolar cells with ChR2 allows the retina to respond to external light and, more importantly, conveys visual information to the brain in the absence of photoreceptors. Targeted ChR2 expression leads to electro-physiological and behavioral improvements in visual function in three different mouse models of blindness (rd1, rd10, and rd16), with visual responses lasting at least 10 months postinjection (the longest time point measured).70 Finally, the safety profile of this therapy shows exclusive ocular biodistribution, limited immune cell infiltration and inflammation, and an absent systemic immune response as measured through hematology, serum chemistry, and delayed-type hypersensitivity tests. Taken together, these data suggest that virally delivered ChR2 may provide a viable clinical therapy for photoreceptor disease-related blindness.

**CLINICAL TRIALS**

Currently there are 16 clinical trials registered at https://clinicaltrials.gov that feature intraocular gene and drug delivery to the back of the eye (Table 3). AAV constructs are used almost exclusively in ophthalmic gene therapy trials, as the virus is very efficient at transducing both dividing and nondividing cells, such as photoreceptors, and has the potential for long-lasting expression. AAV has several other desirable properties for clinical trials including a long history of clinical testing, such that its safety and tolerability profiles are well-known. No vector-related safety issues have been reported, although there have been issues related to subretinal surgery including retinal detachment, choroidal effusions, ocular hypertension in the immediate postoperative period, and ocular hypertension associated with the administration of topical steroids.72 Measurable efficacy has been reported in most patients from all 5 trials.54,58,59,72 In these trials, a subretinal injection was made near diseased tissue, with precautions taken to avoid injection of healthy tissue, in order to prevent damage to remaining vision. Improvement in visual fields was observed 9 months after treatment in areas consistent with the locus of treatment. This is most likely due to a delivery issue of the vector, and investigation should be conducted to determine how to improve diffusion of the virus to treat the rest of the diseased tissue. A potential approach may be to use a different vehicle to deliver the gene to the tissue that is more easily diffused, or perhaps a different route of injection, such as suprachoroidal, to treat the whole retina.

Other routes of administration for nonviral therapy of retinal diseases have been explored in clinical trials. A nanoparticle formulation of paclitaxel albumin (Abraxane; Celgene, Summit, NJ, USA), which is traditionally used for the treatment of breast cancer, is currently underway to determine whether an intravenous bolus is effective in patients with inoperable metastatic uveal melanoma of the eye (NCT00738361; https://clinicaltrials.gov).

A cyclodextrin-stabilized microparticle formulation of dexamethasone is being evaluated for the treatment of diabetic macular edema. Preclinical and phase I safety studies demonstrated that topical cyclodextrin microparticles were equally as safe as conventional dexamethasone eye drops.73 Phase II and III clinical studies are currently underway to examine the safety and efficacy of cyclodextrin microparticles (NCT01523314; https://clinicaltrials.gov).

**TOOLS FOR IMAGING DRUG DELIVERY TO THE POSTERIOR SEGMENT**

The development of tools for imaging is important in clinical trials because these advanced methods can be used to: detect molecular or physiological alterations that signal the presence of disease; evaluate and adjust treatment protocols in real time; and help streamline the drug development process. Nanotechnology serves as an ideal framework for interfacing optical contrast agents (e.g., dyes) with molecular biomarkers of disease in vivo, because the nanoscale is representative of the scale on which biological interactions occur. Megan Capozzi (Vanderbilt University, Nashville, TN, USA) summarized technologies that are used to detect molecular expression in the retina for the purpose of improving clinical diagnosis, including liposomes, dendrimers, gold nanoshells, quantum
dots, and carbon particles (e.g., fullerene). Contrast agents can be targeted to nanoscale biomarkers using either antibodies or synthetic peptides. Antibody-functionalized fluorescent nanocrystals, termed quantum dots, are capable of targeting anti-inflammatory biomarkers of diabetic retinopathy and uveitis for in vivo retinal imaging in animal models. For example, ICAM-1 was visualized in vivo in the retinal vasculature of diabetic rats using targeted nanocrystals. Multiple fluorophores can be used simultaneously to enable multiplexed imaging of molecular expression in animals.

Hans Grossniklaus (Emory University, Atlanta, GA, USA) discussed how imaging may be translated to the clinical setting by using microbubbles both for delivering drugs and for diagnostic imaging. Microbubbles are micro-sized gas-filled particles (typically filled with oxygen) that enhance visualization of tumor vasculature and blood flow. Different types of microbubbles are currently being used for diagnostic imaging including albumin ( Albunex; Mallinkrodt, St. Louis, MO, USA ); tetradecylfluorohexane (Imagent; Bayer Schering-Alliance Pharma AG, Germany), sulphur hexafluoride (Sonovue; Bracco International B.V., Amsterdam, The Netherlands); Perfluorane lipid microspheres (Leovist; Bayer Australia Ltd, Pymble, NSW, Australia); and Perflutren lipid microspheres (Definity; GE Healthcare, Princeton, Nj, USA). Grossniklaus summarized that since these microbubble contrast agents have already received approval by the FDA, it may be possible to load them with drugs used clinically and used for diseases such as uveal melanoma.

FUTURE DIRECTIONS FOR BASIC AND TRANSLATIONAL RESEARCH AND LESSONS LEARNED

Given the current need for more effective and safe ocular therapeutics, it would be of benefit to reduce the development timeline and increase access to the market for newly developed products that fit this description. In the current research environment, tens of millions of dollars are spent per year to develop a single late-stage product for clinical trials. Subsequent costs to perform clinical trials can increase 10-fold. Several factors account for the prolonged time to market including research and development (preclinical and clinical studies) and business considerations (funding, marketing, manufacturing, etc.). During this meeting, we learned to look beyond ophthalmology for methods that may be translated into potential therapies for diseases of the back of the eye. Toward this end, in order to conduct comprehensive preclinical studies that lead to testing of concepts in humans, it may be wise to identify a collaborator or an industry sponsor with whom the risk/benefit of drug development can be shared. Funding for drug development can also be secured through government grants or private venture capitalists, once preliminary studies demonstrate efficacy/advantage over currently unmet medical needs. Meetings with the FDA should be conducted as early as possible to discuss the appropriate regulatory strategy and route for drug approval. To prepare for clinical studies, animal models used to demonstrate preclinical safety and efficacy should be selected based on their ability to predict pharmacokinetics and efficacy in humans. Computational modeling should be used throughout the preclinical and clinical stages to help reduce time and costs. Clinical research organizations that specialize in ophthalmology may be the best place to conduct early clinical trials.

As a case study outlining the lessons learned by investigators from startup companies that specialize in drug delivery, Steve Hutcherson (VisionairX, LLC, Richmond, VA, USA) pointed out that even when delivery has been demonstrated with well-controlled studies of safety and efficacy, one must still find customers for the technology. The company Visionary Therapeutics developed DPTgatifloxacin with the intent of entering partnerships with companies that could use the DPT platform to enhance the value of products through lifecycle extension. Citing that investors do not always appreciate the value of novel drug delivery methods, these scientists/innovators found it difficult to make investors understand the decreased risk and increased benefit of their new drug delivery systems for the back of the eye. Hutcherson exhorted the audience to think simply and inexpensively, and to crack the “tough nuts” (gene delivery, large proteins) with transformative technologies.

The next frontier in translation of drug and gene ocular therapeutics must involve more networking amongst research scientists, clinicians, industry representatives, regulatory agencies, technology transfer offices, business consultants, and venture capitalists. Vision loss from retinal disease is growing in numbers, and cost effective methods to share data in small, highly intensive forums such as these (e.g., SERC) are needed. Most notably, at the 2012 meeting knowledge sharing was not confined to ophthalmology and vision scientists alone, as findings from other fields, such as oncology, respiratory disease, and medical imaging, can add value and perhaps help shorten the route to market for new therapies that treat retinal disease. The translation of knowledge from other fields challenges the current ophthalmic drug development environment to create regulatory guidelines and processes that sponsors can use to navigate the drug development process, and to lower the overall burden of time and cost for bringing a novel therapy from the bench to the bedside.

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References

Drug and Gene Delivery to the Back of the Eye


