



# Retinal Gene Therapy 1998: Summary of a Workshop

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The 1998 Workshop on Retinal Gene Therapy evaluated the potential of gene therapy in treatment of retinal disease. Academic, industry, and private foundation representatives attended. Topics included: determining which retinal diseases are likely candidates for gene therapy, specific retinal degenerations and nonspecific neuronal survival mechanisms, design and use of viral and retroviral vectors in achieving regulated gene expression, animal models of retinal degeneration and associated therapies, human trials, and alternatives to gene therapy. The discussion of human trials explored the justification for moving from animal models to human testing, patient population concerns, lessons learned from previous human gene therapy trials, and the role of industry in support of basic and clinical research.

On February 21-22, 1998 the Foundation Fighting Blindness sponsored a workshop focused on retinal gene therapy. Forty-four scientists from academia, industry and government participated in eleven topic-driven sessions intended to cover all aspects of progress toward a gene delivery approach to treating retinal disease. The format of the workshop was designed to foster a free discussion of the issues at hand with a minimum of prepared presentations. A Primary Discussant provided a succinct summary of each topic, highlighting major areas of progress, controversy and/or opportunity. The majority of each session was an open exchange of ideas by all participants on the topic and any related issues. Additionally, Secondary Discussants were asked to provide additional facts and opinions to facilitate this dialogue. Appendix 1 contains the Agenda for the workshop, including the participants and their assignments. These proceedings are arranged in the order of the topics covered in the workshop and summarize the issues covered, areas needing more attention, and any consensus views reached in a session. The reader should note that the open-format led to many issues being discussed out of the order shown in the Agenda, but within the context of the specific issue at hand. Because this report represents primarily the authors' views of the major issues raised at the workshop, direct quotes from participants have been avoided.

## DNA VIRAL VECTORS

*Contributing Opinion: Jean Bennett, M.D., Ph.D.*

For any vector system designed to deliver genetic material to retinal cells, there are at least four desirable biological properties: First, the appropriate cell type should be targetable; that target could be either the affected cell or a neighboring cell if, for instance, the gene for a secreted therapeutic agent were being introduced. Second, gene delivery should be efficient. That is, given current vector titers and the limited volumes likely to be safely injected into various retinal spaces,

sufficient copies of the passenger gene must enter a sufficient number of target cells to allow expression of the therapeutic agent in a reasonable fraction of the retina. Third, vector mediated expression should be stable for reasonably long duration. This will be particularly important for most genetic diseases where prolonged protection or rescue rather than a permanent cure is the goal and where the opportunities for frequent re-inoculation are limited. Fourth, the intrinsic toxicity of the vector should be low. This includes pharmacological toxicity, local and systemic immune responses to the passenger gene product, the input vector, or to any genes expressed from the input vector. This session surveyed the DNA vector systems currently being used in the retina with reference to these key parameters.

**Adenovirus** infections in humans are widespread and cause a significant fraction of common colds. The virus contains a moderately sized double-stranded DNA genome. By deleting nonessential DNA, initial adenoviral vectors could package about 8 kb of passenger DNA. Additional inactivation of several early viral genes created replication-defective virus vectors that could be grown and concentrated to the high titers necessary (since input volumes will be limited) for transduction of retinal cells. Transgene expression usually occurs within 24 hours of inoculation making studies in animal models convenient. Although these vectors maintain their ability to enter cells of some ocular tissue such as the retinal pigment epithelium (RPE), they do not efficiently transduce mature wild-type photoreceptors. They do, however, transduce developing and degenerating photoreceptors in animal models, suggesting their utility for delivering genes to diseased photoreceptors. Recombinant adenovirus carrying the wild-type cDNA for the  $\beta$ -subunit of cGMP phosphodiesterase has been used for transient rescue of degenerating photoreceptors in the *rd* mouse.

Recombinant adenovirus are relatively immunogenic due to the host response to both the input virion itself and to the production of numerous viral proteins required for infectivity. However, the relative immune privilege of the eye may allow transient adenovirus-mediated gene therapy. Because

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recombinant adenoviral DNA does not stably integrate into host chromosomal DNA, passenger gene expression is transient in ocular tissue. Typically, cells of the differentiated neural retina support adenovirus-mediated gene expression for 1-3 months. This may be of sufficient duration for specific short-term gene therapeutic intervention, if the immune response can be further modulated.

**Adeno-associated virus** (AAV-2) commonly infects humans but causes no known disease. It is a small single-stranded DNA virus that produces very few proteins of its own and is therefore relatively non-immunogenic. Without co-infection with a wild type helper virus (usually human adenovirus), wild type AAV integrates stably at a specific site in human chromosome 19 and establishes a latent infection. In contrast, because the viral *rep* protein required for this site specific integration is deleted in recombinant AAV (rAAV), this property is lost in current AAV vectors. However, rAAV *in vivo* appears to slowly integrate at random chromosomal locations over periods of weeks or months, depending on the target cell. This leads to long term, perhaps permanent, transduction. In rodents, photoreceptor transduction persists for at least 1.5 years. Unlike recombinant adenovirus, rAAV appears to be able to transduce normal photoreceptors as well as retinal ganglion cells and the RPE. Initial results have shown rAAV-mediated photoreceptor rescue in several animal models of RP using gene augmentation or delivery of neuroprotective genes or ribozymes.

Currently, rAAV has two major disadvantages: First, its small size limits passenger DNA to about 4.8kb. Although suitable for most genes currently under consideration for retinal therapy, only limited space remains for regulatory elements that may be essential for fine-tuned control of expression. Second, if rAAV integration is truly random, the theoretical problem of insertional mutagenesis exists. A less important problem is the lag of 3-5 weeks before full rAAV mediated gene expression is seen in rodent photoreceptors. This makes preclinical studies more time consuming than with other vectors and impossible with some rapidly developing animal models of retinal degeneration. New, more efficient rAAV packaging protocols are available that circumvent some early problems of low viral titers and low level contamination with wild type adenovirus helper.

**Encapsidated adenovirus minichromosomes** (EAMs) are a recently developed adenovirus-based vector packaged into an adenovirus virion but lacking all normal viral genes. EAMs maintain many positive properties of their parent adenovirus while reducing, but not eliminating, immunogenicity. EAMs can be produced at titers equivalent to adenovirus and have similar infectivity properties. Expression still appears to be transient in photoreceptors. Their cloning capacity of 36kb should be more than sufficient for all of the known retinal genes and should allow sophisticated regulatory elements to be included. EAMs also offer the possibility of delivering multiple gene for ocular therapy. One remaining problem is that the current packaging system requires a replication competent adenovirus to provide EAM with virion proteins. Complete removal of this contaminating

adenovirus prior to administration has proven technically difficult. Therefore, the testing of "clean" EAMs has yet to be done in the retina. Nevertheless, a  $\beta$ -subunit cGMP phosphodiesterase-carrying EAM has transiently rescued photoreceptors in the *rd* mouse.

Other DNA delivery techniques are not as well studied in the retina. DNA encapsulated by various liposome preparations has generally low transduction rates but, improvements in liposome formulations and cell specific receptor targeting using ligand complexed liposomes should allow more efficient retinal cell transduction. The transient duration of liposome delivered passenger gene expression remains a problem, but, as site directed DNA integration processes become better understood, expression persistence should improve. Alternative viral vectors, such as herpes viruses and vaccinia virus, are being developed, but their genetic complexity and pathogenicity currently pose major technical hurdles.

## RETROVIRUS VECTORS

*Contributing Opinions: Alfred Lewin, Ph.D. and Fredrick Gage, Ph.D.*

Most of the RNA viral vectors currently being tested for gene therapy are based on the murine leukemia retrovirus. Retroviruses can stably infect a wide variety of cell types, but are not capable of infecting terminally differentiated, post mitotic cells such as those of the retina. In contrast, lentiviral vectors, based on HIV and SIV (human and simian immunodeficiency viruses), encode proteins such as *vpr* that permit nuclear import of the viral genome without concomitant mitosis.

**Lentivirus** vectors are frequently pseudotyped, that is, prepared with the coat protein of another virus. In this way the cell type specificity of lentiviruses, CD4+ T-cells, is circumvented, allowing lentivirus vectors to infect a wide variety of cells. Lentiviruses containing the glycoprotein of the Vesicular Stomatitis Virus (VSV-G) have been prepared and tested successfully in rat retinas. Using a rhodopsin promoter, only rod photoreceptors expressed a reporter gene. Infection after a single inoculation spread throughout the retina and was stable for at least 3 months. The requirement for chromosomal integration to obtain gene expression suggests that lentivirus vectors should lead to long duration photoreceptor transduction. Thus, although not yet studied in the retina to the same extent as DNA vectors, both lentivirus and rAAV vectors share a number of positive attributes for retinal gene therapy. In preliminary studies, a lentivirus vector expressing the  $\beta$ -subunit of rod cGMP phosphodiesterase under rhodopsin promoter regulation preserved rod photoreceptors for 6 months (3 rows of nuclei), at least in the vicinity of the injection site in the *rd* mouse.

There remain several technical disadvantages of lentivirus vectors. First, any HIV-based vector system must be demonstrably free of wild type HIV before use in humans will be feasible. More recent lentiviral-based vectors have deleted substantial segments of the original HIV genome, thus reducing further the risk of producing an infectious virus. Second, at current titers, 30-100  $\mu$ l of recombinant virus would be required

to treat an entire human retina. Recently, virion stability and concentration techniques have improved markedly, so that this volume problem should be resolved. Finally, there is concern about the integration of HIV-based vectors. Integration appears to be randomly located, so that a possibility exists for integration-induced mutagenesis. Long term animal safety studies for lentiviral vectors (and for rAAV) aimed at documenting tumor incidence in transduced tissue will be required to resolve this potential safety problem.

Several issues of more general applicability arose during this session. The problem of obtaining appropriately regulated expression of the therapeutic gene was discussed. It is likely that more sophisticated (and very likely larger) promoter elements than currently tested will be needed in order to balance transgene and endogenous gene expression over the long term. Chromatin structure was recognized as an important regulator of gene expression, reinforcing the idea that proximal promoter elements are not the only important consideration. The sites at which viral vectors integrate into the genome often influence expression of the therapeutic gene. Thus, next generation promoters may contain regulators of open chromatin, such as locus control elements. It may be desirable under some circumstances to regulate up or down the level of a therapeutic gene product in a transient manner. Pharmacologically inducible or repressible promoter elements are available and could be included for this purpose. Additional or concurrent administration of such inducing agents may be complicated in the eye. Continued study of retinal cell specific promoters was considered essential for the progress of gene therapy.

### ALTERNATIVE GENE THERAPIES

*Contributing Opinion: Matthew LaVail, Ph.D.*

Retinal gene therapy, in its most direct sense, refers to either gene replacement for genetically recessive diseases or inactivation of a specific dominant negative allele, for example by expression of a ribozyme against retinitis pigmentosa (RP) causing rhodopsin mutant. However, in the context of a degenerating retina, the question of whether nonspecific neuronal survival strategies would be useful is an important topic. This approach may be particularly relevant when the genetic etiology of a retinal disease is not well defined, as in most forms of macular disease. Two broadly defined approaches have been identified: retinal targeting of neuronal survival factors, including neurotrophins, growth factors and cytokines, and retina specific expression of anti-apoptotic factors.

Interest in neurotrophic factors as therapy for retinal degeneration has grown out of several reports of successful pharmacological intervention in animals. Currently, CNTF (ciliary neurotrophic factor) appears to have the widest spectrum of response, exhibiting the ability to slow degeneration in nine different genetic models of retinal degeneration in four different animal species. The success rates for parallel application of BDNF (brain-derived neurotrophic factor) or bFGF (basic fibroblast growth factor) have been less pronounced. There are two practical problems currently

with this drug-based approach: First, CNTF and most other similar acting proteins do not cross the blood-retinal barrier and must be therefore be introduced by intraocular injection. Second, their half life is on the order of a few weeks, so that repeated administration would be necessary to treat any chronic retinal disease. For these reasons, persistent expression of the gene for a neurotrophic agent, particularly for CNTF, in the appropriate retinal cell type seems especially attractive. Although CNTF gene expression mediated by recombinant adenovirus has shown some photoreceptor rescue in *rd* mice, a wider range of improved vectors with cell type specific promoters needs to be evaluated to fully test this concept. Perhaps more importantly, the prolonged effects of elevated levels of neurotrophin expression in the retina are unknown, but preclinical toxicology using CNTF protein in animals is currently underway. Finally, although the receptors for many neurotrophins and their signaling pathways are being unraveled, precisely how this class of neuronal survival agent achieves retinal rescue remains unclear.

The fate of some, but perhaps not all, photoreceptors in several animal models of retinal degeneration is through apoptotic cell death. As the variety of molecular mechanisms leading to apoptosis are revealed, it is becoming clear that a multitude of potential gene therapy strategies could emerge for retinal diseases. Particularly interesting is the recent report of anti-apoptotic gene p35 protection from retinal degeneration in *Drosophila*. Less clear is a report of *c-fos* mouse knock-out protection from light damage to the retina because lack of *c-fos* does not appear to protect from a RP-causing mutation in mice. In other studies, introduction of the anti-apoptotic gene *bcl-2* as a transgene or in a viral vector has led to some protection of the *rd* mouse retina from degeneration. As newer vectors and promoters affording high level, cell specific retinal expression become available, this general hypothesis for treating retinal degenerations will be more critically tested.

### ANIMAL MODELS

*Contributing Opinions: Gabriel Travis, M.D. and Gustavo Aguirre, D.V.M., Ph.D.*

The development of viable retinal gene therapy regimens is critically dependent upon the availability of suitable animal models. In addition to spontaneous mutations identified in three species, numerous animals with mutations in genes affected in human retinal degenerations have been created through transgenic technology. These include both animals expressing mutant forms of rhodopsin and rds/peripherin, and animal with null mutations in several genes important for photoreceptor function, for example, rhodopsin, transducin [ $\alpha$ ], rom1, arrestin, and rim protein (ATP-binding cassette transporter; ABCR). Although most of this work has been done in mice, transgenic rats and pigs with rhodopsin mutations that cause photoreceptor degeneration are available as well. An important advantage of the larger animal models, either transgenic or naturally occurring, is the relative ease of vitreoretinal manipulations and the better surgical modeling of the human retina. This must be balanced against their increased cost and longer generation times. Larger animal models serve as an ideal intermediate species for testing gene therapy strategies

developed in the smaller rodent models.

Small animals such as rodents have much higher absolute rates of retinal degeneration. An interesting and apparently robust observation, true for many mutations, is that the kinetics of retinal cell loss varies inversely with the life span of the animal. Thus, a phenotype that takes decades to appear in humans, and months to years to appear in larger mammals, is frequently visible within weeks in rodents with an apparently analogous genetic defect. This phenomenon increases the potential rate of data acquisition in smaller animals. One disadvantage of all animal models identified or created thus far is that none exist in a non-human primate. Since only primates contain a fovea and macula, non-primates, including pigs, cannot be true models for human macular degenerations.

A potential problem with many existing transgenic models of human retinal degeneration relates to their pattern of gene expression. Over-expression of some wild-type genes, such as those for rhodopsin, has been shown to induce photoreceptor degeneration. The effect of over-expressing a mutant protein is likely to have even more severe effects. Reduced expression of the wild-type allele in heterozygous nulls has also been shown to kill photoreceptors. Consequently, many so-called dominant retinal degeneration models probably reflect the summation of haplo-insufficiency, reduced expression of the normal gene, and a direct toxic effect of the mutant gene product. These effects may manifest different susceptibilities to gene therapy.

Considering ways to achieve appropriate expression levels of both wild-type and mutant alleles in developing an animal model for a particular human retinal degeneration is therefore necessary. To a first approximation, the retinal expression pattern of an animal with dominant disease is 50% of the normal expression level for both the wild-type and mutant genes. Even in this case, a specific mechanism appears to exist in retinal cells to reduce levels of nonsense mutant mRNAs, thus further complicating the picture for an important genetic subclass of mutations. Clearly, an over-expressing mutant transgene on a wild-type genetic background does not represent an accurate molecular model of the corresponding human disease, even if the mutation itself is faithfully duplicated. The availability of knock-out mice into which specific mutant and wild type alleles can be introduced by breeding will greatly aid in developing a second generation of better animal models of retinal degenerations. The goal is to have a sufficiently well-designed model so that if a particular form of gene therapy slows the retinal degeneration, it is likely to predict a beneficial effect in humans with the corresponding genetic lesion.

## MECHANISMS OF RETINAL DEGENERATION

*Contributing Opinion: Fulton Wong, Ph.D.*

Two frequently asked questions about inherited retinal degenerations are: (1) What are the mechanisms of photoreceptor death associated with retinal and macular degeneration? (2) Why does rod cell death lead to cone cell death? At present, there is only limited information available to answer these questions, however, the following issues were considered in this mechanistic context:

### *Why is photoreceptor cell death not autonomous?*

Although genetic mutations are the cause of many retinal degenerations, many cases of photoreceptor cell death appear not to be cell autonomous. For example, in chimera studies, wild type or rescued rods die along with mutant rods. In cases where mutations are in rod-specific genes, such as rhodopsin in RP and ABCR in macular dystrophies, the cones die also. Furthermore, in these diseases, inner nuclear and ganglion cells die too. While the underlying mechanisms of these “secondary effects” are unknown, possibilities include:

- (1) a mutation may cause toxic effects that spread in the retina;
- (2) death of some photoreceptors may lead to the loss of neurotrophic factors needed for survival of other cells;
- (3) cell death may lead to changes in mechanical forces on surviving retinal cells;
- (4) loss of integrin-mediated contact with extracellular matrix may occur.

Whatever the (primary) mechanism by which a mutation affects cells expressing the mutant gene, it seems that the effect triggers a variety of interacting retinal mechanisms that lead to wider degeneration.

*Apoptosis in genetic photoreceptor cell death*—In several different models, photoreceptors die by apoptosis. This finding leads to the following questions:

- (1) Is apoptosis the only mechanism of photoreceptor death in RP?
- (2) Will inhibition of apoptosis effectively treat RP?
- (3) Is apoptosis important in macular degeneration?

Apoptosis refers to a special form of cell death that shows characteristic features. Although some of these key features are observed in photoreceptor death, justifying the use of the term loosely, some are not. Therefore, “photoreceptor apoptosis” should be better defined in relation to well-studied models of apoptotic cell death.

Since the initiation phase of apoptosis can be triggered by a multitude of signaling mechanisms, and different mechanisms may mean different signals for different cells, intervention at this stage of the photoreceptor cell death process would appear to be difficult. Without knowledge of the triggering signals, and without the means to target specific cells precisely, genetic intervention may even lead to undesirable effects.

Potential targets for modulation at later phases of apoptosis may include the following:

- (1) *Bcl-2*; Over-expression of *Bcl-2* can slow photoreceptor degeneration in some models; it may also show rescue effects on retinal ganglion cells;
- (2) Caspase inhibition;
- (3) Inhibition of apoptosis proteins, such as CrmA, NIAP and p35 (in a *Drosophila* rhodopsin mutant



line, expression of p35 rescued photoreceptors, as judged by histology and ERG. However, its potential in mammals remains to be determined).

*The mechanisms underlying photoreceptor cell death are complex*—Understanding the relationship between a mutation and its attendant mechanism of retinal degeneration is further complicated by the fact that “one gene does not equal one mechanism”. Even within a single gene, different mutations may lead to distinct phenotypes. Secondary effects, such as altered oxygen tension, altered vascular supplies, and temporal effects on different stages of disease may modify the phenotype. Until some of these complex interactions are understood, how can we attempt to keep photoreceptors (especially cones) alive? Some possibilities discussed include:

- (1) replace the defective gene in some cases, e.g. the  $\beta$  subunit of phosphodiesterase ( $\beta$ -PDE);
- (2) provide missing endogenous factors;
- (3) provide exogenous factors;
- (4) provide constitutively active receptors or signal transduction molecules;
- (5) inhibit apoptosis by either stimulating anti-apoptotic pathways or inhibiting pre-apoptotic pathways;
- (6) modulate gene expression by specifically down-regulating expression of mutant genes;
- (7) in the future, achieve gene replacement or targeted gene correction.

## TARGET HUMAN RETINAL DISEASES

*Contributing Opinion: Richard Lewis, M.D.*

The features of any human retinal disease that would suggest it as a viable candidate for gene therapy include:

*Prevalence*—The disease must be common enough in the general population to make an interventional trial worth devising. In addition, within a given single disease category with a single mutant gene, the large genetic heterogeneity of point mutations and molecular rearrangements often encountered require that sufficient individuals with the same selected mutations be identifiable in the population.

*Significance*—The disease must be of sufficient clinical severity to warrant genetic intervention, especially when no other pharmacological or environmental manipulation is feasible.

*Mechanism*—To minimize the number of individual trials, diseases with similar mechanisms of retinal degeneration should be clustered. Sequential trials (i.e., distinct clinical trials on different forms of retinal diseases) therefore should involve novel/distinct mechanisms of disease, rather than different diseases caused by the same mechanism. For example, a trial on an early onset, rapidly progressing form of rhodopsin mutation causing RP should not be followed by a trial on the same clinical form of RP caused by another rhodopsin mutation.

*Time Course*—If a disease causes visual impairment rapidly, then early identification of patients at a salvageable stage is required. If the course of the disease is slow, how do you measure the outcome of any intervention in an experimental time frame?

*Assessment*—By what methodologies and objective endpoints does one monitor both the clinical disease and the change in natural history by intervention? Electrophysiology? Psychophysiology? Biochemical markers? Any of these “assessments” must be sensitive, reproducible within patients, reproducible between patient groups, and reproducible between institutions enrolling patients of the same genetic and molecular type(s).

*Genetics*—The design for treatment will depend on the mechanism(s) of the genetic disease. Treatment for loss-of-function mutations might be substantially different from the treatment of disorders in which the mutant protein has a substitution that renders it cytotoxic. For recessively inherited diseases, the complexity of genetic compound heterogeneity present in an outbred population such as that in the United States may also create difficulties.

Several other points related to these issues were made:

With respect to identifying target diseases, the number of patients recruitable for clinical trials may be influenced by the disease incidence, by the number of new cases that appear in the population at a given time, and by the availability of presymptomatic diagnosis, as can occur with younger siblings of older affected identified individuals. Obviously, the number of individuals with any one specific mutation, particularly a compound heterozygote for a recessive disease, would lead to these individuals being classified as “rare” and “very rare,” even in a population the size of the United States.

For certain disorders, such as Stargardt’s disease and some forms of RP, genetic heterogeneity of recessive alleles may make it difficult to find numerous individuals with the same pair of mutations. These difficulties lead one to ask whether there might be some global therapeutic intervention that will provide a more universal “intervention” for recessive diseases? However, such patients are not always necessary, since, for example, patients with a recessive disease and two predicted complete loss-of-function alleles are equivalent at the functional level.

Do we know enough about both the clinical definition of selected diseases and the natural course of untreated disease, for example in  $\beta$ -PDE mutations in recessive RP, or in choroideremia or X-linked RP, to know how we will interrupt the natural course of the disease with gene therapy?

Is it feasible to think that X-linked diseases in which an affected male has only a single (“null”) copy of a mutant gene, such as X-linked RP or choroideremia, might be more suitable targets than a dominant negative single allele, even when a normal allele is present?

Are sufficient numbers of relatively common disease mutations necessary? If one could demonstrate “proof of principle” in a few individuals with a very rare disorder and

achieved positive outcomes, would this allow us to focus on much more approachable forms of the disease(s)?

As clinicians, our first admonition is to “do no harm.” Under what circumstances could we demonstrate that vectors are not harmful? In response, it was suggested that initial clinical trials for safety might use normal volunteers, such as terminally ill individuals in which retinal electrophysiologic characteristics could be monitored and pathologic tissues retrieved within a reasonable time.

Is there a relative risk or advantage to clinical intervention with early central disease (such as Stargardt’s disease) versus patients with an initial peripheral disease (such as certain forms of rapidly progressive retinitis pigmentosa or choroideremia) in which central vision is historically spared until late? Under what circumstances would the approach of either of these classes of diseases be ethically responsible?

### PROOF OF PRINCIPLE

*Contributing Opinion: John Flannery, Ph.D.*

The “proof of principle” question, whether gene therapy for retinal degeneration in animal models has shown sufficient success to justify moving to human studies, is not easy to answer primarily because the definition has so many levels and variations. The question posed in perhaps its strictest terms is, “Have current gene therapy studies demonstrated rescue of functional vision in a genetically valid animal model (see above) in a human-like eye for the years or decades of rescue likely to be required for human retinal disease?” The answer is no at each level and it may remain so for a very long time. Clearly we need interim milestones that will allow a safe but reasonable avenue towards human trials. No consensus opinion was reached in this workshop, but several questions that can be considered elements providing proof of principle emerged from these discussions.

Do current gene delivery systems retard retinal degeneration? The answer is yes, at least in a few animal models (the *rd* mouse and in one transgenic mutant rhodopsin rat) for limited duration using adenovirus and AAV vectors and, soon to be published, for lentivirus and EAM vectors as well. Clearly, a broader spectrum of degenerating animal retinas must be tested using a variety of genetic approaches for longer periods (at least one year in rodent) to firmly establish this basic proof of principle.

Have present systems exhibited any recovery of retinal function? At best, preservation of residual retinal function, not recovery of lost function, is currently observed. The postmitotic, terminally differentiated state of photoreceptors in particular suggests that only rods and cones not already dead or dying in a degenerating retina can be rescued. This means that one aspect of more complete proof of principle experiments must be to attempt to preserve functional photoreceptors in a severely degenerated animal retina in order to more accurately mimic the expected point of clinical intervention in human retinal disease.

Have current gene transfer systems been compared systematically? No, there have been no side-by-side

comparisons of any two vector systems in the retina. Such studies will be complex because of the need to balance effective titers, but they will be needed to firmly establish optimal gene delivery techniques.

Are very long term studies (5-10 years) that mimic the rescue required for human retinal disease necessary for proof of principle? Such studies, although likely to be required for large scale human trials, are probably not necessary for initial human studies, assuming no overt toxicity is encountered in shorter term preclinical animal studies. Clearly, a larger animal model with a longer life span than rodents will be needed. Presently, the Irish Setter model for recessive retinal disease and transgenic pigs with mutant rhodopsin are likely candidates.

Are current assays of retinal rescue sufficient for proof of principle? Biochemical assays of affected retinal gene products and morphological analysis of preserved photoreceptors are ultimately insufficient to demonstrate preservation of visual function. Additionally, they are improbable assays in human trials. ERG analysis, particularly simultaneous recordings of both the treated and control eyes in animal models, is better, but does not fully satisfy a strictly interpreted proof of principle. For that, behavioral tests of preserved vision in animal models of retinal degeneration are likely to be necessary.

In spite of the numerous ways that current proof of principle experiments can be made more rigorous, there was an consensus that early results for retinal gene therapy in animal models are very encouraging.

### PATIENT POPULATIONS

*Contributing Opinions: Alan Laties, M.D. and Samuel Jacobson, M.D.*

*Problems attendant to patient recruitment*— Patient populations exist under several categories. Best known is the patient registry maintained by the Foundation Fighting Blindness. However, it is generally agreed that this represents a very weak instrument. The data are often suspect or incomplete, and there is a tenuous relationship between the registrant and Foundation, leading to a lack of any effective route for patient contact or recruitment toward clinical trials. Far more valuable at present are the patient rosters of individual clinicians who care for patients with inherited retinal degenerations. In the main these are replete with well characterized patients. Thus the phenotype is defined, and in many instances there also exists worthwhile information on genotype.

Of equal importance to clinical trials is that there be a reliable venue for patient contact, plus a personal relationship to enhance the prospects for successful recruitment. Not to be neglected is the fact that logistical considerations also favor recruitment of subjects for trials from the clinical roster of RP patients. Patients are more likely to return to a clinician who has already cared for them, and who is likely to be located in the same city or region.

All in all, the information above leads to a straightforward conclusion: center-based or clinician-based clinical trials are

the most likely to be effective.

*Problems related to ethics and confidentiality*—General discussion took place on several topics: relationships to institutional review boards, patient rights, the need for information sharing or actual patient sharing among centers, problems of patient confidentiality, and the practical difficulties of constructing databases that are at once scientifically useful and accessible to appropriate investigators, while at the same time guaranteeing patient anonymity. The majority of these issues have currently workable local and national guidelines based on previous human trial experience. However, attention must also be paid to the nature of the consent forms. One helpful suggestion was that consent forms include boxes to permit several check-offs with ascending levels of permission. By this means what is and is not permitted in the handling and distribution for an individual sample is clearly denoted. The patients wishes can be readily ascertained and acted upon.

### OTHER HUMAN TRIALS

*Contributing Opinion: Nicholas Muzyczka, Ph.D.*

The aim of this session was to attempt to “learn by example” from other, ongoing human gene therapy trials. The gene therapy community’s experience with cystic fibrosis (CF) trials involving viral vectors providing CFTR (CF transmembrane conductance regulator) gene product to the lung epithelium seemed to contain the most relevant lessons and was therefore the focus. A summary of several such trials was presented. An initial lesson was that it is important to have an internal clinical control if possible. For CF, where nasal epithelium has been used as a model for lung epithelium, it was possible to use one nostril as a double blind control for the other. The outcome measures in the CF trials were sufficiently ambiguous that this sort of internal control approach was essential. The relevant question is what would be an appropriate control for retinal gene therapy. Would it be possible to use one eye as an injection control for the other treated eye or would this be viewed by clinicians and patients alike as unethical, impractical, or simply too risky? For the eye, the clear consensus was that an injected control eye was too extreme and that comparative clinical measures between treated and untreated eyes (ERGs, visual fields, and visual acuity) must suffice in the initial human trials.

The second problem was how to demonstrate that the vector or expression of the transgene was present in the treated patient. The CF community ultimately decided on the use of a biopsy strategy coupled with PCR because the amount of CFTR produced was too low to make Western blots a viable option. Clearly, neither biopsy nor direct biochemical testing are options for the retina.

CF investigators also struggled with the question of how to determine whether there had been a clinically relevant outcome, short of waiting 30 years to see if the patient lived longer than the 35-40 year average lifespan of a CF patient. For this purpose they settled on two kinds of tests, the so called potential difference measurement (PD) and the frequency of *Pseudomonas* infections that are common to CF patients. PD measurements could be made on a regular schedule but are

notoriously variable from patient to patient. In this respect, it was important to set up standards for establishing baselines and ensuring consistent measurements between laboratories. It was emphasized that effective communications among the CF Foundation, the NIH/LB, and the Clinical Centers were important for coordinating this process. The point was also made that neither the PD nor *Pseudomonas* markers were validated, i.e., no one knew whether either assay correlated with a positive outcome for CF patients. These issues (which remain to be resolved by the CF groups) are important for retinal therapy as well, particularly establishing an agreed upon set of clinical criteria for treatment efficacy. On a positive note, electroretinograms (ERGs) are a noninvasive method for tracking disease progression and there was a consensus that, when simultaneously compared to the untreated eye, ERGs will most likely be the primary measure of clinical outcome. On the negative side, since retinal biopsy to measure vector or therapeutic gene levels are not currently feasible, it may not be possible to absolutely correlate any positive patient outcome with expression of the potentially therapeutic gene being tested.

Another important lesson was that as broad a series of preclinical toxicity/safety trials as possible should be generated. In the case of the Adenovirus/CFTR vectors, the inflammatory response in rodent models was largely overlooked, and did not appear to be worrisome even in primate preclinical trials. Nevertheless, this became a major issue when recombinant adenovirus vectors were inoculated into human lungs and eventually lead to cessation of the trial.

The final point emphasized was that it is not necessary to focus only on genetic therapies that are tailored to a particular genetic disease. This issue was raised in response to a number of preceding comments throughout the workshop that argued for a therapeutic approach specifically designed to treat a particular genetic disease, i.e. a ribozyme strategy or gene replacement strategy rather than a more generally applicable strategy like CNTF gene therapy. This sort of nonspecific therapeutic strategy is currently widely supported by the CF community. Among other things, CF groups are trying agents such as butyrate to raise the expression levels of defective CF genes that retain partial activity. Their hope is that this approach would be useful for a broad spectrum of genetic CF defects and may ultimately be simpler to accomplish than CFTR gene replacement. Clearly an analogous argument could be made for retinal gene therapy approaches.

### ROLE OF INDUSTRY

*Contributing Opinion: Kim Brazzell, Ph.D.*

The pharmaceutical industry has a record of strong interest in ocular therapy and gene therapy. The major point of discussion was whether these interests could merge into active support of retinal gene therapy. It was the consensus of the private sector participants that they would be willing collaborators with academia and the Foundation to help solve some of the devastating clinical problems associated with retinal degenerations through these newer approaches. Several basic issues first need to be resolved. The key question needing to be addressed is the identification of those diseases for which

gene therapy could provide both the most benefit to the patient and have the highest probability of being commercially viable. Countering this economic argument, there was a strong opinion that curing blindness should not be limited by how much money a company can make. In the final assessment, however, the realities of business must be understood by the scientific community at large - preserving vision and making money will always be preferred over preserving vision and losing money. In spite of this economic hurdle, early, small human trials retain interest to the private sector both for future larger scale therapy as well as to gain entry into the field of retinal applications for gene therapy.

Typically, commercial concerns regularly evaluate the numerous research opportunities available, all of which have potential patient benefits. They must choose those opportunities that offer the most from the anticipated research investment. At least some research projects must generate revenue or there will be no research and development funds to support new projects. One maxim holds that the more patients helped, the more revenue generated. For retinal disease, therefore, the problem will be to identify a specific class of retinal defect or genetic type that is sufficiently prevalent to interest industry. This is likely to be difficult based on the preceding discussions of the heterogeneity of retinal diseases. Alternatively, a sufficiently general therapy, independent of precise clinical manifestation or genetic etiology, could treat a larger fraction of patients. Approaches such as survival factor or anti-apoptosis gene therapy, as discussed above, appear to provide that possibility. Examples of this strategy are Regeneron's interest in CNTF pharmacology and retinal therapy, and the natural extrapolation to CNTF gene therapy as discussed above.

Finally, the role private foundations could play in helping to define which disease(s) should be tackled first was discussed. Clearly, a balance needs to be forged among the scientist's desire to move the technology forward, the clinician and Foundation's desire to help as many patients as possible and the private sector's requirements. Not addressing the commercial aspects at an early stage could jeopardize the ultimate success of bringing ocular gene therapy effectively to the patient. Although academic institutions with foundation help may be able to carry out Phase I and Phase II clinical trials, they do not have the resources, expertise, and experience needed to move a retinal gene therapy product to the market place. If industry is not involved, it is unlikely that the technology will be of any benefit to patients other than those in the Phase I/II trials and this would be a tragedy. Foundations have a clear role in supporting such early clinical proof of principle and could provide an effective interface between individual scientist/clinicians carrying out these trials and industry. This will be a necessary component of a complete strategy for bringing retinal gene therapy to a broad patient population.

#### **ALTERNATIVES TO *IN VIVO* GENE THERAPY**

*Contributing Opinions: David Valle, M.D. and Raymond Lund, Ph.D.*

Despite the intellectual appeal of treating inherited retinal degenerations at the level of the affected gene, the reality is that this is a daunting challenge. In aggregate, genetic retinal degenerations are extremely heterogeneous: at least 20 genes are involved and the absolute number of individuals affected by defects in any one of these genes is small. Moreover, each of the genes is likely to have a unique set of therapeutic requirements including localization, magnitude and regulation of expression, as well as gene-specific mechanisms of molecular pathology. For all these reasons, it seems prudent to search for alternatives to gene therapy, particularly those that might generalize to several forms of inherited retinal degenerations.

Transplantation has the potential to be efficacious for multiple inherited retinopathies, particularly when combined with early diagnosis by DNA methods. Transplantation may be utilized to replace damaged cells or to provide cells that have been manipulated *in vitro* in order to serve as a source, for example, of growth factors or of key metabolites. Most research in this area has focused on transplantation of the RPE. Experiments in the RCS rat, where the primary defect is known to involve RPE, have shown preservation of photoreceptor function by transplantation of RPE cells at age one month. Stimulated by these and other observations, there are currently 60-80 human patients with a variety of retinal degenerations who have received RPE transplants. No clear cut evidence of efficacy has been observed in these early human experiments and there is clearly need for additional work in animal models to improve methods and evaluate outcomes.

Lessons learned so far have identified potential advantages and disadvantages of RPE transplantation. The potential advantages include:

- (1) transplantation is a general method, although still disease-specific;
- (2) transplantation could be useful for a variety of strategies (e.g. replacement of damaged cells or as a source of trophic factors etc.);
- (3) transplantation has potential for maintaining but not restoring host retinal cells.

The potential disadvantages include:

- (1) problems related to the sources of the cells (fetal, cultured, or postmortem);
- (2) transplantation requires early intervention;
- (3) immune considerations;
- (4) the danger of systemic toxicity or other problems (e.g. infection).

Very little experience with photoreceptor transplants is available. Theoretical advantages include:

- (1) it also would be applicable to many (but not all) forms of retinal degeneration;
- (2) it may be beneficial later in the course of the disease (vs. RPE transplantation);
- (3) photoreceptors may be less immunogenic than



RPE cells.

Theoretical disadvantages include:

- (1) restoration of visual function would require elaborate re-wiring (synapse formation);
- (2) there is currently no proof in animals that it will work;
- (3) the donor cell source is a larger problem as compared to RPE transplantation because of the difficulty of culturing photoreceptors;
- (4) there is a danger of systemic toxicity (e.g. infection).

These considerations have identified several important questions for additional study:

- (1) What are optimal sources for donor cells?
- (2) What is the best technique for delivery, and are other considerations important, such as the use of dissociated cells, gelatin coated cells or consideration of cell polarity?
- (3) Is the transplant environment important relative to the stage of disease, the retinal histopathology, and/or the presence of supplementary factors (growth factors, cytokines etc.)?
- (4) What constitutes "Proof of Principle" for transplantation?
- (5) What are the necessary immune precautions?
- (6) What are the risks of infectious complications?

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We wish to thank the participants who provided independent views of each topic as "Contributing Opinions" to aid in the preparation of this summary.

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### APPENDIX 1.

Agenda for 1998 Retinal Gene Therapy Workshop

Retinal Gene Therapy Workshop  
Emory University Conference Center  
Atlanta, Georgia  
February 21 and 22, 1998

Organized by William W. Hauswirth and Roderick R. McInnes

Sponsored by the Foundation Fighting Blindness

Saturday, February 21

**8:15 PM - Gene Therapy for Inherited Retinal Degenerations:  
Are you Ready?**

Phil Noguchi (Food and Drug Administration)

Sunday, February 22

**08:00 AM - Welcome**

Jerry Chader (Foundation Fighting Blindness)

**08:05 AM - A General Introduction to Inherited Retinal  
Degenerations**

Sam Jacobson - Clinical and Pathological Features  
Ed Stone - Genetic Basis of Inherited Retinal Disease

**08:35 AM - DNA Viral Vectors**

Primary Discussant: Jean Bennett  
Secondary Discussants: Battacharya, Gage, Kumar-  
Singh, Li, Muzyczka

Some Questions: What aspects of DNA viral vectors currently look most favorable in the retina, and why? What are the major problems with these vectors, and what are the most promising approaches for overcoming these problems?

**09:10 AM - RNA Viral and Nonviral Gene Delivery**

Primary Discussant: Rusty Gage  
Secondary Discussants: Bennett, Lewin, Li, Muzyczka, Saperstein

Some Questions: What aspects of RNA viral vectors and nonviral gene delivery currently look most favorable in the retina, and why? What are the major problems with these systems, and what are the most promising approaches for overcoming these problems? How important is regulated expression and how might it be best achieved?

**09:50 AM - Alternative Gene Therapies**

Primary Discussant: John Flannery  
Secondary Discussants: Goff, LaVail, Li, Nickerson, Wong, Zack

Some Questions: Are there useful approaches for more general retinal gene therapy in addition to gene replacement or inactivation of specific dominant negative alleles? Do anti-VEGF, anti-apoptotic factor, neurotrophin, growth factor or cytokine genes offer viable alternative strategies?

**10:25 AM - Break**

**10:40 AM - Animal Models**

Primary Discussant: Gus Aguirre  
Secondary Discussants: Baehr, Farber, Goff, LaVail, Travis, Valle, Wong

Some Questions: What animal models of retinal diseases are most relevant to clinical disease and which are (or will be) the most convincing as preclinical models. Are additional animal models needed?

**11:15 AM - Mechanisms of Retinal Degeneration**

Primary Discussant: Don Zack  
Secondary Discussants: Aguirre, Baehr, Farber, Flannery, Nickerson, Travis, Wong

Some Questions: What information about general retinal biology is needed to better understand retinal degeneration and cell death? How might this data allow more effective gene therapy strategies?

**11:50 PM - Target Human Retinal Diseases**

Primary Discussant: Ed Stone  
Secondary Discussants: Battacharya, Humphries, Lewis, Sieving, Zack

Some Questions: What human retinal diseases should be

first considered for gene therapy, and why? What retinal diseases are (or will be) most amenable to gene therapy approaches? Which need further attention, and at what level?

**12:25 PM - Lunch**

**01:25 PM - Proof of Principle**

Primary Discussant: Pete Humphries  
Secondary Discussants: Bennett, Flannery, Jacobson, Laties, Sieving

Some Questions: What research milestones must be reached prior to a clinical trial? Are additional experimental results needed? What sorts of retinal injection-related or therapeutic gene-related pathology might be expected in humans? Can this be eliminated or minimized?

**02:00 PM - Patient Populations and Clinical Criteria**

Primary Discussant: Sam Jacobson  
Secondary Discussants: Csaky, Laties, Lewis, Saperstein, Sieving, Stone

Some Questions: What patient populations exist that could be used for Phase I and II retinal gene therapy trials. What ethical and logistical problems can be anticipated? What clinical assays exist for gauging the effectiveness of a retinal gene therapy trial? How could they be employed effectively in a Phase I or II trial? What problems exist?

**02:45 PM - Other Human Trials**

Primary Discussant: Ric Boucher  
Secondary Discussants: Csaky, LaVail, Muzyczka, Noguchi, Verma

Some Questions: What lessons can be learned from other human gene therapy trials?

**03:20 PM - Role of Industry**

Primary Discussant: Marty Glick  
Secondary Discussants: Brazzell, Gonda

Some Questions: What is the current role of industry in retinal gene therapy? What should be done to enhance commercial interest and to increase academic-commercial interactions?

**03:55 PM - Break**

**04:10 PM - Alternatives to In Vivo Gene Therapy**

Primary Discussant: Ray Lund  
Secondary Discussants: Chader, Csaky, Laties, LaVail, Valle

Some Questions: What is the status of research on transplantation alternatives to retinal gene therapy? Is *ex vivo* therapy a viable alternative? Are the risk/benefit ratios for alternative therapies predictable and, if so, how do they impact on an overall strategy for treating retinal disease vs. gene therapy?

**04:45 PM - Workshop Overview**

Primary Discussant: Inder Verma  
Secondary Discussants: All participants

Some Questions: Is the field on sufficiently firm scientific footing to bring retinal gene therapy to the clinic in the near future?

**05:25 PM - Discussion**

**06:00 PM - Workshop Adjourns**