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The Proceedings of the First International Symposium on Translational Clinical Research for Inherited and Orphan Retinal Diseases

Washington, D.C.
November 5–7, 2004

Mark O. M. Tso, MD, DSc and
Morton F. Goldberg, MD

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The Proceedings of the First International Symposium on Translational Clinical Research for Inherited and Orphan Retinal Diseases

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PREFACE

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AND GORDON GUND, AB‡

The mission of the National Neurovision Research Institute is to accelerate the translation of laboratory-based research into clinical trials for treatments and cures of retinal degenerative diseases.

In an unprecedented move to accelerate the translation of laboratory-based research into clinical trials for treatments and cures of retinal degenerative disorders, seven governmental agencies and two private foundations joined forces in support of The First International Symposium on Translational Clinical Research for Inherited and Orphan Retinal Diseases. The symposium, held in Washington, DC, from November 5 to 7, 2004, was sponsored by the National Neurovision Research Institute, Inc. (NNRI), a subsidiary of The Foundation Fighting Blindness, Inc. (FFB). Supporting the event both financially and administratively were the following organizations:

- National Eye Institute (NEI)
- Office of Orphan Diseases, National Institutes of Health
- Office of Orphan Products Development, Federal Drug Administration
- National Institute of Neurologic Diseases and Stroke
- National Institute of Aging
- National Institute of Communicative Diseases and Deafness
- National Heart, Lung, and Blood Institute
- Alcon Laboratories
- W.K. Kellogg Foundation

The Symposium attracted more than 170 specialists from 12 countries. The men and women represented ophthalmologic clinical research, basic neuroscience research, pharmaceutical industries, biotechnology companies, governmental organizations, venture capital enterprises, the legal profession, and nonprofit agencies. Sixty-one international experts made presentations during six formal sessions. They also led 14 breakout discussions and a poster session. After the symposium, enthusiastic responses by the participants and their various communities strongly encouraged the publication of the proceedings to share the information and to provide a substrate for current basic research and clinical endeavors.

The NNRI appreciates the support of the editor of *Retina* in publishing these proceedings as a supplement to the journal. The editors of these proceedings sincerely hope that the information provides new perspectives on the novel investigative, administrative, and financial approaches required for successful therapy of inherited and orphan retinal diseases.

Background

The FFB was founded in 1971 as the Retinitis Pigmentosa Foundation, Inc., to provide seed money for investigators to initiate innovative research in inherited retinal diseases. The FFB has supported many pilot projects, which have then advanced to meet the stringent funding requirements of the NEI. The FFB and the NEI are informal collaborators and, together, are major forces in developing therapies for inherited retinal diseases.

Since 1971, the FFB has directly provided close to $150 million in research funds and has helped attract more than six times that amount from the NEI and other major funding agencies. These research initiatives by the FFB, the NEI, and others have led to many major developments in the field, mostly at the fundamental and laboratory level. However, human therapies have recently begun to emerge following proof of principle from in vitro studies and animal models, and the FFB realizes that clinically relevant work is rapidly evolving. A large gap still exists, however, between potential therapies in the laboratory and therapies proven to be both safe and effective in humans—and, therefore, routinely available to patients in the marketplace. The NNRI was founded in 2003 to help bridge this gap. The NNRI is a nonprofit, wholly owned subsidiary of the FFB.

In the United States, orphan diseases, by conventional definition, refer to ailments affecting fewer than 200,000 people. The economic reality of developing new treatments for such a small population, no matter how seriously affected, has prevented sufficient translational clinical research from being conducted. With

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the exception of possible slowing effects on the progression of some of these diseases by vitamin A and a long chain fatty acid (docosahexaenoic acid), no clinically valuable prophylactic or therapeutic intervention is currently available for most patients with hereditary orphan retinal diseases. Nonetheless, recent progress in laboratory research provides a powerful beacon of optimism that has stimulated initiatives for clinical trials aimed at prevention, treatment, and cures of these retinal diseases.

Goals of the Symposium

The goals of the NNRI include the development of bridges of communication among scientific, clinical, governmental, pharmaceutical, financial, and commercial communities to encourage clinical trials of candidate drugs and drug delivery systems for orphan retinal diseases. This symposium was developed as an educational tool to enhance this effort and to accelerate the initiation of Phase I and Phase II clinical trials of new candidate drugs, devices, and other innovative treatment strategies. Success in such small-scale clinical trials should encourage large, definitive (Phase III) trials by reducing the risks and expenses inherent in these major undertakings. Part of the role of the NNRI is to assist in fund-raising for Phase I and Phase II efforts.

In addition, the Symposium enabled experts from scientific and medical communities to exchange information with representatives of pharmaceutical and biotechnological companies, governmental and regulatory agencies, philanthropists, investors, and nonprofit organizations. Together, these experts were able to review opportunities in drug discovery and commercialization of products for such orphan retinal diseases as retinitis pigmentosa, Stargardt disease, Usher syndrome, macular degeneration, and related disorders. The Symposium provided learning opportunities and interactive channels among specialists in widely different but potentially collaborative fields. In this process, the NNRI further encouraged new investigators to perform innovative research in orphan retinal diseases by stimulating their interest in the potential benefit of new, and, as yet, unproven therapies. The NNRI also created a model of collaboration among the pharmaceutical industry, governmental agencies, and nonprofit organizations to overcome delays in drug discovery and drug commercialization.

The Format of the Symposium

Under the direction of the NNRI Board of Directors, the symposium was planned by a panel of specialists and experts listed in the acknowledgement. These individuals met in person and by electronic means to plan the format and select presenters. The plenary and breakout sessions were open to the public.

The Symposium was organized in a sequence similar to the process of translational drug discovery. It started with several clinically oriented ophthalmologists describing recent advances in the understanding of orphan retinal diseases. Next, basic scientists summarized the pathogenesis of these diseases and possible new drug delivery systems suggested by in vitro and animal models. Reports were made regarding exciting therapeutic attempts using new drug discovery systems, clinical trials with vitamin A and docosahexaenoic acid, pharmacologic drug screening, high-throughput screening of chemical compounds, and genetic studies (especially with *RPE65* as a model gene delivery approach).

The program also included discussions of the conditions necessary for successful implementation of clinical trials, such as networking of patients who have orphan retinal diseases and appropriate study designs. In a parallel approach, recent clinical trials of nonophthalmic neurologic diseases were also discussed.

Governmental regulatory issues involved in clinical trials were described, with specific advice for organizers of trials for orphan retinal diseases. Intellectual property, licensing of compounds, and involvement of the pharmaceutical industry in clinical trials were also reviewed. In the final sessions, commercialization of therapeutic agents by the pharmaceutical industry and the support of venture capitalists were analyzed. Bottlenecks in the translational drug discovery were described, along with techniques to minimize holdups.

The Aftermath of the Symposium

During the Symposium, participants interacted intensely, emphasizing a multidisciplinary approach to translational clinical research. A network of experts began to establish a community for subsequent collaboration. At least eight research projects have already developed because of these face-to-face interactions. The Symposium further stimulated interest for additional research programs, such as high-throughput screening of drugs, genotypic study of patients with inherited retinal diseases, and development of a database for international collaborative networks for patient registries.

Orphan retinal diseases, with their low prevalences, may not undergo rapid scientific or clinical advances unless the relatively small international communities of committed scientists and clinicians cooperate by using open-source networking and other collaborative

techniques. Indeed, important scientific or clinical achievement often depends on an increased number of people working with each other in a specific area. The greater the interaction among increased numbers of scientists and clinicians, the greater the productivity is likely to be. The Symposium has increased the size, and hopefully, the functionality of this collaborative community.

The editors of these proceedings think that the concentrated material provided herein will increase translational clinical research in orphan retinal diseases and will offer hope to patients with these blinding diseases. Eventual commercialization of successful therapies will require participation of a large number of people and of diverse organizations, including those represented here. The editors regret that, because of space limitations in this special supplement of *Retina*, it is not possible to include every presentation that was made at the Symposium or to include the names of all of the people who contributed to the many research or business successes.

The First International Symposium on Translational Clinical Research for Inherited and Orphan Retinal Diseases achieved its goal—to be a model of collaboration among governmental agencies, academic institutions, pharmaceutical and biotechnological industries, venture capitalist companies, private equity investors, and nonprofit organizations. We thank everyone who participated.

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CLINICAL PROGRESS IN INHERITED ORPHAN RETINAL DISEASES

Inherited and Orphan Retinal Diseases: Phenotypes, Genotypes, and Probable Treatment Groups

RICHARD G. WELEBER, MD

An orphan disease is defined in the United States as a medical condition affecting fewer than 200,000 individuals. Six hundred orphan diseases affect an estimated 25 million Americans, or 9% of the population. Eighty percent of these rare diseases are genetic. Virtually all inherited retinal dystrophies and degenerations—except for age-related macular degeneration (AMD), which is quite common—fit the definition of an orphan retinal disease. Although individually rare, orphan retinal diseases collectively represent a major cause of untreatable vision loss and blindness.

The functions of many of the genes discovered for orphan retinal diseases were previously unknown, and represent new pathways in cell biology. These rare diseases become models of disease mechanisms in pathophysiology, which lead to insights into more common diseases. Orphan diseases, thus, provide platforms for the development of new therapeutic strategies that can be applied to diseases that are more common.

Among the orphan retinal diseases in which treatments based on gene discoveries are likely to occur are retinitis pigmentosa (RP) and allied disorders, choroideremia, Stargardt disease, and other *ABCA4*-related retinopathies.

Retinitis Pigmentosa and Allied Disorders

Retinitis pigmentosa and the allied disorders comprise a group of genetically determined dystrophies and degenerative conditions of the retina. This group of diseases includes more than 150 gene disorders, approximately 100 of these genes have been cloned. The diseases encompass all different inheritance types. These diseases can affect the retina alone or can be part of a syndrome.

RP is the most common of the diseases, with a worldwide prevalence of approximately 1 in 3500 people. Other diseases, such as Stargardt disease and *ABCA4*-related dystrophies are also prevalent. The number of people in the United States with these conditions has been estimated at 100,000 for RP; 5,000 for Leber congenital amaurosis (LCA); 16,000 for Usher syndrome, a form of RP with congenital deafness; 20,000 for cone–rod dystrophy; 6,000 for choroideremia; and, conservatively, 30,000 for Stargardt disease and other *ABCA4*-related retinopathies. Some of these disorders, such as LCA, cause blindness at a very young age. Because of their severe consequences, these early onset diseases may be among the first to receive treatment, despite their low prevalence.

Nonspecific treatments using approaches such as nutritional factors, neuroprotection, cone-cell survival factors, antiapoptosis therapy, stem cell or tissue replacement, or even an artificial retina may be useful for several types or classes of disorders. Most of the rare genetic disorders, however, will require gene-specific treatments for durable cures. Even gene-specific treatments may lend themselves to certain generalizations. For instance, replacement of a normal gene would be suitable for autosomal recessive diseases and dominant diseases from haploinsufficiency. However, for dominant negative diseases, knockdown of the messenger RNA by a ribozyme or RNA interference (RNAi) is necessary. This approach can be generalized for multiple different dominant mutations of a specified gene, by knocking down the messenger RNA of both alleles and inserting a normal gene whose messenger RNA is protected from such targeted degradation.

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Eventually, treatments should be developed for all of the retinal dystrophies, but it is likely that the more severe phenotypes will receive the highest priority for clinical trials, because the benefit of treatment is great enough to support the risk of intervention. The basis for developing treatment strategies must be a solid scientific understanding of the disease at the cell biology and biochemical levels. Animal models must be available to guide and test the treatment strategies, and success needs to be demonstrated by alleviating the disease in the animal models.

Treatments could be focused on the phototransduction cascade, which has many components under genetic control, such as rhodopsin, transducin, cyclic guanosine monophosphate phosphodiesterase, and even the cyclic guanosine monophosphate-gated channel itself. Arrestin, rhodopsin kinase, retinal guanylate cyclase, and guanylate cyclase activating protein are necessary to deactivate components of the cascade and to reactivate other components for the next cycle of light-induced phototransduction. It is no surprise that, with mutation of any of these gene products, genetic disease can occur in phenotypes varying from LCA to RP to cone–rod dystrophies. Knockout, transgenic, and naturally occurring animal models to test treatment strategies are available for many of these disorders.

Once rhodopsin is bleached and the chromophore is converted to the all-*trans* form, it must be reconverted back to the 13-*cis* form. This conversion takes place within the retinoid cycle, mostly in the retinal pigment epithelium (RPE). A number of gene products are involved in this translation, defects in these genes also cause a wide spectrum of genetic diseases, including Stargardt disease, RP, and a number of other diseases. Some of these phenotypes are reviewed here.

Leber Congenital Amaurosis

Theodore Leber described LCA 135 years ago as a group of inherited disorders characterized by severe vision impairment from infancy. The fundus seemed normal initially, but, over time, it showed mottling and degeneration. There was considerable discussion regarding whether this represented a retinal aplasia or an early onset of retinal degeneration. It is of interest that, in 1916, Leber's writings indicated that he appreciated that this congenital amaurosis merged with other childhood-onset forms of severe retinal degeneration.

Although LCA is usually autosomal recessive, it can also be dominant, although this is rare. Eight genes are known to cause uncomplicated LCA (*GUCY2D*, *RPE65*, *CRX*, *TULP1*, *AIPL1*, *CRB1*, *RPGRIP*, and *RDH12*). None of these genes, at least in the United States, is present in more than 10% of LCA cases, and 50% of cases cannot yet be resolved at the molecular level. In Sweden, LCA accounts for 10% of blindness and has been estimated at 5% of all retinal dystrophies. Although LCA can be complicated by developmental delays, deafness, and seizures, none of the eight known genes is associated with a complicated phenotype.

The electroretinogram (ERG) tells us a great deal regarding LCA. Dr. Franceschetti first demonstrated that the ERG is markedly abnormal in LCA patients 50 years ago.[1] The ERG, which is the recording of the electrical events in the retina associated with stimulation from brief flashes of light, has become an extremely important test for LCA and for all other retinal diseases. By modifying the stimulus parameters, we can tease out responses of rods and cones, responses generated by photoreceptors, and responses generated by middle retinal neurons.

One specific form of LCA is caused by mutation of the gene encoding RPE65. The RPE65 protein isomerizes conversion of all-*trans*-retinal ester to 11-*cis*-retinol in the RPE and, thus, RPE mutations decrease the effective rhodopsin production. Loss of both copies of this gene causes not only the LCA phenotype but also severe childhood-onset retinal dystrophy and early adult-onset autosomal recessive RP. This form of LCA will be the first retinal dystrophy for which gene therapy will go to clinical trial.

Strategies have been devised for treatment of *RPE65*-deficient animals, including bypass of the defective biochemical step in *RPE65*-deficient mice, by presenting another functional chromophore beyond the block. Van Houser and his colleagues[2] showed that administering *RPE65*-deficient mice systemically with 9-*cis*-retinal, a chromophore substitute, resulted in rapid significant recovery of the ERG. Administration of 9-*cis*-retinal or 11-*cis*-retinal intraperitoneally, early in the course of disease, to $rpe65^{-/-}$ mice, resulted in partial prevention of cone loss, suggesting that the absence of the 11-*cis* chromophore may play a role in the early cone degeneration observed in this animal model.[3] Future studies will be needed to determine whether such a strategy of intermittent administration of the native chromophore or an alternative chromophore might be clinically safe and efficacious for patients with *RPE65*-deficient retinal dystrophies.

The second successful strategy for therapy of RPE65-related disease is gene replacement. Gene replacement has been demonstrated in Briard dogs homozygous for a defective *RPE65* gene (see the paper by Hauswirth elsewhere in the Symposium proceedings for further discussion of these studies and of the

Consortium project to treat *RPE65* deficiency in humans).

Retinitis Pigmentosa

Retinitis pigmentosa is the most common of the hereditary retinal disease phenotypes and is part of numerous syndromes (e.g., Usher syndrome). Some of the genes involved in RP can also cause cone–rod dystrophy. The hallmark symptoms of RP are nightblindness, which can have its onset at any time from birth to late adulthood, and visual field loss, which can occur at any age, and as one of several types.

Approximately half of all RP cases that are *not* part of a syndrome occur without a family history of the disease. These may be recessive, sometimes X-linked or, rarely, a new dominant mutation occurring for the first time. Of the approximately half of all cases that are multiplex (affect other family members), most are either dominant or recessive (20% each), but a significant portion (10%) are X-linked. By far the most common causes of dominant RP are mutations of the genes for rhodopsin and peripherin/human *Retinal Degeneration Slow* (*RDS*) gene.

In the United States, mutations of the gene for rhodopsin accounts for approximately 30% of autosomal dominant RP. A significant proportion (12–15%) of these mutations is from a single mutation, Pro23His, occurring nowhere else in the world. This form of RP is of relatively moderate severity and, therefore, it might not be the most suitable candidate for early gene-intervention trials. Other mutations that affect rhodopsin, such as the Pro347Leu mutation (5% of autosomal dominant RP), produce more severe forms of RP and might be more suitable candidates for early intervention. Mutations of peripherin/RDS account for approximately 8% to 10% of autosomal dominant RP.

Other genes that cause dominant RP (*RP1*, *CRX*, *NRL*, *RGR*, *IMPDH1*, *PRPF3*, *PRPF8*, *PRPF31*, *RP9*, *CA4*, and *FSCN2*) account for 15% to 20% of the remaining cases. Recessive RP can be caused by perhaps as many as 60 genes. Interestingly, the gene for Usher syndrome type 2, *USH2A*, accounts for approximately 4.5% of people with autosomal recessive RP, and it seems to arise from a single mutation.[4]

X-linked RP is a particularly severe form of RP, and it is caused by one of two known genes. Seventy-five percent of the cases are caused by a defect in the gene *RPGR*. Although some animal models exist for this disease, there are several reasons, related to molecular genetics and cell biology, why X-linked RP arising from mutations of *RPGR* may not be one of the first retinal diseases to be treatable. A second gene for X-linked RP, *RP2*, accounts for approximately 25% of the cases that are currently definable by molecular means.

Choroideremia, Stargardt Disease, and Other *ABCA4*-Related Retinopathies

Choroideremia is an X-linked disorder that causes a severe phenotype. This disease is associated with diffuse atrophy of the RPE and choriocapillaris and, eventually, total vascular choroidal atrophy. Currently, there is no good animal model for this dystrophy, which limits the ability to develop a therapy.

Stargardt disease and other *ABCA4*-related retinopathies are of particular interest because they are close to benefiting from therapeutic intervention. Stargardt disease was first described 95 years ago by Professor Karl Stargardt. A similar later-onset fleck retinal dystrophy was described in 1962 by Professor Franceschetti as "fundus flavimaculatus." We now know that this latter condition results from mutations of the same gene as Stargardt disease and that the two phenotypes can even be seen among siblings who share the same mutations. Stargardt disease affects 1 in 10,000 children, who typically experience loss of visual acuity and central scotomas, with various rates of progression. The carrier state for disease-producing mutations in *ABCA4* is roughly 1 in 50 individuals. The gene *ABCA4* is located on the short-arm of chromosome 1 and encodes the gene product ABCA4. The phenotypes that can be seen with mutations of *ABCA4* include RP, cone–rod dystrophy, Stargardt disease, fundus flavimaculatus, and a small percentage of AMD. The cone–rod phenotype can produce marked loss of ERG cone responses at an early age, with much more severe loss of vision to follow, making such patients candidates for early intervention.

ABCA4 is a flippase that transports all-*trans*-retinal out of photoreceptor outer segments. Loss of function allows the all-*trans*-retinal to react with phosphatidylethanolamine to form the Schiff base adduct N-retinylidine-phosphatidylethanolamine, which accumulates in the photoreceptor outer segments. When the shed outer segments are ingested by the RPE, the N-retinylidine-phosphatidylethanolamine is converted eventually to a toxic vitamin A–based fluorophore (A2E). The accumulation of A2E contributes to lipofuscin in the RPE and causes cell dysfunction and death. This process is driven by light, and light-deprived animal models demonstrate less degeneration. Although extreme light deprivation has yet to be tested clinically, it is unlikely to be an effective prophylactic measure for humans.

We now know that A2E-laden photoreceptor outer

segments ingested by RPE play a role in lipofuscin accumulation, cell dysfunction, and apoptosis in *ABCA4*-related disease. Phenotypes include Stargardt disease, cone–rod dystrophy, RP, and approximately 1% to 2% of AMD. Although mutation of *ABCA4* accounts for only a small percentage of AMD, A2E accumulation does have a significant role in common forms of AMD. Inhibition of RPE65 by 11-*cis*-retinoic acid has been shown to decrease A2E accumulation,[5] and it is hoped that drugs that lead to reduction of A2E accumulation could be a therapeutic strategy not only for orphan diseases, such as Stargardt disease and other *ABCA4*-related diseases, but also for AMD.

Conclusion

Therapies for early onset retinal dystrophies are likely to be developed first because of the severe consequences often associated with lifelong vision loss. One example in which a therapy is under development is LCA, which affects approximately 5,000 people in the United States, many starting at birth. Promising treatment strategies are in testing phases in mice and dogs deficient in a gene related to LCA.

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The National Eye Institute: Translational Clinical Research Initiatives on Inherited and Orphan Retinal Diseases: Personal Observations

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Molecular medicine, which seeks to therapeutically address the biological mechanisms of disease, is still in its infancy. Nonetheless, its potential to alleviate genetic maladies offers the first real hope for inherited orphan diseases, which have long remained incurable and untreatable.

For more than a decade, the National Eye Institute and The Foundation Fighting Blindness have spent considerable resources toward isolating mutant genes for retinal diseases. We have also made progress in understanding the underlying pathologic mechanisms that result from mutant genes. With that knowledge, we have begun to contemplate rational, molecular-based therapies. Translational research describes the effort to realize the therapeutic potential contained in basic laboratory findings. We are seeing the first glimmers from this work, and the lessons we have learned are helpful in planning future endeavors.

The first lesson is that it is difficult to predict what research will bubble to the surface. The effort to develop a gene transfer therapy for *RPE65*, for example, did not come from a formalized initiative to develop gene therapy for Leber congenital amaurosis. Rather, it arose out of hard work and some serendipity: the *RPE65* gene was cloned in rodents and canines, giving translational research efforts two platforms to test gene transfer techniques. The vision research community, with its very talented gene therapy experts capable of developing novel vectors to transfect the retinal pigment epithelium (RPE), found that the *RPE65* gene is small enough to fit within an AAV vector; most of all, despite the severe visual impairment that results from Leber congenital amaurosis, the morphologic structure of the retina remains intact. Looking backward, we see that these ingredients came together to create the likelihood for success, but we could not have planned *RPE65* gene transfer a priori. The lesson here is that we must pursue all avenues of disease research and be prepared to translate what emerges. Only then can we leverage the best opportunities to develop and commercialize safe and effective therapies (for more information on *RPE65* therapy, see the article by Hauswirth elsewhere in this Symposium proceedings).

The second lesson is that translational research takes time. For example, Dr. Matthew LaVail of the University of California, San Francisco, and his colleagues began evaluating neurotrophic agents as possible therapeutic candidates for retinal degenerations in 1991. The development and application of the encapsulated cell technology device to deliver ciliary neurotrophic factor took longer than a decade of work by two different biotechnology companies; we are only just now completing a Phase I clinical trial. In many cases, the slow progressive nature of these diseases will require lengthy clinical trial investigation to establish therapeutic value. Although thoughtful scientific planning can avoid unnecessary delays, time is the one constant we cannot significantly alter.

The third lesson is that clinician–scientists are becoming more valuable than ever. The vision research community needs to redouble its efforts to develop the next generation of clinician–scientists for translational research. We must ask ourselves some very basic questions. How do we attract talent? How does the National Eye Institute increase the ranks of clinician–scientists in an era of flat budget growth? Can allied nonprofit groups and associations increase their contributions to this endeavor? How do we preserve research time for clinician–scientists in an era of shrinking clinical revenues? To address these and other issues, the National Eye Institute will convene the first David Cogan Clinician–Scientist Symposium. The symposium will honor Dr. Cogan and consider the history and future of these rare and valuable members of the vision research community.

The last lesson is regarding resources that we must establish to aide in translational research. We need phenotype definitions that are mechanistically based on clinically applicable underpinnings from cell biology and physiology. We need to establish and adopt clinical trial outcome measures.

As molecular therapies reach clinical trials, we must be able to draw from a well-characterized, geno-

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typed patient population. Genotyping is one of the biggest resource challenges we face. We need state-of-the-art clinical facilities to administer and evaluate novel investigational therapies. These are not easy resources to come by. In some cases, funding is a challenge. Other efforts will require sharing what has, until now, been considered proprietary intellectual capital. We can work through these issues to ensure that we do not hamstring research progress. I see many opportunities to develop new and novel therapies. It will take time and resources, but we have a good start and many things working in our favor.

Challenges Associated With Clinical Trials for Inherited and Orphan Retinal Diseases

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The final common pathway on the road to successful therapy of patients with various inherited retinal diseases will be the proper selection of patients and the judicious application of various outcome measures. There are many challenges to manage.

Patient Selection

Considerations related to patient selections include disease stage; patient age; confounding variables, such as systemic diseases and environmental factors; natural history of the disease; genetic heterogeneity; and additional retinal changes.

Stage of Disease

Patients with earlier stages of disease have less secondary change in the retina, less scarring, and less change of the retinal pigment epithelium. More tests may be available that are applicable to monitoring these changes, providing more primary and secondary outcome measures. However, the selection of patients at different stages of disease affords us a better opportunity to ascertain the spectrum of disease that may respond to therapy.

Patient Age

Young children may be less able than older patients to consistently and successfully participate in psychophysical tests. Moreover, Institutional Review Board approvals can be more difficult to obtain for studies of young children.

Confounding Systemic Variables

We need to consider the control of potential confounding variables, such as diabetes, hypertension, thyroid disease, and the use of various medications, vitamin, and herbal supplements.

Environmental Factors

Environmental factors, such as sunlight exposure and cigarette smoking, have impacted other studies. These need to be considered in terms of randomization of patients.

Natural History of the Disease

The natural history for deterioration of visual function in a particular disease needs to be known with some degree of accuracy. Although we are making progress in this area, information is painfully lacking. In addition, in terms of cost, a disease in which 50% of visual function will be lost in 15 years presents a very practical problem in terms of treatment trials and the expense of monitoring the patients.

Genetic Homogeneity

Some treatment trials will require the use of genetically homogeneous patient populations.

Retinal Changes Not Specifically Related to Photoreceptor Cell Degeneration

A patient with retinitis pigmentosa (RP) may secondarily have optic nerve atrophy. Although function cannot necessarily be ascertained by paleness of the optic nerve, the potential problems for retinal cell transplantation, gene therapy, and so on, must be considered. Similarly, if gene therapy is tried in a patient with evidence of diffuse disease of the retinal pigment epithelium, the genes that are transfected into the photoreceptor cells may not be able to be sustained by the retinal pigment epithelium. Some patients have changes not only in the retinal pigment epithelium, but also in the choroidal circulation. How can photoreceptor cells be rescued without an underlying circulation to maintain these cells?

Another issue is the variation in clinical presentation of RP. There are at least four different patterns of phenotypic expression in RP patients. Unless patients are randomized along the lines of these phenotypes, this could represent a major limitation in treatment trials. The phenotypes include the following:

1. Patients with the first of these phenotypes present with diffuse disease of the retina, very restricted peripheral fields, and a nondetectable electroretinogram (ERG). These patients would

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not likely be reasonable candidates for therapy with neuroprotective factors.

2. A second phenotype is characterized by a more regional pigmentary degeneration of the retina, especially inferiorly, with a corresponding superior field loss. The ERG response in such a patient is not nondetectable, but it is more reduced than expected from the clinically apparent regional disease. A patient with this phenotype will eventually develop disease that is more diffuse, a more-marked deterioration of ERG function, and loss of both visual field and central vision.
3. Patients in the third category, with certain mutations in the rhodopsin gene, seem similar to patients in category 2. Both show an inferior retinal predilection for disease and superior field loss. However, the in patients in the third category, the ERG is considerably better preserved, remaining relatively stable for several years.
4. Finally, the fourth phenotype, referred to as a *delimited form*, shows a sharp demarcation of the retinal pigment changes, ring scotoma, and substantial ERG responses.

Because of these different phenotypes, it is important to randomize patients along the lines of recognizable phenotypes, because there could be a different response to various therapeutic procedures among the various phenotypes.

Addressing these patient-related issues requires careful, experienced clinical input at all stages of a therapeutic trial. The selection and use of outcome measures are also important considerations for clinical trials.

Outcome Measures

In evaluating intervisit variability, how much do we know regarding the various outcome measures that will be used to determine improvement? Perhaps the outcome measures that we need to select will relate to the stage of the disease and maybe even to the type of therapy being considered. It is also relevant to know whether the outcome measure is able to measure cone function, rod function, or both, successfully, and to know with what relative degree of sensitivity.

Electroretinography

One of the standard outcome measures used to follow degenerative retinal disease has been full-field ERG. This test can be used in children and adults, and at both early and later stages of disease. In an early stage patient with RP, the ERG may show newly reduced rod function but normal cone function. If we could intervene at this stage in the disease course of a patient with mild night-blindness, perhaps the night-blindness could be reversed. Without intervention, cone function will deteriorate. The ERG can be useful at any stage of disease. It can be used to demonstrate almost nondetectable rod function and only minimal cone function.

Which patients will have more intervisit variability in the ERG? In collaboration with Dr. David Birch, we looked at ERG measurements in a group of normal patients during a 2- to 6-year period. To document a statistically significant increase in the ERG over time, some of the ERG components may need to show a 100% change, and, even for a decrease, you may need to show a 50% loss. Our recent data show that in the short-term (not 2 to 6 years but within a couple of weeks) when the ERG is measured 2 or 3 times, the intervisit variability is approximately half, 50% for an increase or a decrease. It is important to obtain intervisit test variability for the duration of treatment that will be used, because long-term intervisit ERG test variability is different from short-term intervisit variability. Surprisingly, only a few studies of this type have been performed. This indicates that strategies that aim at monocular therapy in principle are to be more coveted than those that have to rely on binocular therapy, if one is using this testing modality. The other eye, of course, can be used as a control. Nevertheless, if one uses monocular therapy, one still should know about the interocular difference of the outcome measure in controls. For ERG testing, the intervisit variability can be appreciable. The 95% confidence interval can be approximately one third.

Static Perimetry

A widely used psychophysical test determines static perimetry thresholds when a test target is held at a fixed position and its intensity is increased. The device that we use is a Tubinger perimeter. This requires that patients are able to fixate. Very young patients may not be able to participate in this test, but with compliant patients at an appropriate stage of disease, static perimetry testing can provide valuable information. It can demonstrate that rods are seeing a test light and that thresholds are elevated by a specific amount.

Kinetic Perimetry

With kinetic perimetry, another psychophysical test, test targets move inward from the periphery. This test also requires fixation. It can be used at various stages of disease, but the end stage can be more difficult to measure. Patients with RP have different types of field loss,

different stages of field loss, and different patterns of field loss. It is probable that patterns of loss in one field will change at a different rate than in another field. Independent of the patterns of field loss, on average, RP patients lose 50% of their remaining visual field approximately every 7 to 8 years.

Designing the Testing Protocol and Interpreting Results

For clinical trials, if we select the correct patients at the correct stage of their disease, it might not take hundreds of patients and millions of dollars to obtain results. One could argue that obtaining unambiguous results from a few patients may be more advantageous than obtaining controversial results from large numbers of patients at the end stage of their disease.

How much testing needs to be done? Are the tests interchangeable? How much information is gleaned from doing multiple tests? To completely depend on only one outcome measure is to potentially lose valuable information on treatment interventions. For instance, an ERG may show no recordable rod response and a reduced cone response. However, by obtaining a psychophysical measurement from the patient, the rods may be observed to be mediating the threshold. Thus, one may lose information if locked into using only one type of measurement.

Lastly, it is also important to question whether a few-microvolt change in an ERG measurement is likely to have an impact on the quality of life of patients. With this in mind, we may need to place more emphasis on nonquantitative outcomes. I assess quality of life by looking for something as simple as shin bruising. If our care prevents patients from running into objects, then we have accomplished something important. To lose sight of this concept would be to lose focus regarding the purpose of this Symposium.

Photoreceptor Cell Rescue in Inherited and Orphan Retinal Diseases: Disease-Specific Requirements

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A question that we, as researchers, must ask in developing any therapy for retinal disorders in which photoreceptors degenerate, is whether the patient has any photoreceptor cells to be saved. The answer depends on the gene defect causing the degeneration and on poorly understood cellular and environmental factors. Three examples of retinal degenerative diseases illustrate the range of photoreceptor survival observed in patients. They are dominant cone degeneration, retinitis pigmentosa (RP) with a *PDE6B* gene mutation, and recessive RP with an *RPE65* mutation.

Dominant Cone Degeneration

Dominant cone degeneration is caused by defects in the *GCAP1* gene. The *GCAP1* gene normally promotes the replenishment of cyclic guanosine monophosphate (cGMP), an intracellular messenger essential in the phototransduction cascade in rods and cones. Dominant *GCAP1* mutations induce an overproduction of the second messenger cGMP, leading to the early death of cone photoreceptors. Rods seem relatively unaffected.[1]

Patients with dominant cone degeneration lose central visual acuity and color vision, and become photophobic. Mild symptoms typically start in the teenage years. By approximately age 40 years, vision is decreased to less than 20/200 (legal blindness). Microscopic evaluation of retinas from autopsy donor eyes of patients who were older than 40 years shows that although the majority of cone photoreceptors are absent, some have survived despite the metabolic defect.[1] If a gene or cellular therapy were available to maintain cones in this disease, it could be beneficial throughout life, even at a late age, because some cones are still available for recovery.

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Retinitis Pigmentosa, with a *PDE6B* Gene Mutation

The second example is recessive RP caused by mutations in the *PDE6B* gene. This gene encodes the β-subunit of rod cGMP-phosphodiesterase, which is normally found in rod photoreceptors. The enzyme modulates the levels of the intracellular messenger cGMP in response to changes in light. With no functional *PDE6B*, rod photoreceptors have abnormally high levels of cGMP, which leads to rapid death of the rod photoreceptors and a slow secondary death of the cone photoreceptors.

The rapid loss of rods results in patients having no night vision, even in early childhood. The loss of cones results in a gradual decay of daylight vision, leading ultimately to blindness. Although there are no reported autopsy donor eyes from patients with this gene defect, clinical findings in living patients and from the rd mouse model with defective *PDE6B* prove that rods die very rapidly after birth. Cones follow shortly afterwards.[2] In this example, cell survival enhancement therapy would save few, if any, photoreceptors, unless the patients were treated at a very early age. It is likely that the loss of photoreceptors is so rapid that cell survival therapies might require administration before the age of one year.

Recessive Retinitis Pigmentosa with an *RPE65* Mutation

The third example is RP caused by recessive mutations in *RPE65*. The protein encoded by this gene has a key role in the regeneration of 11-*cis*-retinal, the vitamin A homolog used by rods and cones as the initial molecule to sense light. Without *RPE65*, both rods and cones function very poorly (for more information on the *RPE65* mutation and therapy, see the article by Hauswirth, elsewhere in the Symposium proceedings).

Patients with this mutation have severely reduced rod and cone function from birth; residual vision decreases to blindness between early childhood and early adulthood. However, compelling evidence from the evaluation of the retina in an 11-year-old patient

indicates that numbers of photoreceptors are close to normal, even when vision is severely compromised.[3] Thus, it is possible that cell survival therapy for this disease might be beneficial, even if instituted many years after vision is lost.

Conclusion

In summary, the three genetically defined diseases presented here illustrate three windows of opportunity for administering gene and cellular therapies. In the disease caused by a *GCAP1* mutation, cones die slowly during the lifetime of the patient, with some cones still surviving at age 75 years. All patients with this disorder are potential beneficiaries of gene therapy. In the second disease, caused by a *PDE6B* defect, rod photoreceptor cells are lost in early youth, probably before age four years, and perhaps already at birth. The potential beneficiaries of therapy are fetuses or newborns. The third disease, caused by an *RPE65* mutation, falls in between the other two diseases in terms of the time course of photoreceptor survival, with a large number of cells remaining into adolescence, despite severely impaired vision; patients in roughly the first decade or two of life are the potential beneficiaries of gene and cellular therapy.

These examples also highlight the importance of knowing the time course of cell death associated with each genetically defined form of retinal degeneration when one designs a therapy. To obtain this information, it is necessary to meticulously document visual function in living patients, using methods such as electroretinography or optical coherence tomography. Findings obtained from living patients must be correlated with histopathologic studies of autopsy donors, which could be facilitated by encouraging patients with RP to donate their eyes after death. That is, studies of living and deceased patients are essential for identifying patients and diseases whose photoreceptors could be rescued with therapies that are appearing on the horizon.

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ADVANCES IN BASIC SCIENCES IN INHERITED ORPHAN RETINAL DISEASES

Beyond Basic Research for Inherited and Orphan Retinal Diseases: Successes and Challenges

GERALD J. CHADER, PHD

This symposium was crafted to examine all facets of the movement from discovery in the laboratory to clinical trial. The key element to success is the partnerships among basic scientists, private foundations, the National Institutes of Health, venture capitalists, and industry.

In 1990, the first retinitis pigmentosa (RP)-causing gene mutation was found.[1] Currently, more than 100 genes and 155 chromosomal loci have been identified in which mutations cause retinal degenerations. Steve Daiger's web site (http://www.sph.uth.tmc.edu/RetNet) provides a compendium of all of these genes. Dr. Daiger estimates that we currently know approximately half of the genes for the rare inherited retinal degenerations. This enables us to move toward gene therapy as we continue to seek the unknown gene mutations. Importantly for age-related macular degeneration, we have recently learned of several genes for the disorder.

Many opportunities exist for industry. By knowing a disease gene and its product, we might identify a biochemical pathway that is affected and find a particular drug to control the abnormal result. Think of all the agents sitting on company shelves that could be tested and ultimately used!

We need to develop facilities for large-scale genotyping, such as the Carver Laboratory for Molecular Biology (http://www.carverlab.org). Genotyping should be available to all patients so that we can aggressively devise gene therapy and other gene-based therapies. Patient registries must be developed, not only for clinical trials, but also to reach out to patients when the treatments are available. Dr. Paul Sieving and his staff at the National Eye Institute are diligently working in this area. We hope that we will know all of the retinal degeneration genes within the next decade or so, affording the possibility to treat of all retinal degenerative diseases.

In the preclinical arena and for final animal testing in medical therapy, many good animal models are available for studying retinal degenerations. Natural models include retinal degenerations in drosophila, zebrafish, chicken, rodents, cats, and dogs—covering recessive, X-linked, and dominant forms of RP. Bioengineered models include many transgenic forms of rodent and even pig RP. Better models for age-related macular degeneration are also becoming available. Given that intense light probably leads to an oxidative insult and photoreceptor cell death, models can be created by light damage. Even models for retinal degenerative diseases, such as Usher syndrome are being developed.

With a firm basis in genetics and cell biology and the availability of animal models, potential treatments and cures for the retinal degenerations can and are being designed in the areas of transplantation, pharmaceutical therapy, nutrition/supplements, visual prosthetics, and gene therapy.

Transplantation

In 1988, Li and Turner[2] first showed a delay of photoreceptor cell degeneration after retinal pigment epithelial cell transplantation in the Royal College of Surgeons (RCS) rat. Success with photoreceptor cell transplantation has been less definitive. However, some researchers demonstrated the preservation of light-driven brain responses after photoreceptor cell transplantation in an RP animal model.[3] Thus, proof of principle has been

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established, at least for retinal pigment epithelial cell transplantation, and it is hoped that human clinical trials can be planned.

Another exciting new area of study is stem cell research. Although embryonic tissue is the richest source of stem cells, small numbers of stem cells have also been found in adult tissues. For example, stem cells in the pigmented ciliary margin of the adult mouse eye have been reported,[4] and seem to function as true retinal stem cells. Stem cells that can be transplanted to the retina, where they partially differentiate along a neuronal phenotype, have also been found in the hippocampus, iris, and sclera.

What are the opportunities for commercial companies? We know that stem cells from different sources can be transplanted into the retina and at least partially differentiate. We are beginning to know the genes (e.g., *PTEN* or *6-homeodomain factor*) that control retinal progenitor proliferation. We are beginning to understand some of the extracellular matrix factors in the interphotoreceptor matrix that control differentiation of these cells. Finally, we are uncovering other factors (e.g., lignin) that will allow for correct synapse formation of the stem cell.

Pharmaceutical Therapy

Pharmaceutical therapy includes the use of any agent (e.g., a natural neuron-survival agent or a synthetic drug) to prolong the life and function of photoreceptor cells. In 1990, LaVail and his coworkers first showed that basic fibroblast growth factor, a natural growth factor, could delay photoreceptor cell death in animal models.[5] Since then, a number of natural factors that act as inhibitors of apoptosis have been uncovered (e.g., ciliary neurotrophic factor, brain-derived neurotrophic factor, lens epithelium–derived growth factor, and pigment epithelium–derived factor).

An important aspect of any pharmaceutical therapy for a retinal degeneration is drug delivery. Weekly, monthly, or yearly intraocular injections represent distinct disadvantages to patients who require treatment during a lifetime. Intraocular capsules and inserts that slowly deliver drugs for months to years are coming to clinical trial.[6] Transscleral delivery of drugs directly through the sclera to the retina may also be possible.[7] For example, scleral patches or depots may allow for proteins as well as smaller molecules to diffuse across the sclera into the vitreous and retina. This could provide a noninvasive, safe method for drug delivery for many neurotrophic and antineovascular agents. Edelhauser and his coworkers have examined many of the factors affecting drug delivery through the sclera.[8] The ultimate goal is to reach the target area located at the back of the eye via intravitreal injections/inserts or transscleral delivery.

One interesting question to address regarding pharmacological therapy is whether a combination of drugs is more effective than just one. Van Veen and coworkers have used an elegant explant culture system[9] to show that brain-derived neurotrophic factor and glial cell–line derived neurotrophic factor (GDNF) are more effective together than individually.[10] More work is needed to see whether other combinations of factors are equally or more effective in reducing retinal cell degeneration.

Nutritional Supplements and Retinal Prostheses

Nutritional therapy for retinal degenerations has been controversial, but now must be taken seriously. With mixed success, three human clinical trials have been completed: the vitamin A trial for RP, the docosahexaenoic acid study for X-linked RP, and the Age-Related Eye Disease Study (AREDS) for age-related macular degeneration. Investigators in Paul Sieving's laboratory showed that retinoid analogs could be effective in treating some of the rare diseases (e.g., Stargardt disease) by slowing vitamin A–based fluorophore (A2E) accumulation and the degenerative process.[11] There are many other examples of basic research and progress toward clinical trials on other nutritional agents that could be useful in slowing the course of retinal degenerative diseases.

Visual Prosthetics

Great progress is being made in the development of visual prosthetics. Two clinical trials are currently under way under the auspices of Optobionics Corporation[12] and Second Sight[13] (for more information on visual prosthetics, see the article by Greenberg elsewhere in the Symposium proceedings).

Gene Therapy

Potentially, gene therapy could provide a cure, and not just a treatment, for many inherited retinal degenerative conditions. In 1996, Bennett and colleagues first established proof of principle for such therapy in the recessive *rd* mouse model.[14] In 1998, Lewin used ribozyme therapy to rescue photoreceptors in a transgenic rat with a dominant type of disease.[15] The new, exciting news is that of work on the Briard dog RP model, which has a mutation in the *RPE65* gene and, thus, exhibits a retinal disease process comparable to that in humans with Leber congenital amaurosis. It has been approximately 4 years since the initial dogs received gene replacement therapy, and the results continue to be excellent (for more information on this gene replacement therapy, see the article by Hauswirth elsewhere in the Symposium proceedings).

Gene therapy, including the use of different vectors

and small interfering RNAs, will be one of the most important areas in the future for treatment of retinal degenerations. There has been tangible progress, not just in the slowing of the disease process but also in the restoration of vision, what we might begin to call ″a cure.″ We hope the current Leber congenital amaurosis trial will be the archetype for future neurotechnology trials. GenVec, Inc. is blazing the way with its approved trial for gene-delivered pigment epithelium–derived factor in age-related macular degeneration. The Foundation Fighting Blindness has already had a very productive meeting cosponsored by the Food and Drug Administration and the National Eye Institute, at which close consensus was reached on ocular gene therapy regulatory issues. Thus, the medical therapy path for retinal degeneration gene therapy is clear, and the time is ripe for commercial companies to become involved.

Conclusion

Proof of principle has now been established for several types of therapies for the retinal degenerations. Gene therapy, transplantation, pharmaceutical therapy, nutrition, and electronic implants show promise. With industry help, positive laboratory findings can progress to treatments.

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Cellular Mechanisms of Retinal Degenerations: *RPE65*, *ABCA4*, *RDS*, and Bicarbonate Transporter Genes as Examples

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Our experience with inherited retinal degenerative diseases tells us that there are many ways to kill photoreceptor and retinal pigment epithelium (RPE) cells. The photoreceptors, in particular, are highly susceptible to mutations expressed endogenously, locally, or systemically. In many cases, a gene mutation is expressed systemically, but results in no obvious phenotype in any organ except the eye, where the result is photoreceptor cell death.

Photoreceptors are capable of detecting a single photon. They live in an extraordinarily high oxygen environment, in which there is abundant free radical formation. Several mechanisms for photoreceptor loss are currently understood.

The *RPE65* Gene

Mutation of the *RPE65* gene causes disruption of photoreceptor morphology and function.[1] The RPE65 protein is involved in the visual cycle. The visual cycle brings vitamin A (also called all-*trans* retinol) into the eye and delivers a derivative of vitamin A to the photoreceptor cells, both the rods and the cones. Vitamin A enters the RPE from the choroidal capillaries with the help of retinol-binding protein (RBP). The RBP protein is made in the liver, and the gene that produces it is known to occasionally mutate in humans. In one rare example of two sibling children, who are compound heterozygous for mutations in RBP, their only phenotypes are mild acne, iris coloboma, and slow degeneration of the RPE. They seem to be otherwise quite healthy. Nonetheless, these rare cases underscore the fact that RBP is essential for the delivery of vitamin A into the RPE. RBP is thought to interact with receptors on the basal surface of the RPE. The receptors then deliver the vitamin to a brigade of proteins, many of which are known to have mutations that cause blinding disease. For example, one of these proteins, called LRAT, causes a very early onset retinal degeneration in humans. Mutations in the genes encoding these proteins are all recessive mutations. In principle, similar to RPE65, the malfunctioning proteins could be replaced with normal proteins via gene therapy. The proof of principle for this has been established by the RPE65 consortium, which successfully used gene therapy in affected dogs (for more information on the RPE65 Consortium, see the article by Hauswirth elsewhere in the Symposium proceedings).

The proteins that are involved in the process of changing vitamin A from its all-*trans* retinol form to the 11-*cis* retinaldehyde form are integral in this process. Mutations in these proteins cause impaired vision or blindness. The retinol binding protein (RBP), lecithin: retinol acyl transferase (LRAT), cellular retinaldehyde–binding protein (CRALBP), and 11-*cis* retinol dehydrogenase (RDH5) proteins, among other proteins in the visual cycle, are known to be mutated in certain disorders. Ultimately, the 11-*cis* retinaldehyde is delivered to the rods and cones for use in phototransduction. Rods also express a plethora of gene mutations, including *rhodopsin*, which lead to their demise. However, in the example I have described, the RPE expresses various mutations, but is, itself, healthy; the photoreceptors die as bystanders. Thus, a cell "nonautonomous process" kills the photoreceptors. By contrast, in the case of *rhodopsin* mutations, the process is "cell autonomous."

The *ABCA4* Gene

ABCA4 is a complicated gene, and its protein product, *ABCR*, is fascinating. We call the protein a "phospholipid flippase," because it moves (translocates) a phospholipid from one leaflet of a lipid bilayer to the other leaflet. This phospholipid flippase is found in the margins of the rod and cone outer segment disks, the portion that has a hairpin loop structure because of membrane folding. The function of the flippase is to move a combination of the phospholipid, phosphatidylethanolamine and all-*trans* retinaldehyde, called N-retinylidene-phosphatidylethanolamine (N–RPE), out of the disk membrane. That N-retinylidine-phosphatidylethanolamine complex is produced when rhodopsin is hit by a photon and 11-*cis* retinaldehyde is converted into the all-*trans* form of retinaldehyde. During this process, we

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do not yet know how all-*trans* retinaldehyde leaves the interior of the opsin molecule. Does it come out through the top? If it does, the flippase is not needed. Does some of it go out into the disk lumen, or does some of it diffuse into the plane of the bilayer? Some of it has to leave the rhodopsin molecule through either the bottom or the side. If this were not the case, the ABCR protein would not be necessary. X-ray crystallography suggests that there is an opening in the side through which the all-*trans* retinaldehyde could escape. There is a trapdoor that would probably prevent its exit from the bottom of the opsin molecule.

The answers to these questions are important for recessive Stargardt disease, because we know that mutations in the *ABCA4* gene are responsible for this disease.[2] The problem with recessive Stargardt disease is that the recycling process fails because the flippase is not working properly. As a result, some of the all-*trans* retinaldehyde is not cleared from the outer segment disks, and, eventually, two all-*trans* retinaldehyde molecules become covalently bound to a single phosphatidylethanolamine. The RPE, through its phagocytic mechanism, ingests approximately 1/10 of each rod outer segment per day. When the phosphatidylethanolamine-all-*trans* retinal conjugate is ingested by the RPE, it is converted into a detergent-like molecule, vitamin A–based fluorophore (A2E), within the lysosomes of the RPE. In the presence of oxygen, A2E is oxidized, producing epoxides, which are even more poisonous. Thus, ingestion of this material is like eating a poison pill. Through a bystander effect, the gene mutation expressed in the photoreceptor kills the pigment epithelium, and that, in turn, kills the photoreceptor, in a reciprocal effect.

Thus, patients with recessive Stargardt disease have a vitamin A disposal problem and, therefore, should not take vitamin A. Dr. Paul Sieving, Director of the National Eye Institute, was the first to suggest that the acne medicine Accutane might be effective in treating Stargardt patients. He found that some patients who were taking Accutane for severe cystic acne had a dark-adaptation problem. This was because Accutane inhibits the enzyme that removes a hydrogen from all-11-*cis* retinol. Robert Rando at Harvard University and his collaborators showed that it also acts at the level of *RPE65*, interfering with its activity. As a result, the visual cycle is perturbed and the photoreceptors get less 11-*cis* retinaldehyde; this is good for patients with a vitamin A disposal problem. In this situation, a deficit in one process helps or spares the photoreceptors. Unfortunately, Accutane is very toxic, and long-term use causes liver problems. Analogs less toxic than Accutane are being sought that would slow down the rate of pigment epithelial damage caused by the buildup of A2E.

The *RDS* Gene

Another cellular mechanism of retinal degeneration involves the *RDS* gene, which has a highly pleiotropic affect when it is mutated. A null mutation in the *RDS* gene causes cell death. In the same family, there can be three siblings with the same point mutation who have three different clinical conditions: flecked retinal disease, macular degeneration, and classic retinitis pigmentosa.

To develop a normal photoreceptor outer segment, both alleles of the *RDS* gene have to be fully expressed. This was reported by Dr. Somes C. Sanyal in Holland some years ago, when he published his first paper on the spontaneous mutation of the *rds* gene in the mouse. He showed that when both gene alleles carry a null mutation, no outer segments are formed, whereas one null allele plus one normal allele result in dysmorphic, but functional outer segments. With the *rds* mouse as our genetic background, we performed an informative experiment, by chance, that demonstrated the importance of rods for their partner rods. We were making transgenic animals, trying to rescue the normal phenotype by transgenically giving corrective, normal DNA to mice that lacked photoreceptor outer segments because of a null *rds* mutation. This transgenic gene therapy is performed by injecting the corrective (rescue) DNA into the male sperm nucleus. After the DNA was injected, in one case in which the transgene integrated into the X chromosome, all of the male photoreceptors were normal, having complete outer segments. However, all of the females that were hemizygous for the transgene exhibited patches of photoreceptors lacking outer segments, interspersed with patches of photoreceptors containing normal outer segments. Photoreceptors of females homozygous for the transgene, however, resembled those of the hemizygous males. To our surprise, the females with patches of rescued outer segments lost all of their photoreceptor cells at the same rate as the animals with unrescued outer segments. Therefore, something is required from one population of healthy rods to keep the other rods alive, or perhaps the unrescued rods produce something destructive. This inspires us to think about rod survival factors and cone survival factors; after all, if the rods are kept alive, the cones will be kept alive. We are investigating the predisposing genes and putative positive and negative factors.

Bicarbonate Transporters

A final example of a cellular mechanism for retinal degeneration is the case of a ubiquitously expressed bicarbonate transporter.[3] Made in the kidney and in many other tissues, including the retina and inner ear, the stilbene–insensitive electroneutral sodium bicarbonate cotransporter (NBC3; *slc4a7*), if mutated, causes Usher syndrome (USH) type 2B in the mouse. In the knockout animal, the retina looks normal initially, until two months of age, when the photoreceptors start to die. By six months of age, the superior and inferior parts of the retina have lost quite a few cells. By one year old, essentially no photoreceptors are left. Unlike other mouse models of USH, this is a robust retinal phenotype. Within the photoreceptors, the NBC3 bicarbonate transporter is expressed in the plasma membrane of the photoreceptor synaptic terminals. These photoreceptors have an acid/base problem, and it is apparently killing them.

At the same time, the hair cells in the inner ear in the knockout animals also die. Thus, the animals have an auditory defect, and, interestingly, it involves only the hair cells at the base of the cochlea and not those at the tip. This is also the case with USH2B. The orthologous *SLC4A7* gene in humans maps to 3p22, very close to the USH2B linkage site. We, therefore, think that this mouse is an excellent model for USH2B in humans.

This describes some selected mechanistic examples of how photoreceptor and inner ear hair cells die because of gene mutations. Of course, there are many other examples. Insights such as these, borne out of hard work at the laboratory bench, which will finally provide rational insight into the treatment of inherited retinal diseases.

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Effect of Gene Expression on Cone Survival in Retinitis Pigmentosa

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Our efforts have been directed to understanding the gene expression exhibited in the retina during normal development and disease. We initially used serial analysis of gene expression (SAGE) to rapidly assess which genes are expressed in the retina as a whole. We made libraries of expressed sequences from the retina at several developmental stages. In addition, we made a library from a mouse strain in which the *CRX* gene is mutant, in which photoreceptors do not differentiate. This set of libraries has 800,000 "tags," or bits of sequence from the ends of messenger RNAs expressed in the retina, and comprise the largest collection of tags from a single organ. Essentially, these are complementary DNA tags. The library catalog indicates whether a gene is present or not and when, during development, it might be expressed. Moreover, based on these tags, we chose approximately 1,000 probes to hybridize to tissue sections to investigate where in the retina a gene is expressed. We used tissue sections from the 10 time points in retinal development that were used to make the SAGE libraries.

The SAGE library and associated in situ hybridization images comprise a searchable database that allows a gene to be searched by a variety of characteristics: name; tag sequence; unigene number (which, unfortunately, keeps changing); chromosome location; accession number for the probe; and clusters of genes that might share similarities in their expression profile. The clustering of the genes is based on various criteria, so that genes with certain similarities in their expression pattern can be searched according to similarities based on SAGE tags or similarities based on in situ hybridization data. All of this is available on our web site, at https://bricweb.partners.org/cepko/.

We used the database to search for photoreceptor-enriched genes. Usually, we cannot determine whether rods alone or both rods and cones express a gene. However, in some cases, we have been able to identify rod-specific genes. Recently, we created a mouse model that has primarily cones and very few other cell types. This has enabled us to find many new cone-specific genes. We have also been able to use the SAGE data to create a list of Müller glia-enriched or -specific genes. Müller glia are of interest both in normal development and disease. Müller glia have inward processes that create the inner limiting membrane of the retina and processes that travel upward to surround the photoreceptor cells. Müller glia provide support to the photoreceptors. This could be important in disease.

Müller glia may also serve as stem cells. In the chicken retina, some Müller glia cells divide in response to injury and produce several types of neurons.[1] Findings provided by these new cell molecular profiling studies suggest that Müller glia are multipotent progenitor cells of the adult. Their gene expression looks just like that of the normal multipotent retinal progenitor cells during development in the normal retina. That is, normal retinal progenitor cells are multipotential; they make all of the types of retinal neurons, and they make glia. If we look molecularly for genes in the adult that are expressed in Müller glia cells in an enriched fashion and not in the neurons, the genes we find that fit that profile are also expressed in normal developing progenitor cells. This might inspire investigators to look at Müller glia as a potential source of replacement neurons, either in situ or perhaps in a tissue culture dish.

We have also taken individual Müller glia cells from normal retinas and from the *rd1* retina and asked, "What kind of change occurs in Müller glia cells in the process of disease?" We are still mining these data, identifying genes that change their expression in disease and taking a more precise look at genes that are present only in Müller glia cells.

Electroporation for Making Animal Models

Although we can generate lists of genes expressed in the retina and get fairly high-resolution data by looking at the in situ hybridization, how are we actually going to determine what all of these genes do? One way is to make transgenic animal models of various types. Alternatively, viral transduction can be used to deliver genes to a variety of species. However,

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rather than build a virus (which we have done in the past to assay gene function) or create knockout or transgenic mice (which is both slow and expensive), we are finding it much quicker to introduce DNA plasmids in vivo by electroporation. We use this technique to create models, either by overexpressing a gene or knocking genes down with RNAi (RNA interference). We can also use electroporation as a more efficient way to look for regulatory elements that might direct expression to a particular cell type.

The method of electroporation is simple. One injects plasmid DNA (at least three plasmids can be introduced simultaneously with high efficiency into the same cells) into the subretinal space. This is the same protocol we have used for years to deliver viruses. It seems to work effectively in mice and rats, as long as the procedure is performed between postnatal Day 0 and 7. The procedure can be performed earlier in chicks (i.e., in the embryo during the first two days of incubation), but we have not been able to administer electroporation effectively to mature rodent retinas. Plasmids can probably be introduced in neonates of other species as well.

The DNA is delivered, followed by a few milliseconds of an electric shock administered across the head to open up the pores in the cell membranes. There is no specific uptake mechanism. As long as the pores are open, the DNA can enter, which is why more than one plasmid can be introduced at a time. Our procedure was to perform electroporation at postnatal Day 0 with a plasmid that encodes green fluorescent protein, which is expressed in many cells in the outer nuclear layer. This is very useful for studying rods, because this protocol can be used to introduce genes into rods. Plasmids usually do not integrate with high efficiency, but the expression persists for at least 50 days.

One can also look at loss of function by introducing hairpin RNAs or RNAi. This is a DNA-based method using a human U6 promoter to drive expression of the hairpin. A hairpin can be designed against the gene of interest or against multiple genes, and several hairpins can be introduced to the same genes. The hairpin plasmid is then introduced by electroporation along with green fluorescent protein to mark the cells. It is then possible to see what happens as a result of the hairpin.

We made a mouse mutant for CRX, a transcription factor required for photoreceptor differentiation. The photoreceptors are generated and migrate to their layer, but do not differentiate outer segments or terminals. Similarly, in a conventional knockout for *NRL*, photoreceptors are generated but, in this case, rods do not seem to form. Instead, cells have cone features or, perhaps, are actual cones. To find out if electroporation would allow us to phenocopy the *CRX* or *NRL* mutant phenotypes (i.e., generate the same phenotype as a conventional knockout), we examined the morphology and gene expression after introduction of hairpins to *CRX* or *NRL*. For a control, we took a U6 plasmid without a hairpin, coelectroporated it with a green fluorescent protein-expressing plasmid, and observed green fluorescent protein-positive bipolar cells and many rods in the outer nuclear layer with nice outer segments. When a *CRX* hairpin is introduced, photoreceptors are observed, similar to the conventional knockout, but outer segments are not observed. With an *NRL* hairpin, photoreceptors are located in the outer portion of the outer nuclear layer, which is where cones are located, therefore, presumably these cells now have cone features. They also have shorter and stubbier outer segments, similar to cones. As a control for an adverse event that might occur in a cell during a successful targeting event, we introduced Glyseraldehyde–3–phosphate dehydrogenase (GAPDH) RNAi. Although we have shown that this construct can target glyceraldehyde phosphate dehydrogenase, we do not see any phenotype. An empty hairpin or targeting another gene seems not to hurt the photoreceptors. However, when *CRX* or *NRL* is targeted, one can observe results in photoreceptors. This method allows one to create models of loss of function more quickly. It took us one year to obtain the *CRX* knockout data. With electroporation, we can obtain similar data in a few weeks.

Another approach to obtaining specific regulatory elements to drive expression of a gene in a particular cell type (for a future gene therapy effort) is to use plasmids that have regulatory regions for a gene that is expressed in a specific cell type. We mixed together three plasmids: a rhodopsin promoter with Cyan Fluorescent Protein (CFP); a calcium-binding protein-5 promoter driving Yellow Fluorescent Protein (YFP); and a cellular retinaldehyde-binding protein (CRALBP) promoter driving red fluorescent protein (DsRed). We coelectroporated all three plasmids and observed blue photoreceptors, yellow bipolar cells, and red Müller glia, as we had hoped. This method will allow dissection of regulatory elements, which can be performed more quickly than conventional transgenics. In fact, for many of these cell types, there is no reliable in vitro assay, and in vivo experiments must be used to achieve the proper regulatory elements.

How Can Genomic Methods be Applied to Disease?

If cone death occurs in models in which the diseased gene is expressed only in rods, why are the cones dying? Generally, the rods die before the cones,

although the exact time course is not known. We would like to see what changes in gene expression accompany the onset of cone death. If those changes are common in several different mutants that have different causes of rod death and perhaps different time courses, then, by looking at the commonalities in the different models, we may find some clues for why the cones are dying.

Two models have been proposed for the death of cones in rod disease, a toxin model and a trophic factor model. In the toxin model, the following mechanisms may be involved: 1. The dying rods produce a toxin that kills cones. 2. The collapse of the outer nuclear layer leads to physical pressure on the cone outer segments. 3. Oxygen delivery cannot be reduced enough to keep free radicals under control. 4. Support cells are sick and make toxins.

Possible mechanisms in the trophic factor model include: 1. Rods make a trophic factor for cones. Delivery could be through gap junctions. 2. Support cells normally make a trophic factor for other cells; because the support cells are sick, they cannot supply the necessary trophic factor.

Mouse Models for Retinal Disease

Mouse models are well established for studying retinal disease. They are relatively cheap; they can be engineered to model specific diseases; and their cells can be accessed at any time. They can be used for genomic studies because of coverage of the mouse genome; that is, when sequences are pulled out, we can quickly look up what the gene is and then use the animals for investigations of possible therapies.

The best mouse models for our purposes are those in which cones die despite the disease genes being expressed only in rods. Mouse models in use for retinitis pigmentosa research include *rhodopsin*-null mice,[2] phosphodiesterase-β *rd1* mice (The Jackson Laboratory, Bar Harbor, ME), phosphodiesterase-γ mice,[3] rhodopsin pro23-his mice,[4] and *cyclin D1*-null mice.[5]

Sicinski created the cyclin D1 knockout mouse to look at cell replication;[5] cyclins are important in controlling retinal proliferation. He found that the retina was fivefold smaller than normal in this mutant. In addition, we found that, in the first few postnatal weeks, a whole-mount view of the retina shows dark spots corresponding to holes in the outer nuclear layer. The fascinating thing regarding this model is that the holes in the outer nuclear layer stop expanding, which is very unusual in retinal degeneration. The holes seem to start at approximately postnatal Day 9, progress until approximately postnatal Day 21, then stop. The arrest in progression of the degeneration could provide a clue for stopping death in more progressive models.

The geography of the spread of cone death can be easily visualized by putting a transgene into the cones of the retina. Cones degenerate in a geographic pattern, with the central cones degenerating first, and then progressing peripherally. This pattern suggests a nonautonomous mechanism. There are also local areas in which there is more progression. This geographic spread is very interesting, and we would like to be able to sample, in some cases with microdissections, what is going on within and around some of the peripheral craters.

We wanted to quantify the progression of the rod and cone death for the various models, including the *Rd1* model, and to see whether our strain had the same kinetics of death as were reported originally by Carter-Dawson and colleagues.[6] We also wanted to have rapid ways to evaluate cone and rod death quantitatively in the various transgenic or knockout animals. For this purpose, we performed quantitative reverse-transcriptase polymerase chain reaction on the RNA at various stages. An extremely rapid drop in rhodopsin RNA in the *Rd1* mutant started at approximately postnatal Day 10. This RNA measurement matched very closely with data from a cell-counting protocol. We are also following blue cone opsin by reverse-transcriptase polymerase chain reaction. The drop in the blue cone opsin starts later than the rhodopsin drop, at approximately three to four weeks. We are taking samples from all of the animal models we have gathered to compare time points on the microarrays.

We are trying to categorize the genes that change in expression levels during disease, based on their cellular expression pattern. We are interested, in particular, in genes that changes expression in Müller glia cells, pigment epithelial cells, or rods. One can speculate why these cell types might be important in the survival of cones. To investigate where these changes are taking place in the retina, we use in situ hybridization. After the *Rd1* microarray analysis, we pulled out approximately 300 probes for genes that either increase or decrease in level during disease, then performed in situ hybridization on either the *Rd1* or a wild-type retina at various times during development. In the *Rd1* retina, when all the rods are gone and the cones are undergoing their most rapid death, the *Rd1* gene is highly expressed in the inner nuclear layer and the ganglion cell layer at cyclin–dependent kinase 5 (Cdk5) and its activator p35. In the wild-type retina, there is very little expression of the *Rd1* gene. We are putting all of these genes into bins based on their expression patterns and examining the nature of the genes to determine which genes to investigate further with studies of function.

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Survival Factors for Treatment of Retinal Degenerative Disorders: Preclinical Gains and Issues for Translation Into Clinical Studies

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To advance the therapeutic use of survival factors (also called neurotrophic factors) for retinal degenerative diseases, several key issues must be addressed. These include efficacy (proof of concept), delivery, specificity, targets, and toxicity of the various agents.

Proof of Concept

Proof of concept was initially addressed in the RCS rat, in which we demonstrated that retinal degeneration could be slowed by placing basic fibroblast growth factor (FGF) into the eye.[1] We injected the neurotrophic factor into one eye of the rats on Day 23, when photoreceptors in this animal just begin to degenerate. One month later, we examined the treated and untreated eyes. As expected, most of the photoreceptors in the untreated control eyes had degenerated. In the basic FGF-injected eyes, however, photoreceptors were remarkably preserved.

This led us to examine a number of other classes of survival factors in a different model; a model using light exposure in albino rats.[2] Excess light damages the photoreceptor cells. In this study, we found that photoreceptor cell degeneration was slowed by several agents (acidic and basic FGF, neurotrophin-3 [NT-3], brain-derived neurotrophic factor, ciliary neurotrophic factor [CNTF], and interleukin-1β), all of which work through four different receptor families.

The molecule that has been the most widely studied is CNTF, or Axokine, from Regeneron Pharmaceuticals, Inc. Ciliary neurotrophic factor has been successful in slowing degeneration in 13 different inherited retinal degenerations in 4 different species. It should be noted, however, that relatively few agents have been tested, and only in a few models. There are many challenges and opportunities here for investigating other agents, including pigment epithelium–derived factor, glial cell line–derived growth factor, and rod-derived cone viability factor.

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Delivery

The neurotrophic agents that we, and others, have been using are too large to cross the blood–retinal barrier. Several protective agents small enough to cross the blood–retinal barrier have been found, but, unfortunately, many have significant systemic side effects. An alternative approach is to find a way to provide local and sustained release of the larger, survival-promoting molecules. One approach being tested is encapsulated cell technology, in which the agent is placed intraocularly within a special capsule; encapsulated cell technology was being used in a Phase I clinical trial at the National Eye Institute.

Another approach, using gene-based delivery of neurotrophic factors, has been quite successful in a number of laboratories. The procedure consists of using viral vectors to deliver complementary DNA for a neurotrophic factor. After a single injection of the complementary DNA-viral vector into the eye of an animal, the goal is for cells in the eye or retina to produce the neurotrophic factor, thus, conferring protection against degeneration. Based on the successful slowing of photoreceptor degeneration, we and others have been able to demonstrate long-term expression of the neurotrophic factor[3] for up to 8.5 months of age. In experiments comparing bolus-injected CNTF to gene-based delivery, it is clear that local sustained delivery using gene-based methods is more effective than the bolus-injection method.

Specificity

There is some suggestion that any neurotrophic factor, if delivered in high enough concentration, or if made continuously available, could be protective. This raises the issue of specificity. To test specificity, we overexpressed NT-3, one of the neurotrophins that protects the rat and mouse retina from light damage, in albino animals (the α-A crystallin promoter was used to overexpress NT-3). After 3 weeks of constant light, we found that the number of rows of nuclei in the retina of the nontransgenic animals was reduced from 10 to 1. However, their similarly exposed transgenic littermates had

five to six times that number of photoreceptors, showing that they had been protected by the overexpressed NT-3.

We then transferred the transgene into four different mouse models of retinal degeneration—three with photoreceptor mutations and one with a retinal pigment epithelium (RPE) cell mutation—to investigate whether the overexpression of NT-3 would slow the retinal degenerations. Surprisingly, in no case did NT-3 provide any protection.

Although some agents, such as CNTF, seem to affect a broad spectrum of degenerations and may be mutation independent, it seems that it will have to be empirically determined whether other agents will act in a mutation-independent or a mutation-specific fashion.

Targets and Mechanisms of Action

To understand targets and mechanisms of actions of survival factors, we need to understand the process of cell death, or apoptosis, and the role of neurotrophic factors. This would allow for more effective cell-specific targeting with neurotrophic factors.

There is evidence that at least two of the major classes of neurotrophic factors may actually act indirectly on photoreceptors, perhaps through Müller cells, rather than directly on photoreceptors. The RPE may also be involved. For example, the RPE itself has receptors for three of the major classes of neurotrophic factors: FGF, some of the neurotrophins, and the CNTF receptor family. The RPE has been shown to release seven different neurotrophic agents (FGF 1, 2, and 5; CNTF; leukemia inhibitory factor; pigment epithelium–derived factor; and brain-derived neurotrophic factor), all of which are protective in retinal degeneration mutants. Thus, the RPE is important, not only as a potential mediator of neurotrophic factor action, but also as a target of treatment. This is very important, because the RPE expresses the genetic mutation in a number of retinal degenerations and is clearly involved in one or more ways in age-related macular degeneration. The RPE, in some cases, may simply be secondarily involved, with atrophy occurring after the loss of photoreceptor cells. Even in that situation, to preserve some cone function, it may be essential to protect the RPE from degenerative changes, perhaps with neurotrophic factors, if the RPE is providing some support for cones.

Toxicity

As with the use of any agent, we need to understand the potential for negative effects of agents that are successful in providing neuroprotection. We need to identify the potential negative effects and clarify age-, disease-, and dose-related issues. We also need to determine the impact of each agent on retinal cells and vision, and develop means to regulate negative effects, perhaps by adjusting the dose of systemically administered agents, by removing or changing the number of cells in implants, or by using inducible promoters in gene-based delivery.

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Ciliary Neurotrophic Factor Therapy for Inherited Retinal Diseases: Pros and Cons

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Ciliary neurotrophic factor (CNTF), a naturally occurring neuroprotective protein, is being tested as a treatment for various neurodegenerative conditions, including retinitis pigmentosa. Retinitis pigmentosa is a leading cause of blindness in young adults and affects approximately one million people worldwide. From our work and the work of others, it seems that the effect of CNTF on the retina can be both positive and negative.

Ciliary Neurotrophic Factor in a Mouse Model of Retinitis Pigmentosa

The *rds+/−P216L* mouse is a model of human retinitis pigmentosa. It carries a point mutation in *peripherin/rds* and is, thus, modeled after a naturally occurring human mutation. In collaboration with Matthew LaVail's group at the University of California, San Francisco, San Francisco, CA, and William Hauswirth's group at the University of Florida at Gainesville, Gainesville, FL, we have treated the *rds+/−P216L* mouse model with CNTF vectored with recombinant adeno-associated virus, serotype 2 into the retinal pigment epithelium.[1] A human CNTF-DNA coding construct within the virus contained the human growth hormone signal peptide to ensure secretion of the CNTF protein plus two modifications in its amino acid sequence (S166D/G167H) to heighten its affinity for the α-subunit of the receptor for CNTF. This is a very powerful form of CNTF, perhaps the most potent used in therapy thus far. Expression of the CNTF was driven by a cytomegalovirus promoter or by a chick β-actin promoter.

In our study, 1 eye of each *rds+/−P216L* mouse received a single injection of the recombinant adenovirus vector into the subretinal space. The contralateral, uninjected eye served as an internal control. Injections were made on Postnatal Day 25, and the mice were killed at Postnatal Day 90.

Examination of the control eyes at Postnatal Day 90 showed that only approximately 3 rows of photoreceptor nuclei remained, demonstrating the natural death rate caused primarily by this dominant negative point mutation in *peripherin/rds*. The experimental mice—injected with the recombinant adeno-associated virus to promote secretion of CNTF primarily from the retinal pigment epithelium—showed, in contrast, a very dramatic sparing of cell death. The number of photoreceptor cell nuclei were remarkably higher. However, the morphology of the nuclei had changed somewhat. An even stronger promoter, chick β-actin, which presumably produced even more CNTF, changed the morphology of the nuclei even more dramatically, but with complete sparing of the cells.

Paradoxically, the A waves and B waves of the electroretinograms (ERGs) showed a better response in the untreated than the untreated eye, although the untreated eye had at most half of the number of cells. This was an unanticipated result, and it has been repeated in multiple laboratories. These ERG results were observed for both the cytomegalovirus and the chick β-actin promoter.

Ciliary Neurotrophic Factor Toxicity

We have also looked at the photoreceptor response in normal mice, using exactly the same vector that rescued photoreceptors in the mutant retina, and observed significant cell death in the normal animals. With very high doses of CNTF, presumably, there is a marked toxicity for normal photoreceptor cells, whereas cells that carry a mutation seem to be resistant in terms of cell death. Based on the observations of Theo van Veen and others who have used CNTF in various contexts, it seems possible that proteins in the phototransduction cascade are downregulated when the retina is overstimulated with CNTF.[2] Rhodopsin, for example, is synthesized at a reduced rate in photoreceptors that have been treated with CNTF, therefore, it is not surprising that the ERG response is attenuated. Furthermore, the nuclei undergo a dramatic change (from very condensed chromatin to much more diffuse chromatin), representing a change in the gene expression profile of the photoreceptors. Nonetheless, in the case of *rds+/−P216L*, the photoreceptors survive, and that is one of the objectives of treatment. When mutant mice were injected with axo-

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kine CNTF, no rescue occurred, and there were no negative changes in the ERG.

Decreasing Ciliary Neurotrophic Factor Toxicity

One way to approach the problem of potentially toxic CNTF effects may be to use ophthalmic drug delivery platforms, such as the Neurotech device, which contains encapsulated, bioengineered retinal pigment epithelium cells that slowly release CNTF into the eye. This device has been used by Gus Aguirre's group in dogs that carry a rod phosphodiesterase β-subunit mutation. When the thickness of the outer nuclear layer is compared in treated and untreated dogs, the nuclear morphology in the nontreated animals and the thickness of the layer in the treated animals look the same. Ron Bush and Paul Sieving have used this device in the context of the normal rabbit retina, using 2 different doses (5 ng/dL and 22 ng/dL) of CNTF. They saw relatively little effect in terms of ERG perturbation, although, at the high dose, the treated animals showed a decrease in the ERG at some light intensities. In addition, in the treated animals, there were changes in nuclear morphology at the higher doses, therefore, clearly CNTF has some side effects on the photoreceptors. It is incumbent on us to determine what mechanism is producing these morphologic changes.

Directing Ciliary Neurotrophic Factor Receptor Stimulation

The CNTF receptor is a heterotrimeric protein, made up of GP130, the leukemia inhibitory factor receptor, and the α-subunit, which is not transmembrane, but is membrane anchored. The α-subunit is potentially mobile; it could diffuse, for example, to associate with the bipartite leukemia inhibitory factor receptor, and a leukemia inhibitory factor receptor could be converted into a CNTF receptor. Thus, it is potentially possible for a cell that has only a leukemia inhibitory factor receptor to acquire a full-blown CNTF receptor.

Where are the CNTF receptors located in retinal cells? It has been generally thought that Müller cells have CNTF receptors. If so, and if photoreceptors do not have them, there must be some kind of an indirect interaction, whereby the Müller cells are stimulated first, responding perhaps by producing some factor, and secondarily stimulating the photoreceptors. However, recent evidence from immunohistochemical experiments suggests that the α-subunit of the CNTF receptor is, in fact, located on the photoreceptor cells.[3] The stimulation could, then, be either direct or indirect. The phenomenon is an interesting one that needs to be explored further.

Conclusion

To summarize, CNTF has the potential to rescue photoreceptor cells in inherited retinal diseases such as retinitis pigmentosa. Findings from animal studies indicate that CNTF may also overstimulate cells, downregulate the phototransduction cascade, and cause cell death. To use CNTF as a treatment, it may be necessary to adopt a delivery system that metes out therapeutic doses of the protein.

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Surmountable Challenges in Translating Pigment Epithelium–Derived Factor (PEDF) Therapy From Animal Models to Clinical Trials for Retinal Degenerations

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On the basis of its success in animal models, it seems that pigment epithelium–derived factor (PEDF) may have potential as a neurotrophic agent for treating retinal degenerations, such as retinitis pigmentosa. However, it is not enough to obtain proof of principle through success in animal models. Challenges exist in translating any compound or agent into a clinical trial. I pose five sets of questions in regard to the usefulness of PEDF and the hurdles it will face in moving to a clinical trial:

1. Is the intellectual property secure? Are there no legal problems in pursuing the development of a therapeutic agent?
2. Is the agent marketable? Is there an appropriate target disease, a sufficient patient population, and an adequate calculated return on investment to attract the support of a profit-making company?
3. What is the planned infrastructure? Has a supportive company been identified? Is funding available through venture capital?
4. Can preclinical studies actually be carried out? Is a truly appropriate animal model available? Can the compound really move through an animal model by not only showing efficacy, but also by showing successful delivery and by adequately addressing safety issues?
5. Is the clinical trial feasible? Can patient populations be identified, and can trial sites and participating physicians be enlisted? Are there clear endpoints?

Pigment epithelium–derived factor has several general characteristics that make it a good candidate as a therapeutic agent for retinal degenerations. For the most part, I think that it fulfills the requirements for meeting these five challenges for development. Pigment epithelium–derived factor has been shown to have a number of functions. It inhibits neovascularization by promoting apoptosis in the neovascular endothelial cells;[1] it is currently in a clinical trial, using GenVec adenovector-PEDF as a treatment for wet age-related macular degeneration; it promotes neuronal differentiation;[2] and it acts as a neuron-survival agent by inhibiting apoptosis in neural cells.[3] Interestingly, PEDF also inhibits glial cell growth.[4]

Target diseases for therapy with PEDF obviously include diseases of neovascularization, such as age-related macular degeneration, diabetes, and cancer. Additional target diseases include retinitis pigmentosa, other rare retinal degenerations, and, potentially, degenerative disorders of the central nervous or peripheral nervous system, including Parkinson disease and amyotrophic lateral sclerosis. Finally, PEDF may be effective in treating gliotic conditions, a sequela of many neurodegenerative conditions.

Pigment epithelium–derived factor is a soluble 50-kd protein and a member of the serine antiprotease super family. However, PEDF does not have antiprotease activity in the classic manner.[5] It is generally extracellular and has affinity for glycosaminoglycans and collagen. The receptor binding has yet to be fully characterized, but current laboratory investigations should soon yield more information (S. Becerra, unpublished observations). Many cell types synthesize PEDF, including retinal pigment epithelial cells and Müller cells.

Pigment epithelium–derived factor was originally found to promote a more neuronal phenotype in cultured retinoblastoma cells,[2] which are thought to be of photoreceptor cell derivation, possibly cones. Morphologically, PEDF promotes the growth of long neuriticlike or dendriticlike processes. Biochemically, PEDF upregulates markers, such as neuron-specific enolase, and downregulates inappropriate markers, such as glial fibrillary acidic protein.

The effect of PEDF on neuronal survival has been well demonstrated in cerebellar granule cells from young rats. When PEDF is added to cultured cerebellar granule cells, which would otherwise die by apoptosis during a two-to-three week period, the process is slowed, and, specifically, neuronal apoptosis is inhibited. Thus, PEDF protects neurons from natural, age-induced cell death.[3]

Pigment epithelium–derived factor has also demonstrated the ability to slow insult-induced cell death. Expo-

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sure of cultured cerebellar granule cells to 0.1 mmol/L glutamate in baseline conditions kills approximately half of the cells within a 24-hour period. The addition of PEDF significantly slows the cell death, and preincubation with as little as 1 nmol/L PEDF for 30 minutes before the glutamate addition protects approximately 90% of the cells for a 24-hour period. This demonstrates a rapid effect of PEDF, possibly through an influence on calcium flux.[6]

Pigment epithelium–derived factor also protects against other insults, such as the following: mutation-induced photoreceptor cell death in animal models of retinitis pigmentosa (*rd1*, *rds*) in vivo; high-intensity light damage in vivo; apoptosis induced by oxidative stress (H_2O_2) in retinal culture; low serum stress in cultured retinal pigment epithelium cells; glutamate toxicity in motor hippocampal neurons; and cell death in developing spinal motor neurons. A great many different cell systems have been used to show the neuron-protective mechanisms of PEDF.

Another interesting characteristic of neurodegenerative disease is glial overgrowth. In mixed neuronal and glia cultures from rat brain, PEDF is able to slow glial proliferation.[4] Importantly, PEDF does not kill the glia cells, but stops them from overgrowing. Pigment epithelium–derived factor activates the metabolism of microglia, induces morphologic changes, and blocks proliferation. Astrocyte proliferation is also inhibited, apparently as a secondary effect; PEDF seems to stimulate microglia to produce some type of soluble substance that inhibits astrocyte proliferation.[4]

In summary, good evidence exists to support the potential use of PEDF as an antineovascular agent, a neuron-survival agent, and a gliosis inhibitor.

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Inhibition of Poly(Adenosine Diphosphate-Ribose) Polymerase (PARP) in Experimental Models of Neurologic Diseases: Cell Death Prevention

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The form of cell death that we are investigating in our models of neurologic disease is not necrosis or apoptosis. It does not fulfill the classical criteria for either of those forms of cell death. Poly(adenosine diphosphate-ribose) polymerase (PARP)-mediated cell death falls into category of "other" cell death, as does most of the cell death that occurs in the nervous system. Thus, the assumptions that apply to certain biochemical pathways do not apply to the system we are investigating. Apoptotic and necrotic cell death mediated by PARP is probably one of the more important forms of cell death throughout the entire brain and body.

Using knockout mice and pharmacologic inhibitors, various investigators have shown a significant role for PARP in experimental models of stroke, ischemia-reperfusion injury, diabetic retinopathy and optic nerve transections in the retina, traumatic brain injury, Parkinson disease, multiple sclerosis, and diabetic neuropathy in the nervous system. Inhibition of PARP, and PARP knockout mice, have demonstrated profound protection not only in the nervous system, but in any organ that can undergo ischemia-reperfusion. Inhibition of PARP also protects against diabetes, arthritis, toxic shock, multisystem organ failure, and liver damage, and can be viewed as a "golden bullet."

For the last 25 years, PARP has been known to be "the guardian of the genome." Initially, only one PARP was recognized, residing in the nucleus. Currently, 17 additional PARPs are known to reside anywhere in the cell that DNA or RNA exists. After injury to DNA (e.g., DNA strand break), PARP converts or catabolizes nicotinamide adenine dinucleotide and adds large branch chain ribose units onto various protein acceptors, including itself, to elicit physical changes, deactivate synthesis enzymes, and activate repair enzymes. At some point, PARP dissociates from the broken DNA, repair enzymes move in, and the poly(adenosine diphosphate-ribose) (PAR) units are broken down by the glycohydrolase.

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Poly(Adenosine Diphosphate-Ribose) Polymerase as a Target for Neuroprotection

Intuitively, one would not think of PAR as a target for neuroprotection; however, recently, understanding of the role for PARP has dramatically expanded. In addition to playing a role in genomic repair, PAR is now known to play a key role in cell death, and, at least in sea snails, in long-term potentiation. A paper has recently been published in *Nature* describing how PARP may form the core of mitotic spindles and how the disruption of the PARP pathway can send a cell into mitotic stress and catastrophe.[1]

We are just at the brink of understanding the key roles for PARP. In the central nervous system, a pathway has been identified in which the *N*-methyl-D-aspartate receptors are activated by glutamate, resulting in nitric oxide synthase activation, which produces nitric oxide, leading to the generation of peroxynitrite that oxidizes DNA and causes single strand breaks, which activates PARP. Then, PARP catabolizes nicotinamide adenine dinucleotide. There have been various hypotheses how PARP kills cells. The original hypothesis was that nicotinamide adenine dinucleotide and adenosine triphosphate were consumed in the cell, and therefore, the cell went into energy failure. We now have overwhelming evidence that this is a biomarker for pathologic activation of PARP, and probably does not play a significant role (i.e., it is a biomarker). It may facilitate cell death, but it is not necessary or sufficient to kill the cell. What actually kills the cell is overactivation of PARP, which causes PAR formation. The PAR leaves the nucleus, it stimulates release of apoptosis-inducing factor from the mitochondria, and apoptosis-inducing factor translocates from the mitochondria back to the nucleus—the final commitment point for cell death. The observation of PAR as a signaling molecule and a death molecule is relatively new, and it probably explains why PARP blockade is so protective (V.L. Dawson, unpublished findings).

Other Targets for Poly(Adenosine Diphosphate-Ribose) Polymerase Inhibitors

Other targets for PAR that are involved in cell survival and cell death are being investigated. After experimental strokes in PARP knockouts or in animals treated with PARP inhibitors, the large infarct volumes seen in wild-type or untreated animals were dramatically reduced to 80% to 90% of the original infarct. We used DPQ (3,4–Dihydro-5-[4–(1–piperidinyl)butoxy]–1(2H)–isoquinolinone), but similar results were found with PJ34 and GPI6150 in mouse and rat models. PJ34 protects against 1-, 2-, and 4-hour ischemia, but not against permanent ischemia.

Inhibitors of PARP provide some limited protection against traumatic brain injury. Traumatic brain injury is a very harsh injury involving multiple injury paradigms, therefore, partial protection is significant. Several studies have demonstrated that PARP is activated after ischemia-reperfusion in the retina and that PARP is protective. If transection is performed, protection can also be achieved.

In other models of disease, we mimicked Parkinson disease with the toxin methylphenyltetrahydropyridine (MPTP) and, again, the PARP-knockout mice are completely protected against MPTP toxicity. The Guilford, Inc. compounds provide a partial protection against loss of dopamine neurons after MPTP intoxication. We investigated why the PARP knockouts were so protected, knowing that it could not be solely because of a lack of excitotoxicity. If excitotoxicity is blocked with any of the antiexcitotoxic agents, only approximately 50% of brain tissue is protected. To protect the entire nervous system, the various types of injury that occur over time after primary injury need to be considered. For instance, early excitotoxicity is followed by inflammation, which leads to classic apoptosis. Because PARP is a coactivator for many of the acute neuroinflammatory molecules, blocking PARP blocks acute inflammation, but not the subsequent chronic inflammation, which is important for repair and remodeling in the brain.

Inhibitors of PARP, as well as the knockout mice, have demonstrated protection of the heart against ischemia-reperfusion injury, producing a smaller infarct both in the treated animals and in the knockout animals.

Poly(adenosine diphosphate-ribose) polymerase may also be a very important target for the various diseases related to obesity, which represents an increasingly major health problem in the United States. Diseases related to obesity include type II diabetes, cardiac disease, stroke, endothelial cell dysfunction, and vascular dysfunction, which will lead to diabetic retinopathies and other eye disorders. Poly(adenosine diphosphate-ribose) polymerase is a very good target for diabetes. It spares the β-islet cells in the pancreas and may allow regeneration. It can also reverse cardiovascular dysfunction, and, in a set of studies that were recently published, it also reverses the endothelial dysfunction that leads to diabetic retinopathy.[2]

In one experiment, PARP wild-type animals treated with vehicle demonstrated loss of β-islet cells, but this did not occur in the PARP-knockout animals. When wild-type animals were treated with the Guilford compound or the Inotek, Inc. compound, the β-islet cells were protected against injury. However, the PARP inhibitors do not demonstrate the same amount of protection as is seen in the PARP knockouts.

In terms of pharmaceutical development, PARP represents a good target. Agents that are PARP inhibitory can be administered after injury. These agents are both neuroprotective and antiinflammatory. Drug trials that have been performed by industry, as well as the Phase II studies carried out by Inotek in Europe, have shown that these compounds are relatively nontoxic and well tolerated. Moreover, the protection provided by these compounds is sustained; it does not simply delay cell death.

Problems and Challenges

In addition to its great potential, PARP presents some problems and challenges. The major problem is that there are 18 different PARPs. The current drugs are targeted toward the PARP catalytic domain, which is present in all PARPs. PARP produces the pathogenic, long, branch-chained PARs, but the other PARPs produce very small PARs, which likely have important cellular functions. This lack of specificity in PARP inhibitors may be why mixed results—partial but not complete protection—are being observed with PARP antagonists. One of the goals for industry needs to be the development of a specific PARP inhibitor, particularly a PARP inhibitor. Another problem is that PARPs are important for DNA strand repair. If all of the PARPs were inhibited, DNA repair would be inhibited, and might lead to partial protection but also to a partial sensitivity to cell death. Finally, bioavailability presents a major problem. All of the PARP inhibitors are based on the structure of nicotinamide adenine dinucleotide; they are not soluble. In fact, they are highly insoluble. They cross membranes very poorly, particularly the blood–brain barrier. Some companies are addressing this problem.

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T–Cell–Based Vaccination Against Neurodegeneration: A New Therapeutic Approach

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The work of our group at The Weizmann Institute focuses on investigating why damage to central nervous system (CNS) neurons often leads to irreversible paralysis, and applying this knowledge to development of therapies aimed at repair, renewal, and functional recovery of the damaged nerve cells. We discovered that the immune system plays a key role in the ability of the CNS to withstand damage. This unexpected discovery led us, six years ago, to formulate the concept of "protective autoimmunity," whereby T cells directed to specific self-antigens are recognized as the physiologic fighting force against acute and chronic neurodegenerative conditions.[1] In this context, and because the optic nerve is part of the CNS, we introduced the concept of neuroprotection as a treatment for glaucoma, specifically via a T-cell–based vaccination.[2]

In what seems to be a cruel paradox, the CNS, despite its need for structural and functional plasticity, has only a limited capacity for repair. Both neurogenesis and regeneration in the CNS are severely limited, leaving the system vulnerable to degenerative conditions. In addition, the ability of the CNS to tolerate the activity of the body's regular defense mechanisms is extremely low. We postulated that the factors that impair survival are likely to impair cell renewal as well.

Several years ago, we developed a rat model of the secondary degeneration that occurs after a partial crush injury to the optic nerve.[3] Using this model to assess both primary and secondary nerve damage, we learned that neurons that escape the primary acute insult will, in the absence of intervention, eventually degenerate as a consequence of the injury-induced toxicity that floods their environment. That finding led us to suggest that in glaucoma, as in any other neurodegenerative disorder, at any given time, viable neurons that are embedded in that threatening environment might benefit from neuroprotective therapy.

Numerous factors that participate in the ongoing process of degeneration have since been identified. These factors, which we view as part of the cohort of the "enemy within,"[4] include ionic imbalance, free radicals, nitric oxide, glutamate, growth factors, metabolic deficit, and accumulating self compounds (such as gangliosides, β-amyloid, prion protein, and others). In addition, many studies suggest that neurodegenerative diseases nearly always involve an inflammatory response. Nevertheless, attempts to treat such diseases with antiinflammatory drugs have met with failure. We suggest that the treatment of choice for any neurodegenerative condition is not immune suppression but immunomodulation.

Based on our concept of protective autoimmunity, we view neurodegenerative diseases, including glaucoma, as systemic and local, rather than purely local in nature. This would imply that immune-related factors, if functioning physiologically, are part of the maintenance and support system of the CNS (including the eye), but when malfunctioning, they contribute to chaos and degeneration.[5] According to this view, autoimmune disease can be regarded as the result of a malfunctioning immune system.

The cell-based immune system is a form of T cell immunity to self-antigens and functions to protect nerves from damage. Our findings indeed suggest that protective autoimmunity is a spontaneous, physiologic antiself immune response that protects the CNS against degenerative conditions (the enemy within).

According to this view, autoimmune disease can be regarded as the result of malfunctioning autoimmunity. Furthermore, it is suggested that tolerance to self should be viewed as the ability to tolerate an autoimmune response without developing an autoimmune disease.[6] It follows, then, that T-cell specificity to self-antigens is needed not to launch an immune attack on the individual's own tissues, but to facilitate the homing and the local reinforcement and activation of autoimmune T cells.

Because of the multifactorial etiology of neurodegenerative diseases, it is unlikely that a monotherapy targeting a specific causative factor will be fully effective. Our studies have shown that in cases of acute or chronic CNS damage, regardless of its primary cause, a well-controlled T-cell response directed

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against self-antigens residing in the damaged site represents a multifactorial mechanism of maintenance and repair, which, if not sufficiently effective, can be therapeutically boosted.

Our proposed concept of autoimmunity as a physiologic defense mechanism, and of T–cell–based vaccination with selflike antigens as a means of boosting its therapeutic effect, is based on the following findings:

- T cells directed against peptides derived from myelin basic protein, when passively transferred to mice subjected to a partial optic nerve injury, reduce neuronal loss.[1] In adult rats with severely contused spinal cords, the same T cells show a similar beneficial effect, manifested by reduced posttraumatic tissue loss and better functional ability.[7]
- Passive transfer of T cells can be successfully replaced by active vaccination with peptides derived from relevant self-antigens and administered after the injury, not as a prophylactic but as a therapeutic measure. The success of the vaccination is dependent on the choice of both the peptide and the vehicular adjuvant.[7]

The Underlying Mechanism

Accumulated findings in our laboratory indicate that, for T cells to be significantly effective, they should home to the lesion site and become locally activated there. Once activated, they can serve as a source of neurotrophic factors[9,10] and cytokines. The cytokines shape the microglia in a way that renders the microglia capable of buffering glutamate and producing growth factors without evoking the lethal immune activities associated with killing of microorganisms.[11,12]

Thus, microglia can have a beneficial or a destructive effect, depending on how they are activated and controlled. "Innate" immune activation results in the killing and removal of microorganisms and the secretion of nitric oxide, tumor necrosis factor-α, and cyclooxygenase-2. Activation by controlled "adaptive" immunity triggers both immune and neural functions—delivery of neurotrophic factors and cytokines,[12] removal of growth inhibition (for example, by phagocytosis of myelin), buffering of toxicity mediators, such as glutamate,[10] and activity of antigen-presenting cells.[12]

T-Cell Regulation

If we accept the concept of autoimmunity as the body's physiologic defense mechanism against the enemy within, we need to understand how the body can tolerate the permanent presence of circulating T cells ("autoimmunity on alert") without developing an autoimmune disease, and how the activity of these autoimmune cells is switched on and off.

We discovered that the safe existence of autoimmunity in healthy individuals is made possible by the presence of thymus-derived regulatory T cells. The default activity of these T cells, via cell–cell interaction or the effect of their associated cytokines, is suppressive, as demonstrated by the finding that depletion of these cells increases the ability of rats to withstand injurious conditions.[13] Further studies identified dopamine as the physiologic compound that switches the T–cell–based inhibitory activity on and off.[14]

Therapeutic T–Cell–Based Vaccination

To be translated into a therapy, the inflammatory response must be tightly controlled. This can be accomplished by vaccination with self-related antigens or by downregulating the cells that constitutively suppress autoimmunity. To vaccinate with self-related antigens while avoiding the risk of autoimmune disease, we used weak agonists of self-antigens. Weak agonists, when used as vaccine, can evoke a T-cell response directed against them. However, when such T cells encounter the self-antigens themselves, the T cells can be activated but do not proliferate.[15] A useful weak agonist, capable of cross-reacting with a wide range of self-antigens, is glatiramer acetate (copolymer 1), a synthetic copolymer of four amino acids, which is currently used in a daily therapeutic protocol for multiple sclerosis. When used as a vaccine, copolymer 1 successfully activated the immune system in such a way as to confer protection in cases of partial crush injury in rats, glutamate toxicity in mouse eyes, elevated intraocular pressure in rats, facial nerve axotomy in mice, amyotrophic lateral sclerosis and Parkinson disease in mouse models, and head trauma and psychotic conditions in mice.[5,10,16]

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Animal Models as Tools for Screening Candidate Drugs

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Animal models can be considered in three categories: disease models, molecular homologs, and disease homologs. The model may vary from animals that have a phenotype that is somewhat similar to the disease in the human patient to those that have mutations in the same gene. Whether the models are naturally occurring or induced in the laboratory, the phenotype may be different from that seen in the patient.

Disease models allow the investigation of generic therapies (e.g., trophic factors) to assess photoreceptor rescue. Molecular homologs are appropriate for generic and specific therapies (e.g., trophic factors, gene replacement, or knockdown) used for "proof of principle" studies. The disease homolog is the model in which the mutation is in the same gene, and a disease phenotype is very similar to that which occurs in human patients. Disease homologs are used for generic and specific therapies. They are most applicable for preclinical assessment of therapies directed at human patients.

Naturally occurring models of retinitis pigmentosa and allied diseases occur in mice, rats, cats, dogs, and chickens. Transgenic models include mouse (knockouts, dominant human mutations, etc.) and rat and pig (mutant human rhodopsin).

In comparing the models and the patients, it is important to realize that they may have a very similar phenotype. However, some of the mutations are not similar. The mutation and the phenotype in the animal model must be viewed with some degree of caution; it cannot be assumed that these are truly representative of the disease that is occurring in the patient until the phenotypes are critically examined using the same criteria.

Investigators of neuroretinal degenerative disease are fortunate to have a number of different mutations to study, unlike amyotrophic lateral sclerosis, which seems to have only one primary model system, i.e., the superoxide dismutase mouse model. Neuroretinal disease offers a wide range of mutations that affect almost all of the vital genes involved in the photoreceptor cells and/or retinal pigment epithelium.

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Accessibility of the Retina

In addition to having multiple models to work with, we also have a wonderful ability to assess the consequences of these mutations in the retina. We can use the electroretinogram (ERG) to assess photoreceptor function and correlate improved receptor function with photoreceptor structure. In the normal retina, we can study the components of the ERG in which rod and cone components can be separated and individually examined. When studying diseases that affect the rods, we can mark out completely the rod contribution to the response. In diseases that involve selective cone degeneration, the ERG might show a very normal initial rod response, whereas the component that is contributed by the cones is abnormal or absent. The noninvasiveness and sequential manner of the ERG provides great power for analysis.

In experimental animals, it is also possible to examine the anatomy of the retina and assess disease progress, knowing the physical manifestations of the disease, and determine whether an intervention alters the progression of the disease. The photoreceptors are readily assessed. Using light microscopy, we can also determine quantitatively the nuclei in the photoreceptor layer. Changes in the layer with the photoreceptor nuclei correlate well with changes that occur where the photoreceptor inner and outer segments are located. Interventions may be able to stop the disease at this point and keep the photoreceptor layer viable for long periods.

The retina is an approachable part of the brain; the eye is readily accessible for delivery of compounds. For example, it is possible to inject compounds into the vitreous so that they are distributed within the entire intraocular space, or to inject directly into the subretinal space so that there is direct access to photoreceptors and retinal pigment epithelium.

The retinal pigment epithelium can also be isolated from the eye and grown in primary culture for indefinite periods. We have grown retinal pigment epithelium for one year, at which time we stopped growing it because there was no reason to go fur-

ther. We can use animal retinas to correlate data from objective test results (e.g., optical coherence tomography or functional magnetic resonance imaging) with retinal integrity, and can also monitor the progression of disease and evaluate the success of treatment.

Assessing Therapies for the Retina

The aim of the RPE65 Consortium was to treat the retina with direct gene transfer to the retinal pigment epithelium. One advantage we had was that the dogs had severe dysfunction in the retina, but, at a young age, they had good preservation of retinal structure. We knew that if we could deliver a gene to the correct tissue at the correct time, we would have a long-term model for therapy. As reported by Dr. Hauswirth at this Symposium (see Hauswirth's article elsewhere in the Symposium proceedings), results in Lancelot and the large cohort of treated dogs were dramatic and equally good.

Saving Cone Cells in Hereditary Rod Diseases: A Possible Role for Rod-Derived Cone Viability Factor (RdCVF) Therapy

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Retinitis pigmentosa is an heterogeneous group of inherited retinal dystrophies. It begins with rod degeneration, followed by irreversible and progressive cone cell death, which leads to blindness. We have shown that factors secreted from rods decrease after rod loss, leading to secondary cone cell death.[1] Because many genetic mutations cause impaired visual response of rods, the preservation of cones is an important goal for preserving vision.

We recently identified, by expression cloning, a trophic factor secreted by rods that promotes cone survival.[2] We used a viability assay based on cone-enriched primary cultures from chicken embryos, changing our model from the mouse mutant retina to normal chick embryo retina. Unlike mammalian retinas, bird retinas are cone-dominated and, in culture, cones represent 60% to 80% of the total cell population.[3] Once in culture, these cells normally degenerate during a few days; however, by adding conditioned medium from wild-type mouse retinal explants, spontaneous cell death can be delayed.[1] This model was used to screen potential candidates for promoting cone survival.[2]

A complementary DNA expression library was constructed from five-week-old wild-type mouse neural retina. Purified DNA plasmids from pools of 100 individual clones from this library were used to transfect COS-1 cells. The conditioned medium from cultures of these transfected COS-1 cells was added to cone-enriched primary cultures from chicken embryos seeded in 96-well plates. After seven days in culture, an automated viability assay was conducted, and pools that were associated with a significantly higher number of surviving cells were selected. One specific pool contained twice as many surviving cells compared with controls. This pool was subdivided, and the active fraction from this division was further subdivided, and so on, leading eventually to the isolation of one single positive clone. This clone contained a 502-bp complementary DNA insert encoding a putative 109-amino acid polypeptide. The protein identified from the clone was named rod-derived cone viability factor (RdCVF; OMIM 608791).

Rod-Derived Cone Viability Factor

Rod-derived cone viability factor is a novel truncated thioredoxinlike protein specifically expressed in photoreceptors and found preferentially in the cone extracellular matrix.[2] It has 33% similarity to thioredoxin (OMIM 187700). Unlike thioredoxin, RdCVF does not have detectable oxido-reductase activity. However, an alternatively spliced form results in a longer protein with a C-terminal extension and could have oxido-reductase activity.

Rod-derived cone viability factor expression was shown to decrease as rod degeneration progresses in the *rd1* mouse model of retinitis pigmentosa and to promote cone survival in five-week-old *rd1* mice (no living rods at the chosen age).[2] The effect is inhibited by blocking antibodies through immunodepletion. Rod-derived cone viability factor has, however, no effect on rod survival at an early stage of the disease. Expression cloning also led to the isolation of known trophic factors and other possible candidates, but the strong effect obtained with RdCVF convinced us to concentrate on this factor.

Although RdCVF is a good candidate to target for cone survival in retinitis pigmentosa, a number of issues remain to be addressed. For example, the mechanism of action underlying the cone survival effect needs to be better understood, with identification of a putative cellular receptor and intracellular signaling pathways. There may also be a rationale for preserving nonfunctional rods, because these rods could have a rescue effect on functional cones.

Other Strategies

Many other strategies are currently being investigated, such as ciliary neurotrophic factor delivery, which is already in a clinical trial. Our current strategies, however, concentrate on promoting cone viability by transplantation or by delivery of RdCVF.

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This provides a broad window for late intervention, when all the rods have degenerated and only cones, essential for daylight and precise vision, persist. Other efforts focus on delivery systems for potential viability factors. Injection of a protein does not seem to be a satisfactory option for the long term. Gene-based delivery through viral vectors or using encapsulated cell technology would ensure a more stable and persistent release. Our laboratory is investigating these different options in collaboration with other research teams, such as the one directed by Jean Bennett at the University of Pennsylvania.

Another interesting approach would be to study whether RdCVF could act as a modifier gene, especially in retinal dystrophies with variable expressivity; certain RdCVF haplotypes represent risk factors for developing more severe disease, whereas others could be protective. Current genetic studies are investigating this possibility in photoreceptor dystrophies and in more complex disorders, such as age-related macular degeneration. It has been reported that, in early stages of age-related macular degeneration, there is an early loss of rods (30% loss of rods, whereas foveal cones remained stable).[4] This has been shown both histologically[4,5] and functionally.[6] Our hypothesis is that, in age-related macular degeneration, early rod loss leads to a reduction in RdCVF expression and secondary loss of cones. In this context, RdCVF might be a therapeutic tool to prevent this secondary degeneration. We are currently investigating this hypothesis.

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Usher Syndrome Type 1: Genotype–Phenotype Relationships

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Hearing loss and retinitis pigmentosa (RP) are two of the most genetically heterogenous neurosensory disorders in humans.[1] Regarding nonsyndromic hearing loss, there are at least 100 different genetic loci, of which, 76 have been genetically mapped to a chromosomal address, and genes for 34 loci have now been identified.[1] Syndromic forms of hearing loss are even more genetically heterogeneous. There may be as many as 400 different syndromes with hearing loss as an included feature, and approximately 91 of these genes have been identified.[2] This presentation focuses on the genetics and molecular biology of Usher syndrome (USH), which is characterized by sensorineural hearing loss and loss of vision caused by RP.

Usher syndrome is usually divided into three clinical subtypes: types 1, 2, and 3, which are defined and distinguished by the severity and age at onset of the hearing and retinal phenotypes.[3] There are also atypical classes of USH that do not conform to the clinical criteria of subtypes 1, 2, or 3.

Usher syndrome type 1 (USH1) is characterized by bilateral, congenital profound deafness and RP, with onset in the first decade of life. Affected children have a loss of peripheral vestibular reflexes, which can manifest as delayed developmental milestones, such as late walking. Adolescents diagnosed with USH1 often have difficulty seeing in the dark (nyctalopia), and the vision loss progresses to blindness after a few decades.

Individuals with a diagnosis of USH2 have normal vestibular reflexes, a moderate-to-severe stable hearing loss, and RP with onset in the first-to-second decade. Usher syndrome patients with progressive loss of hearing and variable severity of vestibular function and RP are classified as having USH3. In North America and Europe, it is thought that USH1 accounts for approximately 30% to 40% of USH.[3] It is possible that USH2 and USH3 are significantly underdiagnosed because of the less severe or progressive nature of the clinical features.

Usher syndrome is a recessively inherited trait, and it is genetically heterogeneous (Table 1). There are 7 USH1 loci, 2 of which, *USH1D* and *USH1F*, are linked on chromosome 10. Using positional cloning and positional candidate strategies, we and others have reported that mutations of *CDH23* and *PCDH15* are associated with USH1.[4,9] These genes encode cadherin 23 and protocadherin 15, respectively. Cadherins are transmembrane proteins that have calcium-dependent adhesion domains, which can bond extracellularly with the same cadherin family member on the plasma membrane of the same or neighboring cell.[5] In addition to intermolecular adhesion motifs, cadherins have a plasma membrane–spanning region and an intracellular domain that may have several primary as well as indirect binding partners, including the actin cytoskeleton. Points of contact between cadherins are not static, but can be part of a dynamically regulated macromolecular complex that changes during development or in response to altered physiology.[6]

In the adult inner ear, cadherin 23 was proposed as a candidate for the hair cell stereocilia tip link,[7] which is involved in gating the sound transduction ion channel.[8] We and others have examined this proposition, and the data suggest that there are many different functions of cadherin 23 in the cochlea, but recent data does not support the conclusion that cadherin 23 is the tip link in adult hair cells.[6] However, based on immunolocalization studies, protocadherin 15 has been proposed as a component of inner ear, adult hair cell lateral stereocilia links.[9] Although our current understanding of the functions of cadherin 23 and protocadherin 15 in the retina is still primitive, it is under intense investigation.

Most of the mutations of *CDH23* and *PCDH15* that are associated either with USH1 or nonsyndromic hearing loss are rare and have been reported only once or twice. The R245X mutation of *PCDH15* is associated with USH1 and is the exception. The carrier frequency of R245X is approximately 1% in the Ashkenazi Jewish population and is estimated to account for approximately 50% to 60% of all USH1 in this group.[10] Although R245X accounts for a majority of USH1 among Ashkenazi Jews, there is no higher incidence of USH1 in this group as compared with other populations. So far, R245X is the only common mutation of *PCDH15*.

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Table 1. Usher Syndrome Loci, Map Positions, Genes, and Proteins

Usher Locus	Chromosomal Location	Gene	Protein
Usher type 1			
A	14q32	?	?
B	11q13.5	*MYO7A*	myosin VIIA
C	11p15.1	*USH1C*	harmonin
D	10q22.1	*CDH23*	cadherin 23
E	21q21	?	?
F	10q21.1	*PCDH15*	protocadherin 15
G	17q24–p25	*SANS*	SANS
Usher type 2			
A	1q41	*USH2A*	usherin
B	3p23–p24.2	?	?
C	5q14.3–p21.1	*VLGR1*	VLGR1
Usher type 3			
A	3q25–p25	*USH3A*	clarin-1
B	20q	?	?

Mutations of *CDH23* and *PCDH15* are associated not only with USH1 but also with nonsyndromic deafness (*DFNB12* and *DFNB23*, respectively).[4,9] It is curious that some mutations of *CDH23* and *PCDH15* are responsible for USH1, whereas other mutant alleles seem to cause only nonsyndromic deafness. As yet, the mutations of *CDH23* and *PCDH15* that cause nonsyndromic deafness are missense mutations resulting in single amino acid substitutions. We assume that this type of mutation does not entirely disable the function of these two cell-adhesion proteins. However, the mutations that cause USH are either truncating mutations or missense mutations, which we assume actually do disable the protein. What is surprising is that the retina seems to be more tolerant of these "lesser" missense mutations of *CDH23* and *PCDH15* than the hair cells of the inner ear. For reasons that are not yet understood, cadherin 23 and protocadherin 15 apparently do not need to have perfectly normal function for the maintenance of the retina. However, cochlear hair cells seem to require that cadherin 23 and protocadherin 15 have an unspoiled amino acid sequence to preserve normal hearing.

In humans, one particular change in *ATP2B2*, the gene that encodes for the calcium pump, Plasma membrane $Ca2^{+}$–ATPase (PMCA), can act as a modifier of the severity of the hearing-loss phenotype because of coexisting mutations of *CDH23*.[11] This phenomenon probably accounts for some of the within-family and between-family variation in the hearing-loss phenotype associated with *CDH23* mutations. It seems likely that similar genetic modifiers of the severity of RP associated with USH will also be identified in the future, along with environmental factors that might affect the severity of USH-related RP. Consequently, it is likely that this type of in-depth understanding of the pathophysiology of RP will provide us with new ideas regarding potential therapy.

Animal Models for Usher Syndrome

To make progress in understanding the functions of cadherin 23 and protocadherin 15 in the ear and the eye, and to study the pathophysiology secondary to mutations of these 2 genes, it is crucial to have animal models (usually mice), with mutations in the murine orthologs of the human *USH* genes. Mice with many different truncating mutations of *Cdh23* (waltzer) and *Pcdh15* (Ames waltzer) have been reported. However, these mice are only deaf and do not develop the murine equivalent of RP. The waltzer and Ames waltzer mice show no significant changes similar to the RP of humans diagnosed with USH. Thus, as animal models for USH, they are not helpful for gaining insight into the retinal pathophysiology of USH1D and USH1F in humans. However, the acquisition of in-depth knowledge regarding how waltzer and Ames waltzer mice circumvent retinal degeneration might be exceedingly helpful in devising novel therapies for the RP associated with USH in humans. In this one respect, it would be nice if man were more like mouse.

There are several mutually exclusive and unproven "best guesses" regarding why there is an absence of RP in the waltzer and the Ames waltzer mice:

1. Perhaps with light-stress similar to the intensities experienced by humans in our everyday world, the eyes of the largely nocturnal waltzer and Ames waltzer mice would develop RP.
2. It is possible that the retina of the mouse is significantly different from the human retina and that its function and integrity can be maintained in the absence of either cadherin 23 or protocadherin 15. Maybe the functions of cadherin 23 and

protocadherin 15 are sufficiently similar to each other, such that one of these cadherins can compensate for the loss of the other. If that were the case, a double homozygous waltzer–Ames waltzer mouse should have the potential to develop RP.

3. The genetic background of the waltzer and Ames waltzer mice may have genetic modifiers ("suppressor of RP") that rescue the retina. If this were the case, placement of these mutant alleles causing the Ames waltzer and waltzer phenotype on a different genetic background might result in RP.
4. Finally, it seems plausible that the waltzer and Ames waltzer mutations that have been reported are not actually null alleles resulting in full loss of gene function, but, because of alternative RNA splicing, compensating isoforms of cadherin 23 or protocadherin 15 are produced that are competent to function within the mouse retina. If this idea is correct, a bona fide null allele, such as a gene deletion of *Cdh23* or *Pcdh15*, might result in a mouse that is afflicted with the murine equivalent of the RP of USH1 human patients.

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Retinal Researchers Have Reasons to Be Optimistic

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When the Foundation Fighting Blindness was started in 1971, we had no molecular methods of the sort we have today. DNA technology did not exist in the current form, and molecular biology was mainly the study of the crystal structure of proteins.

Rhodopsin, however, had been purified, by the late Joram Heller, and we were able to gain a better appreciation of what this molecule was really like. During a period of approximately 15 years, Russian scientist Yuri Ovchinnikov and US scientist Paul Hargrave worked very hard to figure out the primary structure of rhodopsin, namely its amino acid sequence. Using this information and his own DNA, a bright young medical student at Stanford University named Jeremy Nathans determined the nucleotide sequence of all of the human cone photopigments and the rod photopigment. This led to the identification of the first retinitis pigmentosa gene by Ted Dryja and his associates.

We now fast forward to the *RPE65* situation. The *RPE65* knockout mouse was published in 1998 by Michael Redmond and his collaborators, and 3 years later, dogs with an *RPE65* mutation were cured through gene therapy by a consortium of scientists from multiple institutions (for more information regarding this therapy, see the article by Hauswirth elsewhere in the Symposium proceedings).

Opportunity and science have progressed at an extraordinary, increased pace in this field. As a basic scientist, I cannot help but be optimistic. I also cannot help but remind you that basic science is important, because all of these clinically relevant advances are founded on basic science. We do learn from the patient, of course. Indeed, the determination in humans of the Stargardt macular dystrophy mutations truly fostered the production of a knockout mouse model by Travis and colleagues. That mouse has also led to great progress. The Stargardt knockout mouse has generated an enormous amount of interest and insight into how this disease works and how we can attack it rationally. A rational attack is much better than a wild guess, whereby you think of a potential treatment, provide a drug, and hope that it will work. However, you could very well be harming some patients while helping others in the process. The more we know regarding the molecular mechanism of a disease, the better off we are.

Looking forward, in my opinion, the first potentially therapeutic molecule worth pursuing with great passion is the rod-derived cone viability factor, which might be instrumental in helping us to understand how to keep cones alive. There is currently an enormous gap in our information. One of the assignments in my other lecture was to talk about pathways that lead to apoptosis (see manuscript 3B in this supplement). The sad fact is that we do not know those pathways. We do not have a single bit of information on what pushes the cell to commit suicide. We know the suicide genes, and talented investigators have used inhibitors and other compounds to interfere with that process, but we want to be more specific than that, in the final analysis. If you administer an antiapoptotic agent and stop apoptosis throughout the body, you can do harm. Apoptosis is a worthwhile process in some tissues, particularly during development. You want it to be taking place. However, you do not want it to be taking place in photoreceptors. The more specific we can be, the better. If we can figure out why a rhodopsin mutation pushes a rod photoreceptor to the brink, we can learn how to intervene. What is it that happens between the time that the first misfolded or dysfunctional protein is made in the cell to the time years later when the photoreceptor finally throws up its hands and jumps off the cliff? We need to know this information so that we can intervene in the process in a rational way. Maybe it is different for a photoreceptor cell than it is for a cancer cell that is being attacked by the immune system.

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THERAPEUTIC APPROACHES IN ORPHAN HEREDITARY RETINAL DISEASES

Drug Delivery Systems for Treating Orphan Retinal Diseases

VINCENT H. L. LEE, PHD

Targeted drug delivery to alter the course of orphan retinal diseases will spark a revolution in ocular drug therapeutics. Investigators involved at all levels need to think creatively and act boldly to explore nonconventional approaches to replace the notoriously inefficient methods that have been used to administer topical solutions. Success will require a well-coordinated, multidisciplinary effort, beginning with the design of drugs that satisfy the stringent requirements of retinal diseases regarding drug transport and potency. Medicinal chemists and protein engineers can apply knowledge derived from genomic and proteomic tools to identify and profile drug targets unique to a given disease state. Moreover, proteomic tools can be applied to fingerprint as well as monitor disease progression, including the early detection of retinodegenerative diseases to retard the deterioration of the affected tissue.

Few, if any, drugs are sophisticated enough to reach their intended targets on their own. A suitable drug delivery system is critical to achieve clinical efficacy and safety. Three features are desirable in a drug delivery system for orphan retinal diseases. First, the drug itself should be long-acting, preferably for the entire lifetime of the patient (although this is unrealistic). Second, the delivery platform should not interfere with light transmission and, hence, vision. Third, the delivery system should operate by a feedback mechanism that is responsive to disease progression, releasing drug at a rate and duration characteristic of the disease state at hand.

Nanosystems comprise a promising drug delivery platform that applies to the entire spectrum of exploratory approaches to targeting drug delivery to the back of the eye. These approaches include topical instillation of eye drops or placement of inserts in the conjunctival sac, direct injection of a formulation into the subretinal space or the intravitreal cavity, or injection into the blood circulation. Nanosystems are attractive drug delivery platforms, not only because of their minuscule size, which allows them access to a previously unreachable space in the eye, but also because they can potentially perform a number of tasks central to the design and evaluation of innovative delivery methods. Inevitably, these methods will be linked to the development of new biomaterials that match the microenvironment in which drug is needed. The delivery system will respond to a biomarker unique to the underlying disease state to survey the progression of disease, and it will transmit this information to a server, which then triggers the formulation of the dosage regimen. Each of these elements itself presents a challenge. Perhaps the most formidable of the challenges is to develop new biomaterials to supplement the two that are in clinical use—poly(lactide-coglycolide) and poly(anhydrides). This is a huge undertaking, which should, appropriately, be funded by a consortium of pharmaceutical manufacturers, the ultimate beneficiaries.

In 2003, Bourges et al evaluated the kinetics of polylactide nanoparticle localization, as well as encapsulated drug release, within intraocular tissues after the injection of 5 μL of a formulation comprised of 100-nm nanoparticles.[1] Although the tissue distribution observations were not quantitated, the nanoparticles seemed to be able to home in on the retinal pigment epithelium. Moreover, the drug delivery system persisted in the retina four months after a single injection, suggesting that continuous drug delivery is feasible. Further work should focus on quantifying the kinetics of nanoparticle deposition and disposition in retinal pigment epithelium cells and on evaluating the

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influence of disease progression on the pharmacokinetic behavior of nanoparticles after intravitreal injection.

It is important to learn from our past experience of drawing wrong conclusions on drug disposition in posterior-segment tissues on the basis of findings in anterior-segment tissues after topical solution instillation. This experience serves as a fine example of how dogma can blind us in the way we formulate and test hypotheses. In considering drug delivery to the eye, the dogma was based on several beliefs:

1. The cornea is the principal, if not the exclusive, pathway of drug access to the interior of the eye after topical drop instillation.
2. Given that the resulting drug bioavailability in the anterior segment tissues is less than 1%, it is virtually impossible to expect that clinically relevant drug levels would ever be attained in the posterior segment tissues.
3. Although drug can penetrate the conjunctiva and sclera after topical drug administration in the eye, it is unlikely that drug would survive the powerful influence exerted by the vasculature perfusing these tissues to reach clinically relevant levels in the compromised posterior segment tissues.

For these reasons, we and other investigators seldom measured drug levels in ocular tissues beyond the lens. Consequently, we missed promising opportunities to identify characteristics that would favor drug access to the posterior segment tissues after uptake into the conjunctiva. Then a possible retinal protective effect of brimonidine after topical drop instillation for treatment of open-angle glaucoma was unexpectedly discovered.[2] Such a possibility was supported by the detection of significant brimonidine concentrations in the choroid and retina, as compared with that achieved with another antiglaucoma drug, levobunolol.[3]

How does brimonidine find its way from the front of the eye to the retina? This drug molecule probably engages a more specific mechanism than passive diffusion for uptake into epithelial cells and transport across epithelia.[4] Most drugs are transported by passive diffusion, being driven by the prevailing concentration gradient.

The time has come for us to take advantage of the diverse endogenous transporter population to effect a radical change in the way drugs are developed, tested, and marketed. In my research laboratory at the University of Southern California, we were able to map the distribution of transporters in the eye, notably the conjunctiva.[5] Surprisingly, conjunctival epithelial cells have the same set of transporters as intestinal epithelial cells, although the mix of transporters is different. Moreover, conjunctival epithelial cells are capable of undergoing endocytosis, one of the requirements for facilitating the uptake of nanosystems.[6]

The availability of animal models that mimic the disease state is essential to the evaluation of innovative drug delivery systems. Such models must lend themselves to rapid screening for drug efficacy and safety. Clearly, this requirement has already posed an enormous challenge in conducting ocular pharmacokinetic studies, because the conventional rabbit model is no longer a suitable model for neurodegenerative disease. However, the transgenic mouse model is. Toward that end, it is imperative that exquisitely sensitive detection methodology be developed to assay the lower drug levels in mouse eye tissues.

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Challenges in the Pursuit of Therapeutic Product Development

WENG TAO, MD, PHD

Neurotech's lead program using encapsulated cell technology (ECT) for retinal degeneration is NT-501. The concept behind ECT is simple—to chronically deliver a low dose of a neurotrophic factor into the eye to prevent degeneration of photoreceptor cells. Factor-secreting cells are encapsulated in a semipermeable capsule, which isolates them from the local environment and minimizes immune rejection while still allowing therapeutic agents produced by the cells to diffuse through the membrane. The capsule is approximately 6-mm long with a small loop at one end to mainly anchor the capsule in the vitreous cavity and to the sclera.

The capsule behaves like a small factory, actually producing neurotrophic factor within the eye for long periods. The capsule prevents direct contact between capsule cells and host cells. It also prevents any large molecules, such as antibodies, from entering the capsule. Essentially, it is an immune-isolating capsule and allows long-term survival of its cells.

The lack of an effective delivery system has been an obstacle to the development of effective therapies for retinal degeneration. The blood–retinal barrier prevents most drugs introduced topically or systemically from reaching an effective dose on the retina. Neurotech is attempting to meet the need for an effective delivery system with the development of the ECT. However, we have a number of technical and financial challenges to overcome before this system is actually on the market.

In terms of cell biology, we must establish that the cells can actually live in a capsule for a long period. The molecular biology needs to be understood also, because we want to genetically modify cells to secrete the factor. Can the factor be secreted at adequate levels? Can the secretion be consistent and stable over time? Immunology represents a major issue because we are transplanting a foreign human cell into the host. Will immune rejection occur? Is the nutrient in the eye sufficient to support the cells? Do we have an appropriate animal model that will give us the data that will eventually translate positively in humans?

Challenges also exist at the practical, physical level. What surgical procedures can be used to minimize potential complications? Will patients and clinicians alike accept this method? Are the materials in the scaffolds, membranes, glues, titanic loops, and so on, compatible with each other and with the host? Are the materials safe in humans and animals? Can we rely on people to make the tiny capsules by hand? Is that practical? Is it efficient? We would need to treat hundreds of thousands of patients. Can the capsules be made by machine? How?

Technical challenges impeding the bringing of ECT to market have been several. During early development, the viability of encapsulated cells was not acceptable. The ECT technology showed great promise in short-term rodent studies, but failed in long-term, large-animal models. Can it be translated into long-term success in humans? This is the challenge we have to resolve. What is causing the nonviability of the cells in the capsule? Is it a metabolic problem? Is it because of host rejection or lack of sufficient nutrients?

Another major challenge encountered in the development of drugs and technology for orphan disease is competition within the company with products that have higher potential for recovering their investment. The success of any product depends on scientists and management understanding its value. Neurotech's NT-501 has been fortunate to receive the support of industry, The Foundation Fighting Blindness, the National Eye Institute, and now, the Food and Drug Administration.

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Strategies for Delivery of Rod-Derived Cone Viability Factor

JEAN BENNETT, MD, PHD

Rod-derived cone viability factor (RdCVF) is one of the most exciting molecules currently being considered for therapy for diseases such as retinitis pigmentosa (RP). Rod-derived cone viability factor has been cloned; we know how to deliver it with viral vectors into existing animal models of disease; and we know how to evaluate its efficacy. Several important decisions exist regarding the design of experiments to test the efficacy of viral vectors.

Leveillard and colleagues have characterized RdCVF isolated from a complementary DNA expression library made from neuroretinas of the wild-type mice, C57Bl/6.[1] Rod-derived cone viability factor has a 33% similarity to thioredoxin. It has a three-dimensional structure, which seems to be important to its function. It is expressed by photoreceptors and present in the matrix surrounding cone photoreceptors.

The first question in developing a gene therapy paradigm in animal models is where to deliver the factor (e.g., to the cone sheath or to the retinal pigment epithelium). Another question is how to deliver the protein. We have had encouraging results in pilot studies testing delivery of RdCVF using adeno-associated virus (AAV) in two animal models. Other vectors, such as bovine lentivirus, may work equally as well.

How to regulate expression also needs to be established. The AAV vector serotype 2 (AAV2) targets retinal cells exquisitely—rod photoreceptors and ganglion cell axons leading to the optic nerve—as seen after subretinal injection of virus containing green fluorescent protein. The green fluorescent protein–positive optic nerve fibers extend out from the eye toward the brain, raising concern that the transgene product could possibly be transported to the lateral geniculate ganglion and other structures in the brain.

One problem with AAV2 is that it takes approximately six weeks to reach peak levels of expression, making it impractical to use in animal models with rapidly degenerating photoreceptors. This can be controlled using different AAV subtypes from different animals, including humans and monkeys, dividable into nine different "clades," (i.e., viruses with homologous regions). Different capsids can be cloned to generate replication-defective viruses that have different characteristics in terms of onset of expression and cellular specificity of expression. For example, an AAV2 capsid targets retinal pigment epithelium cells and photoreceptors to a lesser extent than one of the newer capsids, which targets both of these cell types very efficiently and, furthermore, turns on rapidly, within approximately four days. Other viruses specifically target retinal pigment epithelium and other cell types, including Müller cells. It will be interesting to see the results of studies that compare rescue effects after delivery of a therapeutic gene carried by these different recombinant vectors.

Another issue regards pharmacologically regulated gene expression, and this relates to potential toxicity. We do not know yet whether RdCVF has any toxicity, particularly at the very high levels delivered by a viral vector system. If it does have toxicity, we should have a regulatory set of elements to be able to upregulate it or downregulate it, as needed. One available regulatory system relies on two different elements, one that actually binds to the DNA and the other that carries the transgene of interest, but does not bind the activating system and turn the gene on unless rapamycin is present. Lebherz and colleagues described a rapamycin-based dimerizer-inducible viral expression system in the eyes of monkeys that was able to drive expression of the reporter gene erythropoietin for longer than 760 days.[2] They demonstrated dose-dependency and regulability of gene expression by varying the dose levels of rapamycin; high levels of erythropoietin were attained with double doses, whereas half doses produced levels less than those obtained with single doses. This method may be useful for delivering RdCVF in a controlled fashion in humans.

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Adenovector Pigment Epithelium–Derived Factor (AdPEDF) Delivery for Wet Age-Related Macular Degeneration

LISA WEI, PHD

Wet age-related macular degeneration (AMD) is the initial target indication in GenVec's adenovector (Ad) pigment epithelium–derived factor (PEDF) ocular program. If the studies go well, we intend to expand into other retinal degenerative disorders.

Our drug development strategy at GenVec is to identify medical areas of high importance, to try to understand the biology of diseases in the areas, and then identify compelling, relevant, biologic activities suitable for treatment interventions. In the case of AMD, this is PEDF. We test the identified substance for efficacy in preclinical models and for safety in preclinical toxicology studies. In parallel, we try to identify whether there is a commercial pathway that would be profitable.

We sought to identify a protein with therapeutic properties for wet AMD that would 1) protect and/or rescue retinal cells, 2) reverse and prevent abnormal blood vessel development, and 3) stop blood vessel leakage. Ideally, the protein would be delivered directly. However, because proteins are generally unstable and have a very rapid half-life, an indirect model that uses or leverages the protein's gene is used.

We have focused on PEDF, because of its potential to provide a multifaceted approach to stopping vision loss. Pigment epithelium–derived factor was first identified in 1989 as a neurotrophic and neuroprotective factor[1,2] and later shown to promote regression of preexisting abnormal blood vasculature.[3] The latter feature was key to us, because abnormal neovascularization characterizes wet AMD. GenVec and others have shown that PEDF is a very potent antiangiogenic factor. It can prevent choroidal and retinal neovascularization.

It is interesting that PEDF opposes several angiogenic stimuli, including vascular endothelial growth factor (VEGF), fibroblast growth factor, platelet-derived growth factor, and interleukin-8. Most recently, it has also been shown to be an antipermeability factor. Several laboratory groups have found low PEDF levels in vitreous samples of patients with wet AMD compared with patients with nonneovascular disease.

In a rat model of light-induced photoreceptor degeneration, PEDF prevents photoreceptor death and protects retinal function, as determined by electroretinogram analysis.[4] In a VEGF transgenic mouse model with increased retinal neovascularization, PEDF diminishes the neovascularization and prevents further blood vessel growth.[3]

Our delivery approach is to use a second-generation adenovector to produce PEDF protein locally. This adenovector is multiply deleted in three of the key viral genes and, thus, no longer able to replicate. Because PEDF had not been used previously in humans, we also deemed it prudent to use a transit-expressing vector.

Our criteria for translation from preclinical to clinical studies are as follows:

1. The molecule must show strong efficacy in preclinical models.
2. It must demonstrate good safety in preclinical toxicology models.
3. We must be able to manufacture good manufacturing practice material.
4. We must identify a feasible clinical and regulatory plan.
5. We must secure intellectual property protection.

The Phase I clinical trial design for wet AMD using AdPEDF, carried out by GenVec, found AdPEDF to be well tolerated in all of the doses, even up to $1 \times 10^{9.5}$ pu. We saw no dose-limiting toxicities, no drug-related serious adverse effects, no endophthalmitis, and no significant ocular inflammation. Some patients showed some transient anterior chamber flare and cells. Some patients had intraocular pressure increases, unrelated to dose, that were transient and responsive to standard care.

Our AdPEDF program, similar to several of our other programs, shows that GenVec has the capability to take our vector designs and translate them into clinical leads. Internally, we have the ability to manufacture Phase I, II, and III materials. We also have

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Quality Assurance and Quality Control departments. We have submitted several investigational new drug applications in the field of cancer, cardiology, vaccines, and ophthalmology. We now have a biologic master file for our vaccine program, and we have moved forward with several clinical indications for oncology, cardiology, and vaccine programs. We have internal expertise for securing intellectual property rights.

Our next step in the AdPEDF program is to advance to clinical testing in wet AMD patients with less impaired vision. We intend to extend into other ocular indications.

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Clinical Trials With Micronutrients and Mineral Supplements for Retinal Degenerations: A Summary of a Breakout Session

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The topics of this session included studies of the benefits and effects of vitamin A, vitamin E, and docosahexaenoic acid (DHA) on retinitis pigmentosa (RP). Risk factors for age-related macular degeneration (AMD) and the influence of antioxidants were also considered.

In 1993, a paper was published reporting that change, over time, of electroretinogram (ERG) amplitude, was slower in RP patients taking high daily doses of vitamin A than in patients in a control group.[1] The paper also reported that high daily doses of vitamin E had the converse effect; it accelerated the rate of ERG change. Further examining the data, it was found that the entire effect occurred in the fifth and sixth years of treatment, and that there were no differences between the groups in Years 1 through 4. Because the effect in Years 5 and 6 could be because of a reduced sample size in those last two years, a new four-year study was designed. It took three years to recruit for the new study, and there were fewer subjects in Year 5 and even fewer in Year 6 of follow-up. When the data were corrected, adding confidence limits based on corrections for sample size, it seemed that the effect, if there was one, is much weaker than originally thought.

Regarding DHA in RP, studies have shown very intriguing evidence of a strong correlation between ERG amplitude and erythrocyte concentrations of DHA, suggesting that DHA has an important role in photoreceptors.[2] Because patients with RP show low concentrations of erythrocyte-DHA, it has been postulated, particularly for patients with X-linked RP, that daily supplements of DHA could improve their outcome. In a small, controlled clinical trial, no difference in the rate of loss of cone ERG amplitudes appeared between treatment and control groups; however, a small but significant difference did show up in the rate of loss of rod ERG amplitudes.

Another intriguing finding concerned the rates of ERG loss in individual patients. The rate of cone ERG change for each patient correlated with erythrocyte-DHA levels, such that patients with higher levels of DHA had a reduced rate in the loss of ERG function. The results did not achieve statistical significance, but the trend is encouraging, and a larger sample size might achieve more significance.

A recent study also compared DHA plus vitamin A to vitamin A alone in RP patients.[3] No difference was found, during a four-year follow-up period, in the rate of loss of ERG amplitude between the groups. Using visual field scores as a primary measure, there was also no difference in the rate of loss between vitamin A–only patients and patients receiving DHA plus vitamin A. The patients were then divided into two groups based on whether they had used vitamin A before entering the study. In the 30% of patients who had not used vitamin A, those who taking the DHA supplement showed a slower rate of loss than those who were on vitamin A alone. When the rates of loss for the control and treatment groups are compared, among the 70% of patients who had been taking vitamin A treatment for two years or more before entering the study, there was no significant difference between the rates of loss. However, when all of the raw data are considered and reexpressed as differences from the mean, it was seen that most of the effect could be attributed to differences in the baseline measures. Thus, a conclusion can be drawn that there is no effect of DHA supplementation of vitamin A, even for the subgroup that had not been taking vitamin A before entering the study.

Also discussed were results from the Age-Related Eye Disease Study, which demonstrated no trend suggesting that β-carotene intake altered the risk of developing advanced AMD. There was a significant trend, however, for lutein and zeaxanthin combined, at higher concentrations. In the Age-Related Eye Disease Study, subjects received either three antioxidants (vitamin A, vitamin E, and β-carotene); zinc; a combination of antioxidants and zinc; or a placebo. The conclusions of the study were that the placebo group had a higher probability of advancing to end-stage AMD than any of the three treatment groups. The combination of antioxidants and zinc reduced the rate of progression to advanced AMD by 25% during 5 years, compared with the placebo group. Risk reduction was only 17% with antioxidants alone and 21% with zinc alone. Environmental risk factors for

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progression to advanced AMD were also noted. These include smoking, high body mass index, weight, and sedentary lifestyle, among other factors.

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A Randomized Placebo-Controlled Clinical Trial of Docosahexaenoic Acid (DHA) Supplementation for X-linked Retinitis Pigmentosa

DAVID G. BIRCH, PHD

Docosahexaenoic acid (DHA) is an ω-3 long-chain polyunsaturated fatty acid found in all biologic membranes. It is the major constituent of photoreceptor membranes and is most highly concentrated in photoreceptor outer segments. Although it is not possible to measure outer segment DHA levels in patients with retinitis pigmentosa (RP), it is possible to infer fatty acid status by measuring the DHA content of erythrocyte (RBC) membranes. Many studies have reported that RBC-DHA levels are lower than normal in patients with RP.

Docosahexaenoic acid influences membrane fluidity, and several studies now show that it can affect phototransduction parameters. From animal work during the last 20 to 30 years, we know that DHA deficiency can also cause decreases in the electroretinogram (ERG).

Docosahexaenoic Acid Studies in Infants

One of the clues that sparked our interest in the role of DHA in retinal function came from work with preterm infants.[1] Preterm infants form DHA and arachidonic acid (ARA), an ω-6 fatty acid, from precursors at rates insufficient to adequately support the rapid growth and maturation of their brain and retina. Infants who are breast-fed receive both DHA and ARA from breast milk. In contrast, in the 1980s, infants fed commercial formulas received no long-chain fatty acids. Comparing breast-fed preterm infants to formula-fed preterm infants showed that, in their first few weeks of life, the latter group had a precipitous drop in their blood DHA levels. It is not until approximately 60 weeks postconception, when other foods are added to the diet, that DHA synthetic pathways become more efficient in producing DHA from α-linolenic acid (LNA). Therefore, supplementing infant formula with DHA could prevent the drop in DHA in the early postbirth period.

It was of most interest to us that we could detect differences in visual function among infants in four groups: breast-fed infants; commercial formula–fed infants; infants fed formula supplemented with LNA; and infants fed formula supplemented with LNA plus DHA. In the commercial formula–fed infants, the thresholds were further away from adult values than the thresholds in either the LNA group or the LNA plus DHA group. There were similar differences among the groups in visual acuity, with the LNA plus DHA–supplemented infants showing clear benefits. Much of this work (performed in conjunction with Eileen Birch, Dennis Hoffman, and Ricardo Uauy) showed differences in acuity that were small at 36 weeks but increased up to at least 6 months after birth. It seemed that a nutritional source of DHA was necessary for optimal visual development during the early postnatal period in very low birthweight infants.

The findings were not quite as dramatic with infants born at term.[2] In full-term babies receiving commercial formula, a precipitous drop in blood DHA levels (2%–3% of total fatty acid) would still be seen in the first year of life. In full-term babies fed human milk, the DHA level would stay higher. If commercial formula was supplemented with DHA or DHA plus ARA, the DHA level in the blood increased. A similar functional benefit occurred. The infants receiving DHA or DHA plus ARA supplementation had better visual acuities, as measured by sweep visual evoked potentials, than infants fed nonsupplemented commercial formula. These differences in visual acuity were seen at least until 18 months of age.

We have continued to follow these infants as they mature. Because differences related to DHA intake can be measured in IQ and other cognitive functions,[3] we think of vision as a window to neurologic development. There are clear differences in the development of the infants denied DHA during their early weeks.

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Docosahexaenoic Acid Studies in Patients with X-linked Retinitis Pigmentosa

Because DHA played a demonstrable role in the vision of infants, we investigated the possible role of DHA levels in patients with X-linked RP (XLRP), one of the most severe forms of retinal degeneration. We matched patients by age and looked at the relationship between DHA level and ERG amplitude. We found that blood DHA levels correlated with both rod and cone ERG function, such that patients with low DHA had lower ERG response amplitudes. Even more interesting were the b-wave implicit times, which did not vary with age and which were more prolonged in patients with low levels of RBC-DHA.[4]

Based on the observation that patients with high levels of blood DHA seemed to have better ERG function, we decided to conduct a randomized, placebo-controlled clinical trial[5] to test the following three major hypotheses:

1. Daily nutritional supplementation with DHA elevates RBC lipid concentrations of DHA in patients with XLRP.
2. Elevated RBC-DHA levels are associated with a slowing in the progression of ERG measures of retinal degeneration.
3. The RBC-DHA levels in XLRP are inversely correlated to the rate of ERG loss.

The most important measure of the clinical trial outcome would be whether there was a difference in the rate of progression of cone ERG loss between the group that was supplemented with DHA and the group that received a placebo. A secondary outcome would be a relationship between RBC levels of DHA in these supplemented patients and the rate of ERG loss. We focused on patients with XLRP, because of its rapid progression, especially at early ages, compared with dominant RP.

We conducted a traditional randomized, placebo-controlled supplementation trial. We saw the patients twice within eight weeks each year to measure variability. Measures at each visit included ERG, visual field, ophthalmic examination, and fundus photography. Fatty acids were analyzed to monitor compliance. In addition, a number of analyses associated with biologic safety of long-term DHA supplementation were conducted.

In this Phase I trial, we randomized 44 patients, 21 to the placebo group and 23 to the DHA supplementation group. Overall follow-up was better than 90%. During the four-year duration of the trial, patients in the supplementation group took two 500-mg capsules/d of DHA, which is 400 mg of DHA. We limited the dose, because we were not sure whether higher levels were safe, particularly for young people. Since then, trials for Alzheimer and heart disease have used DHA levels 5 to 10 times higher. Our dose was based on body weight calculations from our infant nutrition studies.

Consistent differences in RBC-DHA levels were found between the two groups. The average level in the DHA-supplemented group was approximately three times that in the placebo group (6.9% versus 2.7% of total fatty acids in RBC lipids). Across nearly all patients (see below for exceptions), the DHA levels were constant, with no real indication that any patients in the placebo group changed their diet or modified their intake during the course of the study. There was a slight trend for DHA levels to decline in the DHA group.

The loss of cone ERG function was our major outcome measure. Our conclusion from the intent-to-treat analysis was that there was no significant benefit on the rate of cone ERG decline and only a modest benefit on the rate of rod ERG loss.

We also evaluated changes in fundus appearance during the four-year trial. We compared fundus photographs from each possible combination of years. Evaluation was performed by four trained judges who, without knowing the group to which the patient belonged, graded the amount of change on a scale of 0 to 3. The DHA-supplemented group showed less change in fundus appearance than the placebo group. Eight (38%) of the 21 patients in the placebo group received a high score (3), indicating a major change in fundus appearance during the 4 years, whereas 2 (9%) of 23 patients in the DHA group were judged to have progressed this maximum amount.

We found no safety issues of concern.[5] Platelet aggregation, lipoprotein cholesterol, plasma vitamins A and E, and total antioxidant capacity were all comparable in the DHA supplementation and placebo groups. We observed no major adverse events.

We concluded that we were able to elevate the DHA levels in patients with XLRP. We did not show benefits in terms of visual outcome that would meet criteria for a change in clinical practice, although there was an indication that the levels of DHA were inversely correlated with the rate of ERG loss. The trial raises the possibility that very high levels of DHA may have a more substantial effect on slowing ERG progression. This remains to be determined in future work.

Docosahexaenoic acid and vitamin A are the most promising of the supplements that have been studied in clinical trials for treating RP. In our studies, we did not require the patients to take vitamin A, although 2 of the 44 patients were using it. Thus, it is difficult to compare our results with a recently published trial in which the two supplements were combined.[6]

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New Paradigms for Drug Discovery

GERALD D. CAGLE, PHD

There was a time when the paradigm in science was to develop a hypothesis, conduct an experiment, gather data, and publish the results. Today's alternative paradigm is to develop a hypothesis, form a company, develop data, and file a patent. The new paradigm supports a role for industry's collaboration with inventors, academic institutions, and government.

The current paradigm for drug discovery includes six main stages:

1. *Target (clinical syndrome) identification:* Basic research, biologic proofs of concepts, and structural chemistry.
2. *Screen to hit characterizations:* Assay development, high-throughput screening, hit confirmation, and hit characterization.
3. *Hit to lead:* Characterization of potency, selectivity, cellular activity, and in vivo proofs of concepts.
4. *Lead optimization:* Identification of candidates that meet criteria and advance to in vivo testing.
5. *Late-stage discovery:* Selection of product candidate via additional in vivo testing.
6. *Development project:* Formulation, pharmacokinetics, toxicology, safety, pharmacology, investigational new drug application, Phase I, II, and III trials, and new drug application.

It usually takes four to six years to move a compound from the beginning of this process to its end. The regulatory process associated with approval of a drug compound (i.e., starting with Phase I clinical studies) adds four to six more years. In total, the whole process takes eight to twelve years, approximately half of the time in the discovery phase, the other half in the development phase. It is an involved process, and no one should think they could race through the process with an orphan drug. Orphan drugs may afford some opportunities for short cuts, but they are small short cuts, reducing the process by not more than approximately one year.

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Screening Existing Drugs for Neurodegeneration: The National Institute of Neurologic Disorders and Stroke (NINDS) Model

JILL HEEMSKERK, PHD

The mission of the National Institute of Neurologic Disorders and Stroke (NINDS) is to reduce the burden of neurologic diseases. Of the 450 diseases under our purview, almost all are rare. Stroke, Alzheimer disease, and Parkinson disease are obvious exceptions. An important part of our mission is to enable the discovery of therapies.

Drug Development Process

The drug development process consists of three stages: 1) identifying a compound with activity relevant to a disease mechanism; 2) preclinical development of the compound to optimize the safety and activity profile; 3) clinical testing to demonstrate safety and efficacy in patients. Preclinical compound development has historically been performed almost entirely in industry.

Preclinical development encompasses extensive work in medicinal chemistry to improve the potency and reduce the toxicity of a chemical compound, as well as all of the standard pharmacology and toxicology testing required by the Food and Drug Administration for a new compound to be tested in humans. Biologically active chemicals of the type identified in typical National Institutes of Health–funded studies must undergo many iterative rounds of chemical modification and optimization to acquire the pharmacological properties needed to become a drug. To bring the potential treatment to patients, it needs to be demonstrated that the compound can reach the target tissue in high enough concentration for a long enough period to be expected to be effective. It must also be demonstrated that the required level of exposure to the compound does not carry a risk of toxicity. The combination of high potency, favorable pharmacology, and low toxicity is a difficult goal to achieve. Even after a compound has been deemed sufficiently safe for clinical study by the Food and Drug Administration, only 1 in 10 compounds that enter clinical trials are successful in reaching the market.

Because the drug development process is so high risk, time consuming, and costly, industry is hesitant to invest in many of the rare neurodegenerative diseases. The NINDS, therefore, works to enable the development of therapies for these diseases. One strategy is to make use of the efforts that industry has already expended during many years to develop and achieve approval of drugs. We have taken this approach and examined existing drugs to determine whether any show promise as treatments for rare neurodegenerative disorders.

The National Institute of Neurologic Disorders and Stroke Neurodegenerative Drug Screening Consortium

The NINDS focused its initial effort on neurodegeneration. We put together a large consortium sponsored through a partnership with the Huntington's Disease Society of America, the Amyotrophic Lateral Sclerosis (ALS) Association, and the Hereditary Disease Foundation. In total, the NINDS sponsored 29 laboratories, each of which tested a collection of approved drugs in their particular model of neurodegeneration (29 assays). We worked with a small company to customize a collection of 1,040 compounds. This collection contained clinically approved drugs and other bioactive molecules with human exposure, such as narcotics and natural product components of herbal medications. Investigators received the compounds as a blinded set. Each investigator tested all of the drugs in their neurodegeneration assays and contributed their data to a central database. Their initial contribution was a list of top "hits" they had identified and confirmed (i.e., compounds that were reproducibly active, showed a dose response, and were the highest potency molecules identified).

The compound collection is still available as the NINDS custom collection. Approximately 800 of the drugs in the collection are Food and Drug Administration–approved drugs. This represents approximately half of the drugs ever approved by the Food

Program Director for Technology Development at the NINDS and Project Officer for Spinal Muscular Atrophy Project and the NINDS High-Throughput Drug Screening Facility for Neurodegeneration.

and Drug Administration for human use. The collection also contains 240 bioactive compounds. A number of those are controlled substances that were included, because they cross the blood–brain barrier. The collection also includes natural products and some neurodegeneration standards, compounds that have been shown in other neurodegeneration settings to be potentially neuroprotective (i.e., creatine, minocycline, N–benzyloxycabonyl–Val–Ala–Asp–fluoromethylketone (zVAD.FMK), and FK506 [Tacrolismus]). These latter compounds, or compounds related to them, have either been in clinical testing or are being considered for clinical testing for neurodegenerative disorders.

The 29 different assays being funded under this program represented a broad variety of neurodegenerative diseases. Because our partners were funding organizations for ALS and Huntington disease research, we had a substantial number of assays in those two disease areas. We also had assays for Parkinson disease, spinal muscular atrophy, Kennedy disease, and other rare neurologic disorders, as well as some assays that represented general neurodegeneration mechanisms, such as apoptosis.

Because we were testing a relatively small collection of drugs, we could use somewhat more complex models than might be used in a higher throughput screen. We did have simple biochemical assays of purified proteins, but we also had cell-based models of mechanisms related to neurodegeneration (e.g., protein aggregation or cell survival in the presence of toxic gene products). We included more complex whole organism models as well, such as worms and flies that expressed mutant gene products. This allowed us to test drugs in the context of the intact nervous system.

Results

Given that many of the assays represented mechanistically related neurodegeneration assays, we expected to see extensive overlap among the active compounds identified by the different laboratories. Although neurodegenerative mechanisms affect different cells in different diseases, there are a number of common themes, such as apoptosis and mitochondrial dysfunction. Instead, we found only limited overlap. The general lack of overlap tells us that each assay has its own properties that respond nearly uniquely to the compounds tested. A full understanding of which are most relevant properties to neurodegeneration awaits further study, and investigators continue to follow up on the results of these screens. However, a few compounds hit in a reproducible way in more than one assay, and those compounds underwent immediate further study.

Future Studies

The hit list produced by the investigators' initial studies included known drugs as well as some natural products and some controlled substances. How do we proceed from the initial data to potential clinical testing?

Obviously, compounds cannot be taken directly from the hit list and applied in the clinic. The first step is to retest the primary hits in supporting assays to obtain more in vitro data regarding the compound. The reproducibility of activity garners more confidence in the compound. For some neurodegenerative diseases, such as ALS and Huntington disease, animal models exist, and some compounds from the screens are now undergoing in vivo testing in these models to gain further proof of principle for their potential activity.

We were lucky to identify a compound that seems to be a reasonable candidate for clinical testing in ALS. Ceftriaxone, identified in assays related to the toxicity of glutamate and mutant *SOD1*, was later shown to have benefit in the mutant *SOD1* mouse model of ALS. Of the cephalosporin antibiotics, ceftriaxone can achieve highest levels in the central nervous system, and these levels are consistent with the necessary activity determined in animal and in vitro studies. Based on these findings, a clinical trial in ALS patients is planned. Whether ceftriaxone has benefit in ALS patients remains to be determined in a clinical setting, but this is an example of the kind of outcome we hope for in these screening studies—to find a novel activity of an existing drug that is safe enough to be given in a chronic setting.

The NINDS is building, under the National Institutes of Health Molecular Libraries Roadmap initiative, a new bioactive compound collection. Our goal is to make the collection available to the research community some time next year.

High-Throughput Compound Screening and Discovery in an Academic Setting

MIN LI, PHD

ChemCORE at The Johns Hopkins University School of Medicine is an integrated robotics and chemical repository unit. It provides combined access to large chemical libraries and state-of-the-art robotic capability. Its goal is to perform high-throughput screening of assays in search of compounds for scientific investigation, clinical use, and commercialization.

Costs and risks of high-throughput screening are reduced when high-throughput screening is performed by an established laboratory, because it is very expensive for an individual laboratory to acquire compounds independently and set up a robotic facility. A goal of ChemCORE is to use its capabilities to bridge chemistry and medicine, and, at the same time, to position the institution competitively for funding and quality scientific results.

Industry or Academia

A major strength of industry is that it can make a huge investment to develop a drug (i.e., $700 million on average). Industry's goal is to obtain a return on its investment; thus, it requires a proven target with a clear therapeutic value. The for-profit objective requires secrecy and "labor in isolation."

In the academic environment, funding is provided by federal agencies and collaborations, which nurture an "open system." Because of limited funding, academia may not be able to provide as much financial support as industry, but it does provide unlimited and free knowledge and expertise. Academia allows the pursuit of targets and structures that are unproven and risky with no guarantee of short-term commercial gain, providing significant opportunity for discovery and therapeutics.

Chemical Core or Typical Core Facility

In the typical DNA sequence core, input is very standard—a DNA template and a primer. The technology is essentially a generic sequencer, and the output is an electronic file. With the ChemCORE process, input can include different targets and biologic systems. The technology requires different assays, different detection, and read-outs. The output is diverse, and the compound structures of interest may have different structural features, requiring participation of chemists with widely specialized expertise.

Challenges

First of all, if theoretical predicted diversity is 10^{60} and there are only 10^7 registered compounds, that means that there is a tremendous diversity of chemical structures yet to be explored, that probably will not be explored in the foreseeable future. Our rationale is that we need to start with a reasonable library size with sufficient diversity, tractability, and renewable supply. At ChemCORE, we focus on a diverse set of 20,000 compounds. In particular, we have 3,000 selected known structures and drug structures.

A second challenge relates to the targets. There are 20,000 human genes; 3,000 G-protein–coupled receptors; 400 ion channels; and 160 potassium channels. To meet this challenge, we focus on innovative assays, discovery-oriented content, and model systems in parallel. Current strategies include 1,000 full-length human protein targets.

A third challenge relates to the chemistry in a diverse hit structure. We have established chemical synthesis agreements with small and midsized specialty companies near Johns Hopkins, and we have recruited 18 chemists from various institutions in the mid-Atlantic region. They participate in the projects through mutually beneficial collaboration and maintain confidentiality regarding results.

Informatics and data mining represent an important domain. We intend to provide real-time data acquisition and analysis, library-oriented linear integration, and, most importantly, an institutional database of knowledge. After we screen an assay, we can provide an index factor to indicate whether the identified compound has been identified in other assays or not. That way, we can facilitate communication among investigators who may have a shared interest and may be potential collaborators.

Hopkins ChemCORE is striving for flagship capability. We would like to have content-rich assays, discovery opportunities, and clinical significance. For example, Hopkins has recently developed and patented technology

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that is able to profile a compound for potassium channel activity for its ability to interfere with the activity. Thus, after an assay is screened, we can provide information regarding whether a compound is likely to influence the potassium channel, which is important in the side effects of QT prolongation.

Current Capabilities

ChemCORE personnel include an automation engineer, assay technicians, chemists, and a project manager. Clients can log onto a secured website, communicate with the project manager, and see the data as they are generated and deposited in their folder. We have various types of hardware and various compound libraries. We receive funding from several sources, including both federal and private sources. Our libraries have produced hits in five assays that were validated with resynthesized compounds. The National Institutes of Health grants were submitted based on the hits. A term sheet has been signed for a state-of-the-art photonics screening platform that is generic for all molecular interaction.

The Consortium Project to Treat RPE65 Deficiency in Humans

WILLIAM W. HAUSWIRTH, PHD

Soon after July 2000, when a Briard dog with the missing RPE65 protein was first treated with gene therapy, a group of researchers from the University of Pennsylvania, Cornell University, and the University of Florida proposed a consortium project to the National Eye Institute. The project was based on data from the studies of the dog, named Lancelot. It proposed to begin, within 5 years, a safe and efficacious adeno-associated virus (AAV) gene therapy trial for patients with the orphan retinal disorder, Leber congenital amaurosis, in which the RPE65 protein is affected. The project was funded in late 2001.

Dogs with RPE65 deficiency are blind at birth and are more severely affected than most humans. Affected humans usually have some vision at birth and tend to be partially sighted for approximately a decade. The dog studies were, in theory, a more difficult test of gene-based therapy than one might expect in a young human.

An AAV, a nonpathogenic human virus, was chosen as the vehicle to deliver a normal copy of the *RPE65* gene (AAV infects approximately 60% of the world's population and is associated with no known disease). To make an AAV vector, the virus' normal gene is removed, and other genes are inserted—in this case, a promoter and the gene of interest, the canine *RPE65* DNA.

In designing the study, we drew on work from the previous 10 years: 150 μL were to be delivered subretinally to distribute the therapeutic gene to the cells of the retinal pigment epithelium. Subsequent study showed that this volume of vector led to only approximately 20% of the retinal pigment epithelium receiving the gene. However, we thought that 20% rescue of the whole retina, especially within the central retina (the macula in humans), would be sufficient for a useful functional change.

Electroretinograms (ERG) from Lancelot, before and after treatment, are described in a report by Dr. Richard Weleber in this supplement. Three months after the eye was treated, the thresholds for detecting light in rod photoreceptors had improved by approximately 100,000-fold (i.e., Lancelot's eye could see input light of 100,000 times lower intensity than he could before treatment.)

Rybaczki-Bullard Professor of Ophthalmology and Molecular Genetics at the University of Florida School of Medicine, Gainesville, FL.

Importantly, Lancelot's improved ERG recordings have been maintained for four years. In fact, there has been a slight improvement over time, which may be related to the fact that, at least in mice, abnormal lipofuscin granules beneath the retina diminish and actually disappear after treatment. This may enhance the function of the retinal pigment epithelium. The cone ERG data are equivalently improved. More than 50 dogs have now been treated. Some received treatment in both eyes; approximately 95% of treated eyes have restored vision, as measured primarily by the ERG. The few failures were probably not failures of the concept, but were likely to have been either surgical failure caused by damage caused during injection or by impure vector.

In addition to the evidence of functional rescue in the dogs, retinal levels of 11-*cis* retinaldehyde chromophore and rhodopsin were restored to nearly normal in the treated eyes. Thus, all of the biochemical, structural, and ERG markers of retinal health indicate that the treated dogs can see. They dogs also run around and act normally. A quantifiable way to document restoration of useful vision would be important, perhaps an adaptation of the Morris water maze for mice. The Morris water maze has been used to compare treated and untreated vision-impaired *rd12* mice.

The Consortium has had several successes. It has completed proof-of-principle experiments for gene therapy in dog and mouse models of Leber congenital amaurosis. It has completed, or is in the process of completing, biosafety studies of the therapy in dogs, rats, and monkeys. The Consortium is also in the process of producing, purifying, and qualifying the AAV vector that is to be used in a Phase I clinical trial to good manufacturing practice standards. Additionally, the Consortium has clinically identified and molecularly confirmed a pool of *RPE65* Leber congenital amaurosis patients from which Phase I subjects will be recruited, and has developed clinical measures of vision for determining therapeutic endpoints. The process of assembling the Investigator-initiated New Drug application, to be submitted to the Food and Drug Administration for the Phase I Trial, is underway. The first patients for the Phase I Trial, all of whom will be older than 18 years of age, are expected to be enrolled in late 2005 or early 2006.

The Role of RPE65 in Inherited Retinal Diseases

DEAN BOK, PHD

The RPE65 molecule was independently discovered and reported in 1993 by Redmond's group[1] at the National Eye Institute and Eriksson's group[2] in Sweden. The National Eye Institute group called it RPE65, whereas the Swedes called it P63. Eriksson's group thought that this molecule was actually the receptor on the basolateral surface of the retinal pigment epithelium (RPE) that mediates the entry of vitamin A into that cell. Some years earlier, it was reported that the receptor binds plasma retinol-binding protein (RBP),[3] a point that remains controversial, because the receptor has never been cloned.

Retinol-binding protein is secreted by the liver and is in very high concentration in the blood, approximately 5 mg/dL. It is noncovalently bound to vitamin A (all-*trans* retinal) and also bound to transthyretin, which transports thyroxin. When the RBP recognizes its receptor, the vitamin A enters the RPE. The RBP then dissociates from transthyretin. Retinol-binding protein is destroyed in the kidney. It might seem that this process is uneconomical, except that so much RBP is present in the blood that it probably does not matter if some is wasted.

It is now generally accepted that RPE65 cannot be the RBP receptor. As Redmond and colleagues have shown,[1] it is not a transmembrane protein. It is a peripheral protein that lies on the membrane surface. After vitamin A enters the cell, it is bound by a protein called cellular retinol-binding protein. Cellular retinol-binding protein is thought to deliver vitamin A to an enzyme called lecithin:retinol acyltransferase. Lecithin:retinol acyltransferase, cloned several years ago, adds a fatty acid to vitamin A, producing a retinyl ester. The ester is insoluble in water and, left alone, accumulates in oil droplets. Rabbit and frog RPE are filled with big oil droplets; in human RPE, retinyl esters are not stored in such quantity and, therefore, oil droplets do not normally accumulate in the RPE.

It was recently shown that RPE65 is a retinyl ester–binding protein.[4,5] It is thought that RPE65 accepts the ester from lecithin:retinol acyltransferase and then presents it to the isomerizing enzyme. In Redmond's knockout mouse, because the protein is missing, the visual cycle is blocked. There is, therefore, no opportunity to make 11-*cis* retinol, which the enzyme would normally do. The hydrogen is removed from 11-*cis* retinol by yet another enzyme, and the 11-*cis* retinal produced in this last step is finally delivered across the surface of the cell to the opsin molecules in rods and cones.[6]

The RPE65 is located in the smooth endoplasmic reticulum of the RPE, where the machinery for the production of the 11-*cis* retinaldehyde chromophore that is necessary for sight is located. Without this regenerative process, one would be born blind, which is essentially what happens in one of the forms of Leber congenital amaurosis. We now know of eight different genes causing Leber congenital amaurosis; *RPE65* is one of them.

In 1998, a paper was published on the mouse *RPE65* gene disruption.[7] In this mouse, the retina looks surprisingly healthy at seven postnatal weeks; under the light microscope, it does not look very different from normal. At 15 weeks, however, the outer nuclear layer (photoreceptor nuclei) has thinned, meaning that photoreceptors are dying. A 15-week-old mouse is the approximate equivalent of age 8 years in the human.

At seven weeks, compared with the wild-type (normal) mice, the photoreceptors of the knockout mice look moth-eaten by electron microscopy. The protein opsin is present but, because the opsin is missing its chromophore, the mice are essentially blind. Their rhodopsin has absolutely no absorbance at the wavelength that reflects the presence of the normal chromophore. If, however, chromophore is added back to these proteins, they become somewhat regenerated, therefore, the protein is probably quite normal but simply lacking its chromophore. In the retina, there is an accumulation of oil droplets in the pigment epithelium. The droplets grow so large that some of the RPE gets bloated with droplets. The droplets are full of retinyl ester, because there is no binding protein for this insoluble molecule.

Redmond and colleagues published their paper on the knockout mouse in 1998. Acland and associates published their now famous gene therapy paper on Lancelot, the *RPE65* deficient dog, in 2001.[8] In a matter of only three years, we went from basic gene discovery to a fabulously successful preclinical trial, whose canine patients remain sighted to this day. An additional 44 dogs have now been treated, and human clinical trials are not

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far off. It is a remarkable story. It was not so long ago that some people could not see the importance of supporting dog colonies for the study of inherited retinal degenerations, when cheaper animals like and mice and rats were readily available. The importance of these larger animal models is no longer in question.

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Cooperation Between Private and Public Sectors Leads to an Intraocular Retinal Implant

ROBERT GREENBERG, MD, PHD

Twenty years ago, an idea was presented for restoring hearing in deaf people. From this idea grew electronic implants to electrically stimulate inner spiral ganglion cells in people who are deaf, because of hair cell degeneration. A device picks up sound, processes the sound, and send signals wirelessly across the skin to an implant. A measure of the impact of the implant is that upward of 80% of users are able to have telephone conversations.

In approximately 1990, at the Wilmer Eye Institute of Johns Hopkins Hospital, two surgeons, Dr. Eugene de Juan and Dr. Mark Humayan, began investigating applying a similar approach to patients with retinal degeneration. Could a signal be picked up with a video camera instead of a microphone, transmitted wirelessly to the retina, and stimulate the inner retina in a patterned way?

First, it had to be established that retinal cells were actually present in these patients and would respond to stimulation. It turned out that a fair number of cells survive in retinal degenerations, including retinal ganglion cells. Next, it had to be determined that these cells were functional. This was tested in approximately 24 patients with retinitis pigmentosa who had no, or barely any, light perception. Under local anesthesia and in an operating room, a 1-mm diameter electric probe was placed as close to the retina as possible and the patients were asked, "Do you see anything?" Even with relatively modest electrical stimulation, they reported seeing something very small (e.g., the size of a pea) in the stimulated area.

The project was born and, with government and private funding, progress was made to develop the ocular implant. In 1999, Second Sight was founded to make a retinal prosthesis. Second Sight is a technical company with approximately 50 employees. It has the regulatory, quality, and manufacturing infrastructure necessary to produce retinal implants. The company works with collaborators, such as Argonne National Laboratory, that provides a special coating, originally developed for military applications, which will be used to protect the electronics of future devices inside the eye.

President and CEO of Second Sight, Sylmar, CA.

Second Sight's first prototypes for the retinal implant are based on the cochlear implant. The implant is attached to the retina with small tacks that are the width of a human hair. In February 2002, an implant was placed in the first patient at the Doheny Eye Institute at the University of Southern California. Currently, 6 patients have received implants. The implant's electrodes are individually controlled and activated; the amount of current required to produce a spot of light is monitored over time.

Originally, the study of these patients was designed to be a clinical trial confined to the clinic. However, the progress of the patients with this relatively crude implant (a 4×4 array of electrodes, 16 electrodes total) was such that we requested and received permission for the patients to use the external device (a signal modulator) at home. Our first patient who took the device home reported after two weeks that he was able to see the windows and doors of his house, as well as large objects. This patient had had no light perception for 50 years. We had not expected the device to produce any useful vision because of the few electrodes involved, and were surprised by its success. We had not accounted for the fact that a patient would scan, moving his or her head from side to side to increase the area of perception.

Psychophysicists are performing studies involving the projection of various images and asking retinal implant patients to locate and identify objects at various visual angles. We have also begun positron emission tomography scanning and electroencephalograph recordings of these patients to obtain truly objective measures of cortical responses to the retinal stimulation.

Second Sight is performing animal studies on a second-generation implant. It is a smaller device with higher resolution (i.e., 8×8 electrodes) and will fit fully inside the eye.

The progress in developing a retinal implant illustrates how a commercial company can provide focused resources that will result in a product with direct benefit to patients. In addition, government/company/university partnerships can often be more productive than any single organization by itself.

Preclinical Assessment Programs to Evaluate Potential Therapies for the Treatment of Orphan Retinal Diseases

TIMOTHY SCHOEN, PhD

The role of the National Neurovision Research Institute, Inc. (NNRI) is to accelerate the translation of laboratory-based research into viable medical treatments and cures for retinal degenerative diseases. To facilitate the prompt development of new treatments, the NNRI recently established four Preclinical Assessment Centers (PCACs). The PCACs provide a mechanism whereby drugs originally developed for other medical indications, such as Alzheimer disease, Parkinson disease, amyotrophic lateral sclerosis, and cancer, can be evaluated for potential use in the treatment of degenerative retinal disease.

Currently, the NNRI has four PCACs: Scheie Eye Institute, University of Pennsylvania, Philadelphia, PA; Wallenberg Retina Center, Lund, Sweden; Johns Hopkins University, Baltimore, MD; and University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA.

Therapeutic candidates are identified through a variety of resources such as biotechnology-related web sites, publications such as *Genetic Engineering News*, and word of mouth. Once a drug candidate is identified, an attempt is made to set up a Material Transfer Agreement between the company that owns it and one of the four PCACs for a primary assessment. If a preliminary screening indicates that the drug is effective in slowing or stopping retinal degeneration, it opens the door to further development. The NNRI has the ability to form joint ventures and partnerships with companies and obtain a return on investment through milestone payments and/or royalties. Even if a particular company is not interested in developing a promising compound, the NNRI may be able to license and develop the drug independently. Conversely, companies wishing to develop drugs on their own may still benefit from other NNRI or Foundation Fighting Blindness resources, such as patient recruitment and scientific advice.

Director, Preclinical Assessment Program at the NNRI in Baltimore, MD.

Examples of Research at the Preclinical Assessment Centers

At the Scheie Eye Institute PCAC, Dr. Rong Wen uses the s334ter rat model to evaluate promising compounds. The s334ter rat exhibits a retinal degeneration very similar to that observed in humans with a particular form of dominant retinitis pigmentosa (RP). Recently, Dr. Wen showed that a single ocular injection of cardiotrophin-1 into the vitreous of s334ter rats resulted in a twofold to threefold rescue of photoreceptor cells. Cardiotrophin-1 is a peptide that acts on the same family of receptors as ciliary neurotrophic factor. At the end of a two-month study, untreated animals were left with just a single row of photoreceptor nuclei. In contrast, rats receiving a single injection of cardiotrophin-1 had approximately three to five rows of photoreceptor nuclei. Furthermore, rats that received multiple injections of cardiotrophin-1 exhibited a tremendous rescue effect, of 8 to 10 rows of nuclei.

At the Wallenberg Retina Center PCAC, Dr. Van Veen evaluates promising neurotrophic compounds using a retinal organ culture technique. Briefly, the technique involves dissecting out the retina and retinal pigment epithelium from *rd1* mice, which exhibit a recessive form of RP. The retina–retinal pigment epithelium sandwich is then placed into tissue culture either with or without an experimental compound. After 21 days, the cultured retina–retinal pigment epithelium sandwich is processed for histology and the numbers of photoreceptor nuclei are quantified.

Both the in vivo s334ter rat model and the in vitro *rd1* mouse retinal organ culture system are useful for evaluating promising neurotrophic compounds, and there are advantages and disadvantages to each system. The s334ter rodent model exhibits a retinal degeneration very similar to that observed in humans. However, certain drugs, when injected into the vitreous of the s334ter rat model, may not be effective because they require a sustained concentration. The retinal organ culture system is able to circumvent this problem because "fresh" drug can be added daily when the culture media is changed. Hopefully, by using multiple in vivo and in vitro models of retinal

degeneration, it will be possible to identify promising rescue compounds that can be channeled toward human clinical trials.

Previous studies by Dr. Peter Campochiaro at the Johns Hopkins PCAC revealed that the systemic administration of a vascular targeting agent, combretastatin, was able to inhibit neovascularization in a rodent wet age-related macular degeneration model. Not only was combretastatin administration able to block choroidal neovascularization, it also caused regression of existing neovascularization. Based on the preclinical studies, a Funded Research Agreement was made with Oxigene, Inc. that provided support for a small Phase I/II clinical trial to evaluate combretastatin in patients with choroidal neovascularization.

Before a human clinical trial can be initiated, the Food and Drug Administration normally requires that safety be demonstrated in a large animal model. Fortunately, the PCAC at the University of Pennsylvania, directed by Dr. Gustavo Aguirre, has several dog models of RP that can be used to assess both safety and efficacy. Currently, gene therapy studies are being conducted in a dog model for Leber congenital amaurosis, an early onset form of RP. The RP dog models are also useful for evaluating candidate pharmaceuticals that have potential for treating degenerative retinal disease. Studies using encapsulated cell technology to provide sustained delivery of ciliary neurotrophic factor into the vitreous of the *rcd-1* dog model revealed that as the output of ciliary neurotrophic factor from the encapsulated cell technology device increased from less than 0.1 ng/d to 5 to 15 ng/d, the number of photoreceptor nuclei in the outer nuclear layer was doubled. In part, because of the research efforts of the University of Pennsylvania PCAC, Neurotech, Inc., in collaboration with the National Eye Institute, initiated a clinical trial to evaluate the safety and efficacy of encapsulated cell technology–delivered ciliary neurotrophic factor for the treatment of RP. The Phase I safety trial has been completed, and Neurotech is planning to move forward to evaluate efficacy in a larger Phase II trial.

CLINICAL TRIALS

Patient Networks for Clinical Trials

A Network of Patients with Orphan Retinal Diseases for Clinical Trials: Goals, Structure, Challenges

RICHARD G. WELEBER, MD

For clinical trials to be meaningful, researchers need adequate numbers of subjects. This is especially challenging for scientists working on diseases in which few people are affected. To help find appropriate patients for whom there are potential benefits, some scientists working on particular diseases have established networked databases in which patients are registered according to characteristics of their disease and numerous other parameters.

Currently, no such network exists that could be used to connect patients who have an orphan retinal disease to the investigators and organizations contemplating clinical trials. Access to affected patients is important in not only the actual designing and carrying out of the trials, but also in the discovery and planning phases when pharmacological companies are making decisions regarding whether to proceed with clinical trials.

The Goal and Process of Linking Information

Patients with orphan retinal diseases are in the care of physicians at medical centers worldwide, and data are collected and cataloged on many of the patients. Considerable phenotypic and genotypic information for patients now exists at The Foundation Fighting Blindness–sponsored centers, but the data are of vastly different types (and degrees of detail) and exist in different formats. How best can the information scattered among many physicians and centers be linked for future access and for what purpose? My initial approach to exploring this topic is to pose a number of questions that must be addressed.

One question concerns the goal of collecting genotypic and crude phenotypic information. Is the purpose of the data collected to enable investigators to contact these patients to invite them to participate in clinical trials? Or, is the purpose to gather significant amounts of phenotypic information for use in scientific studies? Additionally, if genotypic information is collected, how expansive should it be? Would all forms of genetic information be entered into a registry, including sequence changes of uncertain significance for disease? Would phenotypic information consist of visual fields, electroretinogram information, clinical findings, historical data, and facts regarding participation in present or past studies? Would genetic information be designated by the methodology by which it was obtained and the limitations of the techniques used?

Obviously, phenotype and genotype data would need to be standardized. Is this possible? Committees would need to be formed to define conventions for nomenclature and to establish methods for data collection. All participants would need to agree on these conventions and accept the concept of sharing their data.

Infrastructure Development

An infrastructure would need to be designed and created to establish and manage such an international patient network. Needs must be met for entering data, managing data, data upkeep, and periodic review. The registry database would have to accommodate an ever-expanding amount of information.

Questions must be asked regarding who can access the data and for what reason. Would the database be available to virtually everyone seeking information regarding gene mutations in general or specific mutations, or to people seeking information regarding genotype–phenotype correlations? Would an Institutional Review Board (IRB) establish criteria for the process of gaining approval and access to the data? How would the issues of privacy, confidentiality, and data security be handled?

Director of the Oregon Retinal Degeneration Center and Professor of Ophthalmology and Molecular & Medical Genetics at the Casey Eye Institute at Oregon Health Science & University in Portland, OR.

Who would oversee the operations and the functions of the network and the central registry and, very importantly, can the physician/patient relationship be protected in a national or an international network of information? How would intellectual discovery, intellectual property, and authorship issues be handled? These issues are extremely important for industry, and they are important for academics as well. How can state and federal regulations and Health Insurance Portability and Accountability Act (HIPAA) requirements be satisfied? The concept of a registry containing personal information and information regarding genetic sequences raises many issues regarding genetic privacy laws and HIPAA requirements. Privacy laws vary from state to state, and complex issues arise when information is shared among different countries. The physician–patient relationship must be respected and preserved, and this would have to be secured in a way that would preclude investigators from directly contacting patients without previous approval.

A final and major question regards the source of funding for such an effort. Would funding come from The Foundation Fighting Blindness? Would it come from the National Institutes of Health? In another presentation at this Symposium, the National Eye Institute director, Dr. Paul Sieving, discussed the possibility of creating a genotype–phenotype network under the auspices of his institute. How would this affect our thoughts regarding creating the network we are discussing?

A Stereotypic Molecular Data Registry

In the stereotypic molecular data registry containing genotype and phenotype information, the data would reside within the centers in which the physicians care for patients with retinal dystrophies. The information at this level would be confidential, but it could be encrypted and then coded and transmitted with unique identifiers to a central data registry, where it would be then handled as anonymous data. Such an arrangement would require a steering committee, and the review process would probably also involve a committee. Requests for information would come from various sources, for example, industry, academia, private agencies, and interested persons. If approval were granted, the registry would send the data in a way that secures the individual anonymity of the patients. If the recipient intended to contact patients, a mechanism would exist by which the registry would review and approve the request, contact the physicians at the centers, and ask them to communicate with the patients. This is one scheme that provides a framework for answering some of the questions posed.

To summarize, a phenotype and genotype data network of patients with orphan retinal diseases would benefit investigators and organizations considering clinical trials. Many questions must be asked and answered regarding the function, design, and funding of such a network. Patient confidentiality must be assured.

National and International Patient Networks: Standardization of Phenotype and Genotype Definitions

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The key to exchanging information to the benefit of all investigators and patients revolves around the basic principles of cooperation, transparency, and organization. A first requirement for useful cooperation is the establishment of common definitions. Phenotype and genotype definitions must be agreed on and adhered to by all participating individuals and centers. The criteria for identifying genotypes, as will be done in the National Eye Institute/the National Institutes of Health initiative, will be rather straightforward. More challenging will be the defining of clinical phenotypes. Parameters for defining diseases should be as quantitative as possible. Pigmentary degenerations in the peripheral retina, or macular degeneration including drusen, are examples of conditions for which digital photography can be the basis for quantitative definitions of phenotype. Imaging technology is now available to provide useful quantitative data to define phenotypes with an appreciation of the need for standardization for purposes of reproducibility and objectivity. Fundus photography is a useful technique. Psychophysical and electrophysiological tests allow us to measure the response of the entire retina, or to discriminate, measuring particular areas of the retina. The protocols should be very specific and discrete to produce reproducible and quantitative data.

We may encounter difficulty in obtaining demographic data, such as race and ethnicity, because we are not legally permitted to require this information, and in some ethnically diverse populations, patients may not volunteer it.

An issue that is certain to arise as we work together and share patient data is how to handle authorship of studies and publications. How do the various participants in a cooperative study demonstrate that they have contributed to the creativity and the intellectual concept of the particular paper or manuscript, and how is the order of authorship determined? How do we deal with other aspects of intellectual discovery and property? How can conflicts of interest be avoided?

Of course, a major issue regards funding sources to create and maintain a national and international patient network. We have appreciated the support of The Foundation Fighting Blindness and the National Eye Institute in many other endeavors, and we would undoubtedly need their support for such an undertaking.

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Biostatistics in Clinical Trials

Design of Phase III Clinical Trials for Treatments of Orphan Retinal Diseases: An Overview of Considerations

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A well-planned, well-executed, randomized, and controlled clinical trial is the most powerful experimental technique for assessing the efficacy of a new treatment. However, numerous criteria must be considered for the implementation of a retinal disease treatment trial.

Study design is a critical foundation for evaluating new treatments. A consensus for study designs and methodologies is outlined by the Consolidated Standards of Reporting Trials Statement (CONSORT; www.consort-statement.org).

Although the CONSORT criteria apply to clinical trials of orphan retinal diseases, studies for these rare conditions bring numerous additional challenges. For example, small numbers of affected patients pose difficulties in achieving required sample sizes to answer clinically meaningful questions and determine treatment effects. Moreover, there are large variations in both genotypes and phenotypes of retinal degenerative disease. However, with the correct study design and proper study execution, treatment outcomes can effectively be determined.

Clinical Trial Designs

The *parallel* design is the one most commonly used for clinical trials of retinal degenerative diseases. With this design, persons (or a site) receive one study treatment, the advantage being that differences observed between the groups (treatment versus control) are likely because of the assigned treatment. The main disadvantage is that larger sample sizes may be required.

An alternative is the *crossover* design, which provides for administration of two or more study treatments, one after another, in a specified or random order. The advantages of this design are that the patients can serve as their own controls, and smaller sample sizes are usually sufficient. The disadvantage is that crossover studies are subject to biases from possible period effects (i.e., exposures that may be limited to a specific period), carryover effects, and dropouts. This may or may not be an appropriate design for clinical trials of retinal diseases.

Criteria for Clinical Studies

General criteria apply to the design of all clinical trials. An investigator must start with a clear statement of study aims and definition of study outcomes. Sample size must be considered, and eligibility criteria must be determined. It is important to include a comparison (control) group or comparison treatment. Randomization techniques and masking plans for final data analyses have to be determined in advance. Measurements must be standardized, and complete follow-up must be planned for all participants. There must be ongoing monitoring for patient safety and plans for interim analyses with stopping guidelines. All of these issues must be considered in advance of starting the study.

Study Aims and Definition of Outcome Measurements

A clear definition of the study aims should specify the goal of the treatment. For example, is the goal to slow progression or to restore vision? The intervention and comparison groups also need to be specified, as well as the primary, secondary, and tertiary outcome measures. Is the aim of the study to look at long-term or short-term outcomes? The more precisely the aims of the study are defined, the greater the likelihood of achieving study goals.

Possible study outcomes for retinal degeneration clinical trials include full-field electroretinography, static and/or kinetic perimetry, visual acuity testing, and fundus photography, as well as quality-of-life measurements. Precise, quantitative descriptions of the primary study outcomes are needed to provide the basis for sample size estimates. The outcome selected should be clinically meaningful. For example, a surrogate measure might not be as likely to answer the study aims as a direct clinical measure. Investigators should consider whether the selection of functional or

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structural changes would result in more clinically meaningful results regarding patient care, or more scientifically meaningful findings that would contribute to better understanding of the pathogenesis or the prognosis of the disease.

Can the outcome be described in clinically meaningful terms? For categorical measurements, improvement and no improvement must be defined (e.g., by loss or gain in lines of visual acuity). Regarding continuous measurements, mean change between baseline and end of follow-up in some of these outcomes (e.g., central visual field loss, electroretinography, and quality of life between treatment groups) would be compared. Another approach is to compare mean scores between treatment groups at a certain point in time.

The more objective the measurement, the more observation bias is minimized. The degree of variability inherent in a particular measurement must be considered (i.e., more variable measurements are less precise and require larger sample sizes to detect a statistically significant difference between treatment groups). The length of follow-up should be based on the length of time required for a meaningful treatment effect to be observed. This will vary according to the study outcome, and can range from six months to one year, two years, or longer. If multiple outcome measures are used, some combination of outcome measures could be considered, so that success could be defined as success in a certain number of measures.

Sample Size

A number of factors must be considered in determining sample size. These include the following: 1) the expected change in the study outcome in the comparison group; 2) the proportion of patients in the comparison group expected to develop events as defined in the context of the study design; 3) knowledge regarding the natural history of the disease sufficient to make these estimates; 4) the amount of difference between treatment groups that is considered to be clinically meaningful; 5) the estimated variability in the comparison group for a continuous outcome measurement; 6) the desired α-level or type I errors; and 7) the desired power (e.g., 80% or 90%). Decisions regarding sample size should take into account losses caused by participant drop out. Inadequate sample size can result in insufficient power to detect statistically significant differences, which leads to inconclusive results.

Some practical considerations apply to selecting sample size. Can the sample size goals be met? Can recruitment be completed within the proposed period? With diseases that are rare, recruitment can be a lengthy process, therefore, the outcomes for patients enrolled early may be known before enrollment of subsequent patients is completed. The period to complete recruitment may need to be limited at the beginning of the study.

Eligibility Criteria

Eligibility criteria must be very clearly defined and must be specific regarding age range, disease stage, phenotype, and genotype. There must be a clear rationale for each decision made, knowing that the mechanisms of the treatment actions will vary and may affect patient subgroups differently.

Selection criteria have implications for generalizing the results. More specific criteria, such as a narrow age range, same disease stage, and genetically homogeneous population, will increase specificity and may increase the likelihood of detecting a treatment effect. Very specific criteria also decrease the potential for generalization and increase the difficulty in finding eligible patients. Broader criteria will increase generalization potential and increase population heterogeneity. They also potentially mask treatment effects in some patient subgroups, but increase the ease of finding eligible patients. These various factors have to be weighed and balanced.

Comparison Treatments

Potential treatments for retinal degenerations might include gene therapy, replacement of gene product, cell transplantation, pharmacological substances, and visual prostheses. What are some of the options for comparison treatments? If no standard comparison treatment is available, then placebo, sham therapy, or perhaps no treatment could be used for comparison purposes. Issues of ethics and feasibility need to be considered in selecting control groups. If masking is possible, it minimizes observation bias.

Randomization

Randomization is one of the hallmarks of clinical trial design. A fundamental issue in ophthalmologic clinical trials is whether eyes or patients are randomized. The advantage of randomizing patients is that each patient represents an independent observation, meaning that treatment effects for each patient are unique, and there is no risk of crossover effects from one eye to another. The disadvantage is that randomizing patients requires a larger sample size.

The advantages of randomizing eyes are that smaller numbers of patients are needed and the patient serves as his or her own control. However, there are also disadvantages. For example, possible

crossover effects and losses to follow-up affect both the treated and the control groups. In addition, correlations between eyes can complicate the interpretation of the results.

Data Analysis Strategy

A data analysis strategy must be planned at the beginning of the study in consultation with a biostatistician. Will analyses be patient-based or eye-based, and will measurements be analyzed continuously or defined as reaching a particular threshold value? How will subgroup analyses be handled? Will they be considered as part of the initial design? The validity of subgroup analyses may be questioned if they are included ad hoc or once the study has been concluded. Subgroup analyses represent a complex issue that can be incorporated in the design and included in the randomization scheme.

Other Design Issues

Decisions concerning masking of the patients and investigators, particularly the people who are obtaining outcome measurements, must be carefully considered. Standardized measurements must be defined and protocols developed for obtaining them. Protocols must be developed for complete follow-up of patients, including close monitoring and complete documentation of adherence to protocol, adverse events, and safety-related issues. Close attention to these methodological factors will prevent imprecise measurements and bias from influencing the study results.

In summary, clinical trials of orphan retinal diseases are subject to methodological and design requirements similar to requirements for more common conditions, but the smaller number of available patients presents additional challenges, which may include selecting eligibility criteria, finding enough eligible patients, and defining study outcomes. Decisions concerning different study designs will have a direct impact on the required sample size power and the ability of a study to achieve its stated aims. The establishment of a retinal degeneration clinical trial network that includes a group of investigators who can collaborate to resolve fundamental design issues will facilitate the design and successful implementation of clinical trials for emerging treatments.

Genetic Typing in Clinical Trials

Challenges in Genetic Testing for Clinical Trials of Inherited and Orphan Retinal Diseases

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Genetic testing can offer many benefits when planning and conducting clinical trials for retinal degenerative diseases. Advancing technology is making the genotyping process less expensive, more quick, and more widely available.

At the same time, the complex nature of retinal degenerative diseases can make genetic testing a formidable process. Many genes cause retinal degenerative disease; many disease-causing genes are yet to be discovered; and some diseases are caused by multiple genes or even nonmutated genes.

Some benefits of genetic testing as they pertain to clinical trials are:

1. Treatment can be matched to a specific disease mechanism.
2. The gene-specific natural history of the disease can be identified.
3. Interindividual variability can be reduced and controlled.
4. Genotyping may allow presymptomatic treatment.

Genotyping Platforms and Costs

Numerous techniques are available for genotype screening of patients. They include allele-specific amplification or restriction digestion, low-density single-nucleotide polymorphism chips (10,000 or fewer spots per chip), DNA sequencing, medium-density single-nucleotide polymorphism chips (more than 100,000 units per chip), denaturing high-performance liquid chromatography, and single-strand confirmation polymorphism.

By dividing the unit cost of each of these techniques by the unit size, the costs (as dollars-per-base [dpb] analyzed) are calculated as follows. If an allele-specific test costs $3.00 to perform, it would cost $3.00 to analyze one dpb. If low-density single-nucleotide polymorphism chips cost $600 per 10,000 noncontiguous bases, the cost is $0.06 dpb. By this method, DNA sequencing costs $0.15 dpb; medium density single-nucleotide polymorphism chips cost $0.009 dpb; denaturing high-performance liquid chromatography costs $0.003 dpb, and single-strand confirmation polymorphism costs $0.0025 dpb. For a treatment trial for a disease such as malattia leventinese, which is thought to be caused by a single mutation (Arg345Trp in the *EFEMP1* gene), an allele-specific test based on restriction digestion could be performed at a cost of $3.00 per patient. Automated DNA sequencing would cost $12.00 per patient, and a solid-phase allele-detection method would cost as much as $900 per patient. Although the latter method can evaluate many more base pairs per dollar than DNA sequencing or restriction digestion, it provides no benefit in this clinical situation. The added genotypic information can only add irrelevant and potentially misleading information.

The Challenge of Heterogeneity

Several inherited eye disorders can be caused by a number of different genes. For example, the phenotype known as autosomal recessive retinitis pigmentosa (ARRP) is likely to be caused by more than 50 different genes. Such "locus heterogeneity" creates a serious challenge for a genetic test that evaluates many genes simultaneously. The problem is that the carrier frequency of many of these gene defects is so high in the general population that heterozygous sequence changes in affected individuals have little diagnostic significance.

Assume that ARRP is caused by 50 different genes, each causing 2% of the ARRP cases in the population of patients being testing. Because approximately 1 in 6,000 individuals is affected by ARRP, the disease in approximately 1 in 300,000 individuals is caused by a specific 1 of the 50 genes. The Hardy-Weinberg equation predicts that the carrier frequency of an autosomal recessive disease with this prevalence in the population will be 1 in 274 people. However, because there are 50 different genes with this frequency in the population, the cumulative carrier frequency for ARRP is 50 in 274, or almost 1 in 5 people. Thus, one in five normal people would be expected to harbor a true disease-causing mutation in one allele of an RP gene.

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Similarly, one in five RP patients would be expected to harbor a true disease-causing mutation in one allele of an RP gene in addition to the mutations that actually cause their disease. Thus, it must be assumed that any heterozygous changes that are observed during multilocus genetic testing of highly heterogeneous diseases represent one of the true disease-causing mutations in addition to this type of irrelevant discovery.

Consider the following observations that were made during a screening of a large cohort of patients affected with the genetically heterogeneous condition known as Leber congenital amaurosis. We screened 8 different genes for mutations in a cohort of 450 probands.

In one proband, we found six different variations distributed across four genes: *CRB1* (Phe488Ser and Leu753Pro); *RPGRIP1* (Arg598Gln and Gly124Glu); *RPE65* (Ala434Val); and *GUCYD2* (Trp21Arg). Additional information was needed to deduce which of these changes is responsible for the patient's disease. As it turns out, the *GUCYD2* variation is a nondisease-causing polymorphism. It is present in 2% of the entire population, which is too common to cause a disease with a prevalence of 1 in 50,000 people. The *RPE65* variation is also clearly a nondisease-causing polymorphism, because it is present in 11% of the black population. If one had used an entirely white control group, one might have erroneously concluded that this was a disease-causing variant. Thus, it is very important to screen a large control group with the same ethnic composition being tested to avoid this type of error.

Finally, the *RPGRIP* mutations were both found to lie on the maternal allele. This could only be detected by examining the parents of the proband. Having eliminated the *RPGRIP1*, *RPE65*, and *GUCYD* variants, and demonstrating that the two *CRB1* variants were each inherited from a different parent, it was deduced that the latter two variants are the most likely cause of the individual's disease.

To summarize, genetic testing for clinical trials offers many benefits, but the heterogeneous nature of retinal degenerative diseases can make the process challenging. No genetic testing platform is ideal in all respects. Generally speaking, the more focused a molecular hypothesis can be (on clinical grounds) before genetic testing is performed, and the fewer genes that need to be screened to evaluate the hypothesis, the less expensive the test will be, and the less the likelihood of being misled by a variation in a gene other than the one that is truly causing the patient's disease.

Importance of Genotyping in Clinical Trials of Inherited and Orphan Retinal Diseases

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Photoreceptor dysfunctions fall into diagnostic categories according to the type and extent of retinal cells affected. A general summary of the diagnostic categories and their fundamental features follows, ordered approximately according to their ultimate impact on overall vision:

- Partial color blindness. One or two cone types absent or anomalous.
- Stationary night blindness. Poor rod sensitivity, slow adaptation. Little to no rod degeneration.
- Macular degeneration. Degeneration of rods and cones of the macula.
- Cone degeneration. Cones degenerate; rods function well.
- Achromatopsia (rod monochromacy). No cone function, because of congenitally dysfunctional cones or the result of cone degeneration.
- Retinitis pigmentosa (rod–cone degeneration). Rods and cones degenerate.
- Cone–rod degeneration. Cones degenerate faster than rods.
- Congenital retinal blindness. Rods and cones fail before or soon after birth.

Each of these diseases is genetically heterogeneous, with many possible causative genes and numerous causative mutations within those genes. Regarding the question of whether genotyping will be required before therapeutic trials, the answer will depend on the type of therapy under consideration. For example, trials of a gene-specific therapy obviously require genotyping to know that a test patient has the specific gene targeted by the therapy.

If a planned therapy is designed to heal a specific cell type, genotyping may still be very important. It is conceivable that a drug could be developed that enhances the resistance of a specific cell type to any sort of physiologic damage. If such a drug fortified the cones, for example, it might be beneficial mainly to those forms of retinitis pigmentosa caused by rod-specific gene defects, in which cones die after the surrounding rods. The drug would help patients with many but not all forms of retinitis pigmentosa and, thus, genotyping would be necessary to know which patients to treat.

Another cell-type–specific therapy could conceivably involve the retinal pigment epithelium. A small set of disease genes specifically expressed by the retinal pigment epithelium cause a degeneration of photoreceptor cells, because of malfunction of the retinal pigment epithelium. Genotyping would be necessary to identify the patients for therapy to correct the specific retinal pigment epithelium gene defect.

We still do not understand many things regarding the mechanisms of the compounds we are investigating as possible therapies. Furthermore, for many inherited and orphan retinal disorders, only some of the disease-causing genes are identified. It is reasonable to recommend that all therapeutic trials should strive, as far as possible, to genetically characterize the patients in the early safety and efficacy trials. One could then review the results retrospectively to determine whether particular genetic forms of retinal degeneration were helped, and to what extent.

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Clinical Trials in Other Neurological Diseases

Failures and Successes of Clinical Trials for Parkinson Disease Treatments

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In 1817, James Parkinson described the disease that would bear his name as "involuntary tremulous motion with lessened muscular power in parts, not in action and even when supported, with propensity to bend the trunk forward and pass from a walking to a running pace, the senses and intellect being uninjured."

Parkinson disease (PD) is a relatively common neurodegenerative disorder affecting approximately 1% of the population older than the age of 65 years and approximately 4% to 5% of the population older than the age of 85. It is caused by a selective degeneration of dopamine neurons in the substantia nigra. Lessons learned in the field of PD may very well apply to inherited and orphan retinal disease. The opposite will hopefully also be true, that the PD field will gain from the inherited and orphan retinal disease field.

Development of Levodopa, the First Therapy for Humans

One of the major achievements of 20th century neurology was the development of levodopa therapy for PD.[1] This was the first disease in which a specific neurochemical defect was identified in the brain, allowing development of a rational chemical therapy and ushering in the era of clinical neurochemistry.

Dopamine deficiency in PD was described in 1960 by Ehringer and Hornykiewicz, a key event that led to the era of levodopa therapy.[2] In 1961, levodopa was administered in patients with PD,[3] but, throughout most of the 1960s, results were inconsistent. One lesson to be learned from the levodopa experience is that therapies need to be based on mechanistic insight and that persistence is critical. In the 1960s, levodopa therapy for PD was ready to be abandoned. Then, Cotzias and colleagues at the National Institutes of Health did a clinical study in patients that showed that high doses of levodopa could actually produce dramatic improvement in PD. This landmark paper was published in the *New England Journal of Medicine* in 1967.[4]

Before levodopa treatment was established, patients died from PD. Now, levodopa-treated patients live longer, as indicated by normalized Kaplan-Meier survival curves, suggesting that levodopa prolongs life, allowing some patients to live a normal life span.

The side effects of levodopa include dyskinesias and motor fluctuations. In the early 1970s, the advantages of adding a dopa decarboxylase inhibitor to the treatment were discovered, preventing levodopa from being metabolized to dopamine in the periphery and allowing it to enter the brain, thus reducing side effects and gaining better symptom control. The first combination of carbidopa plus levodopa, or Sinemet, became available in 1975. Since then, other methods have been developed to overcome the complications of motor fluctuations and dyskinesias, including continuous levodopa infusion, long-acting levodopa combinations, and, more recently, dopamine agonist (pergolide, bromocriptine, ropinirole, or pramipexole) monotherapy in combination with levodopa. Anticholinergics, such as trihexyphenidyl, have also been developed. Monoamine oxidase B inhibitors, such as selegiline and rasagaline, are also in use, as are catechol-O-methyltransferase inhibitors, such as tolcapone and entacapone, to inhibit the breakdown of dopamine.

Need for Longer-term Therapies

All of the treatments mentioned here are symptomatic therapies, and they are very effective during the initial stages of PD. The typical patient is usually diagnosed within the first year after the onset of symptoms. Symptoms may include minor stiffness, foot-cramping, and tremor. Initially, the patient will do relatively well on Sinemet or levodopa or a dopamine agonist. After three to eight years, complications be-

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Under a licensing agreement between Guilford Pharmaceuticals and the Johns Hopkins University, Dr. Dawson is entitled to a share of the royalty received by the University from Guilford Pharmaceuticals. The terms of this arrangement are being managed by the Johns Hopkins University in accordance with its conflict-of-interest policies.

gin to develop, with symptoms that are eventually not manageable by the medication. Ultimately, cognitive decline ensues.

Neuroprotective or neurorestorative therapies need to be developed to prevent the onset of resistant symptoms and, ultimately, cognitive decline. The course of PD over time includes a loss of dopamine neurons, then the development of symptoms. As the disease progresses, additional symptoms develop, including motor complications, cognitive decline, and, ultimately, death. Initial treatment of PD may be nonpharmacologic—education, support, nutrition, and exercise. Basic science data in animal models of PD suggest that exercise delays the degeneration of dopamine neurons.[5] When these measures no longer control symptoms, pharmacologic therapy is instituted. Ideally, neuroprotective measures would begin at this point. Unfortunately, there are no proven neuroprotective or neurorestorative agents for PD.

At the onset of functional impairment, treatment might consist of amantadine, levodopa, or dopamine agonists. As the disease progresses, treatment may include a combination of levodopa, dopamine agonists, and catechol-O-methyltransferase inhibitors. Ultimately, some of the surgeries, such as deep-brain stimulation or pallidotomy, might be considered.

Future Therapies for Parkinson Disease

How will we treat PD in the 21st century? I think that therapy will be based on neuroprotective and neurorestorative strategies. To develop neuroprotective and neurorestorative therapies for PD, we need to understand the pathogenesis.

Current theories on the pathogenesis of PD focus on genetics. At least 11 genes have been linked to PD. This knowledge has created a renaissance in PD research, allowing the identification of new targets and new animal models. Substantial data also point to the environment and endogenous toxins as factors in PD. Approximately 5% to 10% of cases of PD are attributed to genetics. Conversely, 90% of PD cases are sporadic, with no known genetic component. Currently, the underlying causes of PD seem to feed into a common pathogenic pathway involving oxidative stress, mitochondrial dysfunction, inflammatory processes, apoptosis and cell death, and—a recent area of tremendous interest—protein aggregation, ultimately leading to the symptoms of PD.

As we identify the factors in the pathogenesis of PD, we can begin to identify drugs that might interfere with these pathways. Ultimately, the processes at work in PD lead to neuronal dysfunction and death, then inflammation, and then the dopaminergic deficit and electrophysiologic imbalance of the basal ganglia nuclei, leading to the symptoms of PD.

Trials are now ongoing with compounds that potentiate mitochondrial function: including coenzyme Q10, creatine, and monoamine oxidase B inhibitors. Compounds acting on dysfunction of the ubiquitin proteosome system are still mainly at the basic science level and include activators of UPS, chaperone inducers, overexpression of chaperones, β-sheet breakers, and α-synuclein "busters." Compounds that block cell death include caspase inhibitors, mixed-lineage kinase inhibitors, and poly(adenosine diphosphate-ribose) polymerase inhibitors. Blockers of inflammation include cyclooxygenase inhibitors, minocycline, and peroxisome proliferator–activated receptor-γ inhibitors. Neurorestorative therapies under investigation include glial cell line–derived neurotrophic factor (GDNF), neuroimmunophilins, gangliosides, stem cells, and transcriptional activators. Symptomatic therapies include dopamimetics and deep-brain stimulation.

Clinical Trials for Neuroprotective Therapies

One of the first neuroprotective treatment trials for PD was the Deprenyl and Tocopherol Antioxidative Therapy of Parkinsonism study.[6] Even today, 12 years later, debate continues regarding whether deprenyl is neuroprotective or whether it only provides symptomatic relief. Thus, it was not possible to determine whether the delay in the need for levodopa was because the drug slowed neuronal degeneration or because symptomatic effects masked the ongoing disease progression.

The other published trial of drug therapy and PD used coenzyme Q10.[7] This was a double-masked placebo-controlled pilot study that demonstrated that high doses (1200 mg/d) of coenzyme Q10 were associated with a trend toward a reduced rate of deterioration of motor function from baseline during the 16-month course of the trial. The trend, on post hoc analysis, was statistically significant, but the original endpoint was not statistically significant. In addition, the activity of daily living scores improved significantly, raising the possibility of an unanticipated symptomatic effect, again confounding the interpretation of the study.

In another study, GDNF was directly infused into the putamen of five patients in a Phase I safety trial.[8] Glial cell line–derived neurotrophic factor is a potent neurotrophic factor with restorative effects in a wide variety of rodent models of PD. After one year, there were no serious clinical side effects. There was a 39% improvement in the off-medication motor subscore of the Unified Parkinson Disease Rating Scale and a 61% improvement in the activities of daily living subscore.

Medication-induced dyskinesias were reduced, and positron emission tomography scans of [(18)F]dopamine uptake showed a significant 28% increase in putamen dopamine storage after 18 months, suggesting a direct effect of GDNF on dopamine function.

Current Status of Parkinson Disease Treatment Trials

Based on the results of the coenzyme Q10 study described here, several studies are ongoing using higher doses of coenzyme Q10 and a larger patient population, coupled with imaging to determine whether coenzyme Q10 is a neuroprotective agent. Creatine is also being currently investigated in the National Institute of Neurologic Disease and Stroke–sponsored trial; as is minocycline, which is thought to function by inhibiting caspase activation and also inflammation. There are also ongoing trials with glycosyl–phosphatidylinositol (GPI)–1485, a neuroimmunophilin, which is thought to be a neurorestorative compound. An ongoing trial is being conducted by the Parkinson Study Group with CEP1345, an mixed-lineage kinase inhibitor that is thought to ultimately block apoptosis; this is called the Precept Trial. The endpoints, again, are the Unified Parkinson Disease Rating Scale score improvement and function, along with imaging. The GDNF trial recently completed a double-masked placebo trial. Preliminary data presented at the American Neurologic Association meeting indicated that GDNF did not provide any benefit compared with placebo.

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Clinical Trials of Neuroprotective Agents in Glaucoma

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Glaucoma is a continuum characterized by an accelerated rate of apoptosis and death of retinal ganglion cells. Early in the course of this optic neuropathy, there is loss of optic nerve fibers (the axons of the retinal ganglion cells). Even by the time glaucoma progresses to the stage at which there are observable changes in the retinal nerve fiber layer, optic disk, or visual function, the patient is still usually asymptomatic. Only late in the course of the disease does the patient become symptomatic, and, eventually, blind, if not adequately treated.

The mechanism of optic nerve damage in glaucoma is unknown. Several mechanisms likely contribute, alone or collectively. In the presence of high intraocular pressure, for example, peptides and other chemical signals and electrical impulses are blocked at the level of the lamina cribrosa, a putative site of optic nerve damage in glaucoma. It has also been postulated that optic nerve damage in some glaucoma patients may be related to changes in retinal or choroidal blood flow and ischemia, excessive glutamate stimulation, or inflammatory cytokines.

At present, high intraocular pressure is the only factor in most of our patients that we know contributes to glaucoma. It is the only risk factor that we can currently treat. In contrast, neuroprotection offers the opportunity to prevent optic nerve fiber loss and retinal ganglion cell loss independent of intraocular pressure. Currently, only one multicentered and appropriately powered clinical trial, of the noncompetitive *N*-methyl-D-aspartate antagonist, memantine, assesses neuroprotection in glaucoma.

Memantine blocks the persistent activation of receptors by the excitatory amino acid glutamate. It has a neuroprotective effect in animal models of optic nerve injury[1] and glaucoma.[2] Memantine is being evaluated in two parallel Phase III trials. Each study has enrolled more than 1,000 patients. Of interest, memantine already is Food and Drug Administration–approved for use in the United States for moderate-to-severe Alzheimer disease.

Standardization of efficacy endpoints is essential. Furthermore, the Food and Drug Administration requires efficacy endpoints in its drug approval process:

1. Visual function testing. Regulatory agencies have equated glaucoma progression with standard achromatic visual field loss. With standard automated perimetry, also known as white-on-white perimetry, a white target is projected on a white background, and the intensity of the white target is adjusted until it is detected. Other psychophysical testing, such as retinal ganglion cell selective functional testing with frequency doubling technology perimetry and short wavelength automated perimetry, have potential for evaluating progressive glaucomatous optic nerve damage in clinical trials of neuroprotection. In some patients, these tests can detect a functional abnormality several years before standard automated perimetry.
2. Optic disk and retinal nerve fiber layer assessment. Optic disk assessment, photography, and digital imaging are easily performed. Digital imaging, with confocal scanning laser ophthalmoscopy, can be used to examine the optic disk and the retinal nerve fiber layer. Diagnostic accuracy with the confocal scanning laser ophthalmoscope is comparable to clinical examination of the optic disk by experts, and useful for predicting future visual field loss.[3] However, only a limited normative database is currently available, and change algorithms need validation.

The retinal nerve fiber layer can also be examined with scanning laser polarimetry, which estimates the retinal nerve fiber layer thickness by measuring ocular birefringence. Scanning laser polarimetry can help predict which patients will develop glaucomatous visual field loss.[4] This technology is limited by the relatively high frequency of atypical scans in highly myopic (>5D) and elderly (>70 years old) patients, as well as with those with age-related macular degeneration. At this time, there is no validated algorithm for detecting progression.

Finally, optical coherence tomography can be used to measure retinal nerve fiber layer thickness, by obtaining direct cross-sectional images of the retina. It has a number of limitations for use in glaucoma, including a lack of reproducibility in its current platform, and the absence of validated algorithms for detecting progression.

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Alzheimer Disease: Therapeutic Targets for Clinical Trials

PHILIP C. WONG, PHD

Alzheimer disease (AD) affects more than 4 million elderly people in the United States and is the most common cause of memory loss and dementia. Because of population growth and increased longevity, the number of patients will triple during the next several decades. The prevalence, costs, lack of mechanism-based therapies, and impact on patients and their caregivers of AD make it one of the most challenging of all medical conditions.

Current AD treatments consist of cholinesterase inhibitors, *N*-methyl-D-aspartate antagonists, neurotrophic factors, antioxidants, antiinflammatory agents, hormone replacement therapy, and management of behavioral problems. Most treatments are for symptoms, and only marginally helpful. Therefore, a large unmet need exists for new mechanism-based or disease-modifying therapies.

Mechanism-Based Therapies in Animal Models

Efforts to find mechanism-based therapeutic targets in animal models have been successful, providing hope that pharmaceutical companies will become involved in the development of therapeutic agents (e.g., γ- or β-secretase inhibitors). Human trials for therapeutic agents have included a trial of the AN 1792 Alzheimer vaccine. Although Phase I was not associated with any toxicity, Phase II trials were suspended because of severe adverse reactions (meningoencephalitis) in a subset of patients.[1,2] Passive immunization approaches are being pursued, with the goal of making a vaccine with antigens that do not stimulate T-cell–mediated immunologic attacks.

Therapeutic Targets

During the last decade, genes linked to AD have been identified, allowing investigators to develop model systems to study disease mechanisms and to design rational therapeutic strategies for treatments. Recent findings strongly support the view that one central underlying mechanism of AD is in the abnormal processing of the amyloid precursor protein (APP), leading to the accumulation and aggregation of β-amyloid peptides. The recent discovery of secretases, enzymes that process APP to generate β-amyloid peptides, opens opportunities to evaluate their potential for development of drugs that will inhibit this pathogenic pathway.

The main pathologic hallmarks of AD are extracellular deposition of amyloid plaques in neurons within specific circuits of the brain and intracellular accumulation of neurofibrillary tangles. The main constituents of amyloid plaques are the β-amyloid peptides.

The processing of APP into β-amyloid peptides requires two enzymatic activities: β-APP–cleaving enzyme (BACE1) and the γ-secretase complex. These enzymes, therefore, represent excellent new therapeutic targets for the development of novel protease inhibitors for the treatment of AD. We now know that the β-secretase BACE1 is required to cleave APP to generate the APP carboxy terminal fragment, the substrate for the second enzyme, termed γ-secretase complex, which is required for releasing β-amyloid peptides.

We have conducted a series of proof-of-concept experiments with β-secretase to demonstrate and validate that it is, in fact, a target for development of therapeutic strategies to ameliorate β-amyloid peptide accumulation in AD. β-Secretase is a very unusual enzyme in that it is a type-I transmembrane protein with aspartyl protease activity that is directly involved in the proteolysis of APP, which is also a type-I transmembrane protein. The crystal structure of the enzyme, resolved several years ago, facilitates the design of small compounds to inhibit it.

The other therapeutic target that has been focused on is the γ-secretase complex. Four transmembrane proteins are involved in the γ-secretase complex: presenilins, nicastrin, APH, and PEN-2. Presenilins 1 and 2 are directly involved in AD; mutations are responsible for a large number of early onset cases of AD. More recently, another type-I transmembrane protein, nicastrin, was revealed, and two other multipass transmembrane proteins, APH-1 and PEN-2, were identified through genetic screens. Presenilin is thought to be the catalytic domain of this enzyme. The other three components are cofactors that facilitate the action of the γ-secretase complex. γ-Secretase is an unusual enzyme, in that the cleavage of the APP carboxy terminal fragment occurs within the transmembrane domain to release the β-amyloid peptide.

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β-Amyloid Precursor Protein–Cleaving Enzyme-1 Inhibition

Realizing that BACE1 is abundant in the brain and localized to the hippocampus led us to hypothesize that the profusion of BACE1 predisposes the brain to β-amyloidosis. This BACE1 enzyme is particularly rich in neurons as compared with nonneuronal cells. To demonstrate that it is the major β-secretase in the brain, we and others knocked out the *Bace1* gene in mice and showed that when the gene is deleted, no β-amyloid peptides are detected in neurons. These results validate the idea that BACE1 is the major β-secretase in neurons in the brain.

To further demonstrate that removing BACE1 is beneficial and therapeutic, at least in mouse models, we and others have used the transgenic AD mouse model developed in 1996.[3] Based on AD-linked APP and presenilin 1 mutations, investigators generated transgenic mouse models and produced a mouse that develops age-associated β-amyloid deposits in the brain, as well as learning and memory deficits, as measured by the standard Morris water maze to assess spatial reference memory. These are ideal models to assess mechanisms and therapeutic strategies. Using this mouse model, we reasoned that if, indeed, BACE1 is the key enzyme required for amyloid production, then removing or inhibiting this enzyme will reduce β-amyloid peptides and prevent β-amyloidosis and memory deficits.

To test the hypothesis, we crossbred mice that carry the two AD-linked mutant transgenes with mice lacking *Bace1*. We observed that if the BACE1 enzyme is deleted in these transgenic animal models, β-amyloidosis and memory deficits do not occur in the brain of AD mouse models lacking *BACE1*. The results validate BACE1 as an excellent therapeutic target for development of drugs to inhibit formation of β-amyloid. Currently, both pharmaceutical companies and academic institutions are eager to develop BACE1 inhibitors for the treatment of AD.

Inhibition of γ-Secretase

The γ-secretase complex is more complicated than BACE1 as a target, because it is required to process many key signaling pathways other than APP. Complete inhibition of γ-secretase could be detrimental and lead to adverse side effects. We need to assess these targets to determine whether their activity can be partially inhibited and still produce advantageous outcomes in ameliorating β-amyloidosis. Perhaps a combination approach can be applied (e.g., use of both β-secretase and γ-secretase inhibitors that might partially inhibit one or more components of the γ-secretase complex). To test this approach in the future, we will use knockout approaches to delete one allele of *BACE1* and one allele of components of the γ-secretase complex and ask whether a synergistic effect on reduction of β-amyloidosis can be achieved.

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Partnerships in Pre-Clinical & Clinical Trials

Governmental, University, Pharmaceutical, and Foundation Partnerships to Advance Translational Research in Retinal Disease

STEPHEN J. RYAN, MD

Participants at this Symposium share a common goal, to move research expeditiously through clinical trials to establish the best treatments for patients. To accomplish the goal requires unique partnerships of public and private groups. The National Neurovision Research Institute, Inc. and The Foundation Fighting Blindness perform an essential service by encouraging collaborative research and by functioning as effective public advocates for research into retinal disease. Government, the pharmaceutical industry, and academic institutions also play critical roles. The Food and Drug Administration has the ultimate responsibility for the safety and efficacy of drugs and devices.

On the government level, we look to the National Institutes of Health for national leadership in biomedical research. We particularly applaud the current emphasis on translational research—in addition to basic research—and the sponsorship of workshops and planning events. The National Institutes of Health provides a tremendous service in coordinating varied research and strategic efforts.

As Paul Sieving pointed out in another presentation, the National Eye Institute, similar to the National Institutes of Health, has limited resources, and we cannot look to the government to fund everything. It is important that government agencies work together as partners.

Industry's role is to produce the products to help the patients. It has expertise in research, product manufacture, and performance of clinical trials. An important consideration of industry is whether the number of consumers using a product is sufficient to provide the necessary returns.

Academic institutions provide great strengths in basic research and the conduct of clinical trials, because many of the patients with rare diseases are referred to academic centers.

Research and development of new products involve genomic databases, animal models, support of clinical trials, and various grant mechanisms. At this Symposium, five different types of groups have come together as potential partners in research and development. They are private agencies, the National Institutes of Health, the Food and Drug Administration, industry, and academic research institutions. We and our patients will all be so much greater off for working together.

President of the Doheny Eye Institute and the Grace and Emery Beardsley Professor of Ophthalmology at the Keck School of Medicine of the University of Southern California, Los Angeles, CA.

Fostering Partnerships: A Perspective From the National Institute of Neurologic Disorders and Stroke (NINDS)

KATRINA GWINN-HARDY, MD

The key to successful research partnerships for lessening the burden of neurologic diseases is identifying what the parties have in common. Within the National Institutes of Health (NIH), we (the National Institute of Neurologic Disorders and Stroke [NINDS]) work with organizations that fund and study neuroscience (e.g., the National Institutes of Aging; Mental Health; Child Health and Development; Heart Lung and Blood; the National Eye Institute, and the Office of Rare Diseases). Outside of the NIH, we work with other government agencies, industry, and foundations, with which we have overlapping goals. Research and development are goals we have in common with commercial industry. With foundations and voluntary organizations, we share interests in education, public outreach, and with facilitating and funding research.

The NINDS also provides support for new investigators, who may not be ready to submit NIH grant applications, and for more senior investigators entering the field of neurologic disease from other disciplines.

The NINDS offers many types of research project grants through approximately 98 funding mechanisms. One, the hypothesis-driven and investigator-initiated RO1 grant, represents the traditional research grant. Other types of funding available include R21 grants, for high-risk/high-benefit translational research; R43, R44, R41, and R42 grants, for small businesses; U01 grants, for cooperative agreements; PO-1 program project grants, for multiple projects with a unifying theme; and P50 center grants, often congressionally mandated.

Special initiatives include the following: 1. Request for application. This is usually a one-time solicitation on a particular topic. 2. Program announcement. This is an expression of an Institute's ongoing interest in funding a particular area of research. 3. High program priority. This sometimes provides funding of certain grant applications that do not fall under a specific initiative, but are important to a mission of the Institute. 4. Contract. This is for projects of value to the NIH and/or other government groups.

A unique translational research program at NINDS is the Cooperative Program in Translational Research. This is a program of cooperative agreements that support milestone-driven projects focused on the identification and preclinical testing of new therapeutics.

Investigators are encouraged to monitor the NIH Guide issued every Friday. It describes all of the new requests for applications published during the week and is an excellent resource for staying informed regarding trends and priorities throughout the NIH.

Program Director, NINDS, Bethesda, MD.

Fostering Partnerships: The National Institutes of Health (NIH) Mission

PAUL A. SIEVING, MD, PHD

In keeping with its mission, the National Institutes of Health (NIH) uses public money to improve the health of the nation through medical research. Much of the NIH research portfolio is targeted at basic biologic research in health and disease. The NIH also focuses on translational research and on the clinical trials that emerge. The NIH mission has obvious affinity with the pharmaceutical and biotechnology sector as well as nonprofit organizations dedicated to fighting disease.

Partnerships with the NIH take many forms, including Cooperative Research and Development Agreements, Small Business Innovation Research (SBIR) awards, and Small Business Technology Transfer Research (STTR) awards. Cooperative Research and Development Agreements enable NIH scientists to work with the private sector to jointly develop new technologies or therapies. Cooperative Research and Development Agreements also enable industrial partners to contribute funding and to seek intellectual property rights or exclusive licensing to commercialize projects.

The SBIRs and STTRs were created as set-aside programs for 2.5% of an agency's extramural budget for domestic small business concerns, to assist with research and development of novel technologies or therapeutics that have commercial potential. The SBIRs require that the principal investigator have his/her primary employment with the small business concern at the time of award and for the duration of the project period. Primary employment is not stipulated under an STTR agreement. However, the small business concern must have a collaborative research partner at a university or other nonprofit institution. At least 40% of the STTR research project is to be conducted by the small business concern, and at least 30% of the work is to be conducted by the single, partnering research institution.

The SBIR and STTR programs have three phases. The objective of Phase I is to establish the technical merit and feasibility of the proposed project and to determine the quality of performance of the small business award recipient before providing further federal support in Phase II. Support under Phase I is normally provided for 6 months ($100,000) for SBIRs and for 1 year ($100,000) for STTRs. The objective of Phase II is to continue the research efforts initiated in Phase I. Only Phase I awardees are eligible for a Phase II award. The SBIR and STTR Phase II awards normally may not exceed $750,000 total. However, applicants may propose longer periods of time and greater amounts of funds if necessary to complete the project. The objective of Phase III, when appropriate, is for the small business concern to pursue, with non-SBIR/STTR funds, the commercialization objectives resulting from the Phase I/II research/research-and-development activities. In some federal agencies, Phase III may involve follow non-SBIR/STTR funded research and development or production contracts for products, processes, or services intended for use by the US government. Cooperative Research and Development Agreements and SBIRs are the most obvious and codified partnership opportunities available through the NIH.

Beyond direct contractual relationships and grants, there are more nuanced ways in which the NIH can join forces with public and private organizations. For example, the National Eye Institute has spent much of the last decade cloning the genes of the visual system and, along the way, many nonprofit medical research foundations have augmented its efforts. The cloning of these genes has now given rise to the development of animal models. Here too, many nonprofit organizations have helped in the mission to create and maintain such models. These partnerships have been informal. Often a foundation has supported an RO1 grantee with equipment, postdoctoral salary support, or coverage of travel expenses. The acknowledgments sections of most published papers usually credit, in addition to the National Eye Institute, a nonprofit foundation that has also supported the work. This kind of funding partnership gives a very welcome lift to vision research.

Another informal but no less important partnership that emanates from the NIH research mission can be found in the *N*-ethyl-*N*-nitrosourea mouse mutagenesis project, for which approximately $50 million has been spent. This project systematically seeks to create mutations in the 30,000 genes that comprise the mouse genome. After mutagenesis, the rodents are screened for defects in vision and hearing, as well as for other diseases. From this project, a number of models have been developed. The

Director of the National Eye Institute, Bethesda, MD.

models are then cataloged into public databases that become a publicly available resource for both private and public sector scientists.

The National Eye Institute is interested in partnerships that will augment its existing research efforts. Such partnerships are vital to accelerating the common goal of developing sight–saving therapies for retinal degenerative diseases.

Fostering Research Partnerships: A Perspective From the Office of Rare Diseases (ORD)

STEPHEN C. GROFT, PharmD

The Office of Rare Diseases (ORD) was established in 1993 within the Office of the Director of the National Institutes of Health (NIH). The annual budget of the ORD is now approximately $15.5 million, which is spent on research of more than 6,000 rare disorders. This is a small amount to address a large number of diseases. However, it represents a large increase from 2 years ago, when the budget was approximately $2 million, and this budget increase allows the ORD to participate in many new areas and to begin to cofund selected research activities.

We work with the research community and patient advocacy groups to establish their presence at NIH. We join as many NIH-sponsored research initiatives as possible.

One area of interest within the Intramural Research Program is the Bench-to-Bedside grant program; we fund approximately 10 of these programs a year. Another major component of the ORD is the sponsorship of scientific conferences. Last year, the ORD sponsored 86 conferences in rare diseases, including our participation in this Symposium. The ORD has sponsored more than 550 scientific conferences since 1995. Some goals of our conference programs include the following:

1. Establishing research priorities.
2. Stimulating research interest leading to RO1 applications.
3. Developing program announcements.
4. Establishing diagnostic and monitoring criteria.
5. Developing animal models.
6. Supporting patient and tissue registries.
7. Developing research protocols, collaborative arrangements, and clinical trial plans.
8. Disseminating results to targeted professional and voluntary health organizations.

Progress in research and product development requires the collaboration of many different partners. The ORD coordinates efforts among industry (large and small), the academic research community, professional societies, patient advocacy groups, and the federal government, whose agencies are responsible for reimbursement, extramural and intramural research programs, and regulatory activities. Among the groups, we try to enhance understanding of what is needed regarding research and development. The ORD offers and subsidizes a training program through weekend seminars for the leaders of patient advocacy groups to educate them regarding the NIH and the Food and Drug Administration activities in rare diseases research and orphan products development.

Two years ago, we were given a legislative mandate to establish research centers of excellence in rare diseases — the Rare Disease Clinical Research Network. The goals of the Rare Disease Clinical Research Network are to provide for the systematic collection of clinical information to develop biomarkers and assessment measures, as well as new approaches to the diagnosis, treatment, and prevention of rare diseases. Another important goal is to promote training of new clinical investigators in rare diseases. Activities of the Rare Disease Clinical Research Network include longitudinal studies of individuals with rare diseases, clinical studies, Phase I and II studies, and/or pilot and demonstration projects. The Rare Disease Clinical Research Network provides a test bed for distributed clinical data management that incorporates novel approaches and technologies for data management, data mining, and data sharing across rare diseases, data types, and platforms. Inquiries regarding the programs and centers described here are welcomed and encouraged.

Director of the Office of Rare Diseases at the NIH, Bethesda, MD.

Fostering Partnerships: An Industry Perspective

GERALD D. CAGLE, PHD

Why should academia, for-profit and not-for-profit organizations, government researchers, inventors, and industry collaborate? The answer is that by collaborating, we bring expertise from various sectors. By doing so, we maximize efficiency and synergy in the overall process of discovery and development of new products that will provide meaningful benefits to doctors and patients.

Collaborations allow win/win situations—if they are done correctly. Collaborations, like business developments and joint ventures, need a formal agreement. Agreements should protect the interests of all parties and should be reviewed by legal staff from all sides. Principled negotiations, in which there is a balance of give and take from all concerned, are always best.

When academia, for-profit and not-for-profit organizations, government researchers, or inventors establish collaborative relationships with industry, certain matters must be addressed immediately. One is a confidentiality agreement to protect intellectual property and confidential information. The confidentiality agreement is a necessary antecedent to any discussions that will take place. Collaboration costs, too, should be agreed on. Inventors, academicians, and representatives of any for-profit or not-for-profit organization will incur costs, and a formula should be established by which these costs are returned wholly or in part.

Intellectual property rights need to be protected, and risks (liabilities) need to be identified. Everybody starts a relationship with the absolute surety that a product will result. However, this is not always the case. What happens to the data, for example, if the venture does not result in a product? If I were an inventor or a not-for-profit organization, I would want the data to be returned. These are the kinds of issues that need to be addressed at the very beginning of the collaboration agreement process. Good faith is certainly an essential component by all parties, but the possibility that the desired outcome might not be achieved should be considered.

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Agreements should also be in place to assure that regulatory, ethical, and financial approvals are obtained, as required, by the home institution. Timelines and contract milestones should be clearly identified.

Listed below are six steps of discovery and development, followed by the collaborative parties likely to be involved in each step:

1. Find inventor (Iv), government (G), academic institution (A), and industry (I).
2. Correlate (Iv, G, A, and I).
3. Animal models (Iv, G, A, and I).
4. Preclinical efficacy (G, A, and I).
5. Identify patients with disease (G, A, and I).
6. Clinical trials (G and I).

Research should be done in a number of quarters, and development should be a much more structured and goal-oriented process. To find or discover, correlate, and develop animal models is the purview of all of us. We can work together or work independently to develop these parts of the overall process.

The key to good relationships with industry and among the various groups is to establish a good working relationship with all of the people and organizations involved in the collaboration. I offer the following caveats to inventors and others who may become involved in collaboration with industry:

1. Establishing a collaboration is not a single-step process. It is a process that takes time. You need to get to know people in the companies that serve your field of interest. Ophthalmology is very fortunate in having a number of good companies with whom to work.
2. Know who is going to do what.
3. Commit to communicating back and forth, even to "overcommunication."
4. As time develops and a relationship matures, trust is established. At Alcon, we have worked with some scientists, investigators, and inventors for more than three decades. These are clearly win/win situations, which are the kinds of collaborations we seek.

REGULATORY ISSUES INVOLVED IN CLINICAL TRIALS

Federal Regulatory Issues: Investigational New Drugs in Ophthalmology

WILLIAM B. BOYD, MD

Drug manufacturers and researchers who are investigating new drug products must be aware of federal regulatory issues involving investigational new drug (IND) applications, the new drug application process, and more. In charge of assuring the safety and efficacy of drugs available to the American people is a unit within the US Food and Drug Administration called the Center for Drug Evaluation and Research. Within the Center for Drug Evaluation and Research is the Division of Anti-inflammatory, Analgesic, and Ophthalmologic Drug Products, which reviews numerous applications for investigational new drugs.

Investigational New Drug Application

An IND application is required whenever new drug studies in humans take place in the United States. This includes studies involving off-label indications, unapproved drugs, changes in formulation of an approved drug, or changes in the mode of administration of an approved drug. The IND status provides an exemption that allows a drug to be transported across state lines for purposes of investigation. Either the drug manufacturer or the investigator can sponsor IND applications. Primary efficacy variables are critical for the identification of the effectiveness of the drug product; differences should be demonstrated that are statistically significant and have clinical relevance. It is recommended that clinical trials include multiple concentrations and/or multiple dosing regimens. At least one of the clinical studies should include treatment of patients with the proposed final market formulation.

New Drug Application

A new drug application is the vehicle through which a sponsor formally proposes that a new pharmaceutical be marketed for use in the United States. It includes the data gathered during the preclinical studies and human clinical trials conducted under the IND. The basis for approval is that the drug benefits outweigh the risks in adequate and well-controlled trials.

The new drug application includes the following reviews: medical, biopharmaceutical, statistical, microbiology, chemistry, and pharmacology/toxicology. When the drug approaches approval, a review of the proposed labeling takes place. Each section is crafted with carefully considered information comprising indications and usage, contraindications, warnings, precautions, and so on.

Review Outcomes

The review process results in one of three outcomes: a not approvable letter, an approvable letter, or an approval letter. Common reasons for nonapproval of a drug product are that the application was never submitted; no clinical benefit was demonstrated for the proposed indication (or the benefit does not outweigh the risk); the quality of the product cannot be assured; or the product is not stable during its shelf life. Twenty-eight ophthalmic drug products received approval between 1998 and 2004.

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The Food and Drug Administration's Office of Orphan Products Development: Incentives, Grants, and Special Designations Speed Therapies for Orphan Diseases

MARLENE E. HAFFNER, MD, MPH

The Food and Drug Administration (FDA) is dedicated to promoting the development of products to diagnose and treat orphan diseases through its Office of Orphan Products Development (OOPD). The office was created in 1982 and administers the major provisions of the 1983 US Orphan Drug Act (ODA). It is responsible for approving drug and biologic therapies for patients with orphan diseases.

In the more than 20 years since the ODA was introduced, more than 200 drugs and biologic products have come to market for orphan diseases, compared with only 10 drugs previously. The act established incentives for pharmaceutical companies that allowed them to risk investment in research into treatments for rare diseases. Previously, pharmaceutical firms were reluctant to invest resources into developing such therapies, because of the unlikely financial return on their investment.

Orphan products are drug and biologic therapies that receive orphan designation from the FDA's OOPD. The sponsor of a product designated by the OOPD as an orphan qualifies for certain incentives established by the ODA:

- Tax credits on clinical trial expenses incurred during the investigation of the drug.
- Grant funding by the FDA through the OOPD.
- Seven years of marketing exclusivity for an orphan-designated drug or biologic product receiving FDA market approval.
- The ODA incentives have stimulated considerable interest in the development of products for treating rare diseases.

The OOPD is located within the FDA's Office of the Commissioner (Dr. Lester M. Crawford). A team of OOPD medical reviewers receives and evaluates requests from potential sponsors. The review involves verifying the scientific rationale of the proposal, confirming the rare disease prevalence, and then, when appropriate, designating drugs and biologic products that qualify as orphan products. The OOPD operates separately from the FDA drug and biologics review divisions; it acts as an ombudsman, assisting sponsors.

Orphan Products Grant Program

The OOPD also administers the Orphan Products Grant Program, providing funds to support clinical studies for the development of orphan products. The goal of the orphan grant program is to encourage clinical development of products for use in rare diseases or conditions. The products studied can be drugs, biologics, medical devices, or medical foods.

The Orphan Products Grant Program funds $250,000 to $300,000 in total costs annually, primarily to academic researchers conducting Phase I and Phase II trials. In fiscal year 2005, the program will award $14.4 million to researchers helping to move rare disease therapies from the research bench to the patient bedside. Investigations funded by the Orphan Products Grant Program have contributed to the FDA approval of 39 products to treat rare diseases.

The Food and Drug Administration's Humanitarian Device Exemption

In 1996, the FDA announced a program to make it easier and less costly for manufacturers to bring medical devices for orphan diseases to market. The provisions of the Humanitarian Device Exemption of the Safe Medical Devices Act of 1990 allow a medical device to be approved if manufacturers show that it is safe and has probable benefit to patients with an extremely rare condition. These regulations preclude approval requirements based on costly clinical studies to establish effectiveness. To qualify for Humanitarian Device Exemption approval, the device must be intended for use in the treatment or diagnosis of a disease or condition affecting fewer than 4,000 individuals in the United States each year. Beginning in 1996, the OOPD was given responsibility to grant Humanitarian Use Device designation for sponsors developing such medical devices.

Director of the FDA Office of Orphan Products Development and a Rear Admiral in the US Public Health Service.

Once Humanitarian Use Device designation is received, the device's sponsor is eligible to receive Humanitarian Device Exemption market approval from the FDA Center for Devices and Radiologic Health. Firms must show that devices will not expose the patient to any significant or unreasonable risk, and that the probable benefit of the device outweighs the probable risks. Manufacturers must also show that there is no comparable treatment available and that the company would not be able to bring the product to market otherwise. Since October 1996, the OOPD has received 154 requests for Humanitarian Use Device designation. One hundred four devices have been designated by the OOPD as Humanitarian Use Devices, 38 of which have received market approval.

The ODA is one of the most successful healthcare laws passed in the late 20th century. A direct outcome of the act is that more drugs for the treatment of orphan diseases are available to people who need them. Orphan designation procedures, as well as the FDA drug approval process, have grown and successfully adapted to emerging technologies in drug development.

INTELLECTUAL PROPERTY IN CLINICAL TRIALS

Intellectual Property: Patents and Transfer Agreements Preceding Clinical Trials and Commercialization

TERRENCE P. ROSS

"The Congress shall have the power to promote the Progress of Science and useful Arts, by securing for limited Times to Authors and Inventors the exclusive right to their respective Writings and Discoveries." The Congress implemented that constitutional authorization in Title 35 of the US Code §101,—"Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefore."—patent protection, as stated by the US Constitution.

For an invention to be patented, it must meet three requirements. One, it must have a useful effect or purpose. For example, a drug must be not only safe and efficacious, but it also must produce some sort of improvement. Two, the invention must be novel and it cannot be obvious. It must be a new invention not known, used, or invented by others. The subject matter must be sufficiently different from what has been used or described before so that it is not obvious to a person having ordinary skill in the area of technology related to the invention. Finally, although not stated in the statutes, the patent application must, according to the regulations of the US Patent and Trademark Office, be filed in a timely manner.

In ophthalmology, a case that stands as an example of an *obvious* invention is one of Dr. Samuel Pallin who patented a method of self-sealing cataract surgery. When he sued several surgeons for using his method, the courts invalidated his patent. They stated that "it was obvious what he was doing" and that any surgeon should have realized that simply by changing the surgical incision a better-sealing cataract surgery would be achieved. Pallin's technique was, therefore, not sufficiently novel or different. In fact, the courts and Congress do not like the concept of a surgical procedure being patentable. Congress has even passed legislation stating that patents cannot be obtained on surgical procedures in and of themselves unless the procedures involve a device.

Regarding timeliness, an inventor who delays applying for a patent on an invention risks a finding that he or she has suppressed, concealed, or abandoned the invention. The inventor also risks losing the rights to the invention if it was in the public domain for more than one year before filing the patent. An example of a patent denied because of delay is the case of Mr. Lemuelson, now deceased, who applied in the late 1980s for a series of patents related to the invention of bar codes. Mr. Lemuelson apparently began work on bar codes in the 1950s, but no patents were issued at that time. If they had been issued, his 17-year patent right would have run out before the product was even in use. After others had successfully implemented bar code systems, Mr. Lemuelson pushed for the issuance of his patents and, once obtained, he began to sue under it. This is what is known as a "submarine" patent (i.e., a patent an inventor files on a device or technology not yet developed and later brings to the surface after the patented devices have been implemented). It was ruled that Lemuelson's invention had been suppressed, during which time others had invested money and energy in bringing the product into use. Therefore, his patent was invalid.

Trade Secret Protection as an Alternative to Patent Protection

As an alternative to patent protection, intellectual property (IP) can also be protected through trade se-

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cret rights. A trade secret is any valuable knowledge not known to the public. Trade secret rights are granted by individual states, but many states have adopted the Uniform Trade Secrets Act, which prohibits misappropriation of trade secrets. Although the federal government recognizes trade secret rights, there is no federal remedy enabling one party to take action against another to protect its trade secrets or recover damages. This can pose a problem.

Trade secret rights are different, in a very fundamental sense, from patent rights. Patent rights give the inventor a monopoly for 17 or 20 years, depending on the situation. In return, the inventor discloses the discoveries to the rest of the world so that perhaps others can build on them. Trade secrets are just the opposite. The inventor who discloses the learnings or discoveries to the rest of the world has actually given away the trade secret, so there is no longer any trade secret protection. Trade secret rights are maintained by taking reasonable measures to prevent the secret from becoming widely known or disclosed. Trade secrets concern not the invention itself, but the method by which the information regarding the invention is disseminated. Therefore, trade secret protection can be very useful up to the point when the inventor is ready to apply for the patent. The combination of trade secret protection and patent protection can provide protection for research from start to finish.

United States Versus European Union Patent Processes

It is important for US inventors to obtain a patent in the European Union as well as in the United States. In the European Union, patents are granted on "first-to-file" basis, whereas in the United States, they are granted on the basis of "first-to-invent." The two processes are illustrated below:

- First-to-file process. Inventor A develops an eye drop that cures blindness. Six months later, inventor B develops the same eye drop and immediately files a patent application. Inventor A then files a patent application. Inventor B was the first to file and wins.
- First-to-invent process. Inventor A develops an eye drop that cures blindness. Six months later, inventor B develops the same eye drop and immediately files a patent application. Inventor A then files a patent application. Inventor A will be granted the patent if he or she can document the invention by dated laboratory notes and additional other means. It is not required that laboratory notes be notarized.

Even though the US has a first-to-invent system, an inventor who waits too long risks losing patent rights under Title 35 of the US Code § 102(b), which disallows the patent of an invention that has been in the public domain for more than 1 year before the date of the application for patent. This statutory bar applies to inventions described in a printed publication anywhere in the world or in public use or on sale in this country longer than 1 year before the date of the application for patent in the United States.

Timing of Patent Applications

Applications for drug patents are usually filed at the beginning of the clinical trial—after completion of the preclinical testing, filing for the investigational new drug application, and receipt of approval for clinical trials. This is the best practice, which most researchers, clinics, and companies follow.

At the time of patent application, nondisclosure agreements should be in place, and the process of documentation of the research should have been started. If a university or company is filing the patent application, the people doing the research (employees or independent contractors) should have signed, in advance, an Assignment of Rights for their work. Unlike copyright law, in patent law, rights of individuals conducting the research do not automatically devolve to the employer or university.

In advance of initiating clinical trials, and at each phase of the clinical trials, the IP rights that will govern the results should be reviewed for clarity and completeness and whether these involve exclusive ownership, cross-license, or joint ownership.

For a patent to be filed, the invention must be far enough along that it has "specific utility" (e.g., a patent application cannot be filed for a flying car until there is a blueprint). Patents enjoy a limited lifetime, which is 20 years from the date of filing or 17 years from date of issuance, whichever is later. If you file too early, you risk shrinking the window of time between the Food and Drug Administration approval and the patent expiration during which you can recoup research and development costs.

The Food and Drug Administration process can be extraordinarily long. Congress recently passed the Patent Term Extension Act, the Hatch-Waxman Act, which allows for restoration of one-half the time lost for clinical testing and Food and Drug Administration approval, up to five years. For instance, if the patent was issued at the beginning of the clinical trials, which have gone on for eight years without approval, the patent holder may report that delay to the Patent and Trademark Office and request an extension of the

patent. The Patent and Trademark Office grants a 30-month stay to generic manufacturers attempting to apply for an Abbreviated New Drug Application if the patent holder files a patent infringement suit against the generic applicant within 45 days of being notified. This is a fairly important right given to patent holders in the drug field. It extends the ability to keep a monopoly by keeping generics off the market.

The subject of the patent cannot have been described in a printed publication more than one year before filing. Thus, the inventor must not publish a paper on the subject until after the patent has been filed. If a paper is published, the patent attorney should be informed immediately, because he/she then has one year from the date of publication to get the patent *on file* (not approved). At a professional conference or symposium, the subject of a patent may be discussed, but no written materials, even abstracts, can be distributed. Slides may be shown during a presentation, but may not be included in any conference programs or proceedings publication.

Grant proposals are considered printed publications, because they are available to the public under the Freedom of Information Act. However, the inventor can take precautions to restrict the information available to the public, and, therefore, not risk creating prior art, such as published articles and doctoral dissertations. When submitting a grant proposal, the inventor may "designate, by appropriate markings. . . any portions of [your] submission that [you] consider to be protected from disclosure (45C.F.R.612.8(c))," thus preventing it from being subject to release under the Freedom of Information Act.

Patent Protection During Clinical Trials

The fact that the subject of the patent cannot have been in the public use or on sale for longer than a year before filing raises this question: Are clinical trials a public use or sale? Unfortunately, the correct answer is yes and no. Technically, clinical trials *are* a public use, but there is an exception to the public use bar for legitimate experimental purpose. The factors determining whether clinical trials are for a legitimate experimental purpose are the following: length of the test period and the number of tests; whether the inventor was paid for the testing, raising the issue of public sale; confidentiality agreements of the users; records of the testing; and whether persons other than the inventor performed the testing (control of the test).

Factors qualifying clinical trials for the status of "legitimate experimental purpose" apply only to proving that the invention works for its intended purpose. Tests to refine the invention after it has been proven to work are not considered experimental.

Guidelines for Transfer of Intellectual Property Rights

At some point, the patent holder may have to seek funding from a venture capitalist, government agency, or private foundation. Any funding organization expects the IP rights to be properly secured.

To protect IP, assure that agreements are signed at every stage of the process by all relevant parties. Before transferring rights, make sure the inventors are known, thereby avoiding confusion in the future.

To be considered an inventor, a person must contribute to the *conception*. It is not enough to derive the idea. People are considered joint inventors if they have made some contribution to the conception of the invention, even if they do not physically work on the invention together, or even if each does not make the same type or amount of contribution.

Usually, the people who test the product after the blueprint for the invention has been developed are not inventors; thus, most conductors of clinical trials would not qualify as inventors.

If any of the employees involved in development of the product are independent contractors or work for hire, make sure that agreements are in place before conception to ensure that these workers know that the company owns the IP rights to products developed while they are on the job.

Are there any other inventions directly or indirectly related to the study drug? Make sure that the transfer agreements are secured for all relevant products. Ensure that the rights are secured for not just the patented invention, but also for all notebooks, printed material, and research related to the invention.

Most clinical trials involve partnerships between the company developing the novel method or product and a university or research facility that actually conducts the trial. For *IP owned before the venture*, a joint venture agreement should be drafted that makes it clear that both parties retain the IP rights. It should also be stated that the parties may agree to cross-license some of this IP at a later time. If necessary, the other party may need to be granted explicit rights to use the invention to conduct the clinical trials.

For *IP developed during clinical trials or new ideas that result from clinical trials*, several options exist for protecting and sharing rights. It is important to know the differences and decide which works best for you:

1. Joint ownership, in which all parties agree to jointly own all IP.
2. Cross-license, in which parties agree to divide the IP rights, with each party owning certain rights, and grant each other a royalty-free license to practice and use the inventions.
3. Exclusive ownership, in which one party owns all rights, title, and interest in the IP.

The patent owner must be careful that the transfer of rights is not seen as a sale. An assignment of right is not a sale. However, if the patent holder is selling the rights to a process, the performance of the process *may* trigger the "on sale bar" of Title 35 of the US Code §102(b), in that the process has been offered for sale more than 1 year before applying for the patent.

It is important to remember that in transfer of trade secret rights, confidentiality is the key to protection. Thus, all agreements should contain confidentiality clauses and adequate security.

Conclusions and Caveats

Remember the following six points to protect your IP:

1. Take precautions not to disclose the invention before filing.
2. Do not publish or distribute printed publications regarding the invention before filing.
3. Designate what information is protected when submitting grant proposals.
4. Conduct clinical trials in accordance with experimental use factors.
5. Use available laws to extend the life of the patent for time lost during clinical trials and Food and Drug Administration approval.
6. A patent on a medical procedure alone (i.e., one that does not use a patented drug or device) is not enforceable; a novel device used during the procedure represents an enforceable patent.

Intellectual Property in Drug Development: A Report From a Breakout Session

ANTON HOPEN

Several participants in this First International Symposium on Translational Research for Inherited and Orphan Retinal Diseases, including Lester Kaplan and Jay Foust, emphasized the importance of holding regular meetings with a patent attorney to assure protection of intellectual property. Dr. Kaplan, from a perspective gained from long-term involvement in the field, presented an overview of intellectual property considerations. In drug development, he emphasized that the marketability of a compound is measured in terms of the relative costs of securing and maintaining the drug. He also called attention to the fact that, even when a product may seem to be obvious and unpatentable, a legal expert may disagree and recognize development opportunities. The inventor/entrepreneur should have full disclosure with someone in a relationship that is confidential.

Mr. Foust specified nine guidelines for protecting and developing intellectual property:

1. Obtain a broad-based claim, to protect rights to all aspects of the technology.
2. Protect the commercial product, filing very broad claims to cover all potential embodiments.
3. Consider monopoly pricing power. Does the proposed product have sufficient advantage over existing cheaper products to persuade the consumer to buy the product?
4. Define the drug. What will it and its packaging actually look like on the store shelf?
5. Consider that setbacks in development can lead to solutions that become very valuable.
6. Be careful in making collaborative arrangements. When university and other collaborators are brought in, make sure your company has acceptable interest in the patent, if not ownership. Even if your technology is effective, can you police it? Are you writing patent applications that would have to be enforced against clinicians?
7. Of the various patent claims, "composition of matter" is recommended as the most enforceable and easiest to police.
8. Patent inventorship must be accurate and reflect who materially contributed to at least one of the claims; the "mechanic" who builds the device according to the specifications of the "inventor" is not necessarily a collaborator.
9. Inventorship can be dynamic, and if inventors are added or removed without deceptive intent, the patent will remain enforceable. A situation in which one of the inventors has been left off a patent application must be resolved immediately by the filing of a concurrent patent application by the person who has been omitted.

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LICENSING OF COMPOUNDS FOR CLINICAL TRIALS

Mechanics of the Food and Drug Administration's Form 1571: Investigational New Drug Application

GARY NOVACK, PHD

Fulfilling the mission of The Foundation Fighting Blindness—to find the causes, treatments, preventions, and cures for retinitis pigmentosa, macular degeneration, Usher syndrome, and the entire spectrum of retinal degenerative diseases—involves evaluating compounds in humans. An early step in the process of developing new drugs for patients is the filing of Form 1571 of the Food and Drug Administration (FDA), also known as the Investigational New Drug (IND) application. Form 1571 of the FDA is filed before a Phase I clinical trial begins. An equivalent to this application exists in other countries.

The filing of Form 1571 serves as notification of the intent to begin a clinical trial, as opposed to a request for approval. Thirty days after application, unless FDA has notified the applicant to the contrary, the applicant may ship the drug across state boundaries to test in humans.

The requirements of Form 1571 are basically as follows:

- Rationale for human treatment. This is different from proving efficacy in animals. Although the rationale for human treatment might be shown by efficacy in animals, it could also be demonstrated by showing, for instance, that the compound blocks calcium flow, that calcium seems to contribute to neurodegeneration, and that it is safe.
- Chemistry, manufacturing, and control data. This covers all features of good manufacturing practice. How will the drug be produced? Is it stable? What container is it in? Is it known that the container material will not interfere with the stability of the product?[1]
- Good laboratory practice toxicology studies. A number of pharmacology and toxicology studies are required before a drug can go into clinical trials. These are performed in stages. Certain studies are required for Phase I. Others are required for Phase II and III. Good laboratory practice applies the basics of analytical chemistry. All of the conditions of the study are documented in detail, including such information as the lot number and brand of dog chow fed to experimental animals at all times during the study.
- Phase I protocol and overall plan. This details all aspects of the initial study as well as the plans for subsequent studies. In addition to descriptive information, it includes such requirements as patient consent forms and case report forms.
- Clinical investigator's brochure. This is a confidential document summarizing everything known regarding the compound. It contains the information a physician would need to make a benefit-to-risk judgment when evaluating patients for entrance into the trial.
- Investigator curriculum vitae and signature. This shows that the new product will be evaluated by a qualified investigator who is licensed to practice in the state in which the study is to be carried out.
- Between research and project status (i.e., the start of the development work required for an IND), a number of questions need to be answered. Does the treatment show dose response, time response, antagonism by the antagonists, and structure–activity relationships? Is the compound patent protected? If the compound is protected by patent, then permission/cooperation from the innovator needs to be obtained. If the compound is not patent protected, then a sponsor should be found to perform good manufacturing practice synthesis of the active ingredient. Is the therapeutic dose sufficiently less than the toxic dose?

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- Although the IND Form 1571 is brief, the work required to complete it is substantial. If the IND is submitted without adequate information, it can be put on "clinical hold." A clinical hold is an FDA order to delay a proposed clinical trial. An applicant may respond to the clinical hold, and the FDA is required to respond back within 30 days. It is far better to consult the FDA regarding the adequacy of an IND application in advance of submitting the application, to avoid a clinical hold order. Form 1571 of the FDA is available online from www.fda.gov/opacom/morechoices/fdaforms/FDA-1571.pdf.

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Licensing Compounds: Lessons From ISTA Pharmaceuticals

VICENTE ANIDO, JR., PHD

ISTA Pharmaceuticals is a small company that uses three approaches to obtaining new products. They are licensing, reformulation, and in-licensing.

1. When licensing, ISTA takes a product that is already approved in another country and applies company expertise to the product and formulation development process for the United States, the clinical phases (usually Phase IIb and Phase III trials), and the US regulatory processes.
2. In reformulation, we take a drug that we know already works and find a new application for it. This allows ISTA to put a product with new patient benefits on the market quickly.
3. With in-licensing, ISTA purchases the rights to a drug already in development or on the market. This can be an advantageous arrangement for a small company. Our in-licensing criteria include 1) that the product is being developed in the United States, and 2) that we anticipate sales of approximately $50 million. Currently, only approximately 15 drugs in the ophthalmic marketplace meet both criteria. ISTA considers drugs at different stages of development, but typically prefers products that are in Phase II clinical trials or later. Most ISTA expertise is with drugs at that level and, for our investors, represent the best added value to the company.

ISTA spends approximately $15 million per year in research. For that $15 million, we can complete 2 large clinical trials, 2 smaller clinical trials, and achieve proof-of-principle with a third product, starting it through the regulatory process.

Very few large companies are involved in product development for ophthalmology, and those that are, are usually interested in compounds with potential sales of several hundreds of millions of dollars worldwide. Thus, as a small company, ISTA is in a good position to compete for in-licensing products. Many products being developed by the larger companies pertain to treatments for retinal diseases, therefore other areas of ophthalmology, mainly the front of the eye treatments, are left for companies such as ISTA. We are particularly interested in in-licensing agreements for therapeutic products that were originally developed for treatment of nonophthalmic conditions. Our interest is in developing treatments for ophthalmology. ISTA has in-licensed three products in the past few years.

In licensing agreements, we look carefully at the definition of "product," because we want to be sure that even if the compound is protected by intellectual property rights, the dosage form or topical delivery system we are developing will be within our domain.

President and CEO of ISTA Pharmaceuticals, Irvine, CA.

Proving Inventorship: The Importance of the Inventor/Laboratory Notebook

ANTON HOPEN

H.R. 2795 of the 109th Congress (June 8, 2005) amends Title 35, US Code to eliminate the first-to-invent system and awards the patent to those that file first. If this bill passes, and it is expected to, the following discussion may become obsolete. Although H.R. 2795 will put pressure on inventors to file patent applications as early as possible, it will substantially reduce the costs of district court litigation and completely eliminate interference proceedings at the US Patent & Trademark Office.

An investigator should keep an inventor/laboratory notebook to document all observations related to an invention. This may seem simple and obvious. However, the importance of doing so—thoroughly and routinely, to protect intellectual property and the financial stake of the inventor, company, and investors—cannot be overemphasized.

The inventor/laboratory notebook has three functions in the patent process:

1. The documentation entered in the inventor/laboratory notebook enables the attorney to conduct a thorough patentability search. It allows the attorney to search not only the obvious professional journals, but to locate information in more obscure literature and in unusual resources, such as foreign patent offices.
2. The subject matter in the inventor/laboratory notebook is used for specification of the underlying patent that will seek to protect this technology.
3. In the case of legal situations and litigation, even administratively in the US Patent and Trademark Office, "interference proceedings" may be involved.* The quality and depth of notations and documentation could determine the financial success or failure of a company.

The inventor/laboratory notebook may also have historical value. A researcher's work may turn out to be significant, and others may later want to describe in detail the pioneering work in the field. Filed patents and even a researcher's own publications are distillations of the raw data, details of which are often omitted and may be of value for future examination.

The importance of the laboratory notebook to a patent attorney also relates to the conception of "reduction to practice." In the United States, the patent rights go to the "first-to-invent," even if that person is not the first to file for patent (for more information on this topic, refer to the presentation by Terrence Ross in this *Retina* supplement). Although this system often entails a great deal of expense and effort, it is founded on the basis of equity and fairness. The patent is awarded to the person who first conceived of the invention and diligently "reduced it to practice."

There are two ways to establish reduction to practice. *Actual* reduction to practice, in the case of a medical treatment, is achieved at a point during clinical trials at which the efficacy of the product is proved. *Constructive* reduction to practice is achieved by filing the patent application with sufficient detail to enable one of ordinary skill in the art to reproduce the invention without undue experimentation.

Inventors in the medical/biologic area are in a unique position. Unlike simple engineering inventions, which can be constructed and demonstrated immediately, medical/biologic inventions are complex, unpredictable, and require clinical trials.

Another important concept is *presumption of validity*, which means that a patentee does not have to prove in court that the patent is valid. The accused infringer bears the burden of proving by clear and convincing evidence that the patent office erred in awarding the patent.

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*Occasionally, two or more applications are filed by different inventors claiming substantially the same patentable invention. The patent can be granted to only one of them, and a proceeding known as an "interference" is instituted by the US Patent and Trademark Office to determine who is the first inventor and entitled to the patent. Approximately 1 of the applications filed become involved in an interference proceeding. Interference proceedings may also be instituted between an application and a patent already issued, provided the patent has not been issued for more than 1 year before the filing of the conflicting application, and provided that the conflicting application is not barred from being patentable for some other reason.

THE PHARMACEUTICAL INDUSTRY IN CLINICAL TRIALS

Strategies for Success in Drug Development

WILLIAM M. WARDELL, MD, PHD

In clinical development for bringing a product to market, the term "clinical program" is more accurate than "clinical trial," because one single trial is never sufficient. A full clinical program in a large pharmaceutical company usually requires at least 20 to 40 clinical trials before a drug can be approved. This number is less than it was 10 years ago; clinical programs run by large pharmaceutical companies previously required nearly 100 clinical trials to obtain approval of a new drug application. Every step of the clinical program carries the risk of failure. However, certain approaches can be taken to reduce the risk.

Overview of Clinical Trial Phases I, II, and III

The clinical program is carried out in three phases. Phase I is usually performed in normal volunteers. Its purpose is to check for gross toxicity and to determine what dose can be tolerated. Phase II studies use a few patients and attempt to show proof of mechanism or proof of therapeutic concept. Phase III, with the dose having been established and the concept proven, must show the drug to work in humans (efficacy) and to be acceptably safe.

Because the governing law, passed in 1962, requires clinical investigations (i.e., studies in the plural), the Food and Drug Administration (FDA) and its European equivalent, the European Agency for the Evaluation of Medicinal Products, usually require at least two large Phase III trials to show positive efficacy results and a large enough number of patients to demonstrate safety. For orphan drugs and rare diseases that have no available treatment, the clinical program may be somewhat smaller, and one Phase III trial may be sufficient. The more commercially attractive a therapeutic area becomes (i.e., the larger the potential market and number of patients that could be treated with the drug), the more extensive the size of the clinical program the FDA expects.

The sequence of development can be thought of as follows: exploratory development, consisting of preclinical research through Phase I clinical trials and the early part of Phase II trials (called Phase IIa, which focuses on clinical proof of concept); and full-scale development, which includes the Phase IIb, Phase III, and Phase IV clinical trials (postmarket). In a highly efficient program, a successful Phase IIa trial can lead to an extended Phase IIb study that evolves into a Phase III trial.

Exploratory Development Phase

In the exploratory development phase, several approaches can help decrease risks that might be encountered later. First, one must be very precise regarding the definition of the rationale, known disease mechanisms, targets, and therapeutic concept. Ideally, the disease mechanism is well defined, and the drug has a proven mechanism of action on a specific target on which the drug should plausibly work. The target is usually an enzyme, an ion channel, a receptor, or a gene, and the drug's pharmacologic mode of action will usually be to stimulate a pathway or activate a receptor (in the case of agonist drugs) or to block a receptor or pathway (in the case of inhibitors or antagonist drugs). The preclinical definition of these actions provides the basis for describing the drug's pharmacologic action, and the putative medical effect of these actions becomes the basis for the therapeutic concept.

A clear explanation of how the entire process is to be performed should be written very early, in the form of a product development plan document, because this helps to uncover and solve problems that will be encountered in developing the compound. It also demonstrates to potential investors that the development team understands the process.

Furthermore, in the exploratory development phase, it

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is valuable to have more than a single compound to test in the context of the defined mechanism. There are many examples in therapeutics in which the first or second compound in a series fails, but success is achieved with the third or fourth compound. Success may even have come from a back-up chemical program that was looking for different types of molecules with the same action. Preliminary work should include evaluating the feasibility of different approaches before selecting the exact indication statement for the drug.

The conventional sequence of Phase I to III clinical trials will apply to most drugs, but can be modified in special cases. For example, Phase I pharmacokinetic and tolerance studies are usually performed on normal volunteers in special clinical pharmacology units, but, in the case of cancer chemotherapeutic agents, because of their potential for toxicity, the studies are usually performed in volunteer patients who have cancer.

Full-scale Development Phase

Full-scale development usually begins with a Phase IIb study, which aims to define and achieve the clinical endpoints that will be specified in the drug's labeling. However, if all goes well, a large Phase IIb study that gives excellent results could be used as one of the two required pivotal studies, leaving only the one confirmatory study to be completed at Phase III. If the results are especially impressive and very useful for particular types of patients, some drugs can qualify for approval under the "Accelerated Approval" regulations after just one Phase III (or even a Phase IIb) study, subject to postmarketing study requirements and/or utilization controls in the early years of a product's life.

It is important to identify specifically the indication of interest, and the patients must, accordingly, be carefully selected. For each phase of the program, patients must be recruited who have the characteristics appropriate for the indication. At the same time, the patients should represent a broad enough spectrum to allow for discovering that the drug might act in a slightly different way than expected. Establishing the right dose is very important. If the dose used in the first phases is too low, an excellent compound may fail to show efficacy and be abandoned. Conversely, if the dose is too high, patients may be harmed, which could also cause the drug to be dropped prematurely.

The function of the drug development program is to discover every possible flaw. Given the enormous cost of developing a drug and taking it through the long process to approval, if flaws that cannot be overcome are discovered in the early stages, development should be stopped.

In the full-scale, or late-stage development process (Phases IIb and III), the theories that were verified at the proof-of-concept stage must be tested rigorously. The tests, usually in the Phase III studies, must be defined in great detail, because once each study starts, the design usually cannot be changed. Crucial decisions are made regarding the types of patients, the sample size, the statistical tests, the primary and coprimary endpoints, and so on. There is no room for error. If the trial fails, repeating it would involve not only large costs, but also valuable time, often more than a small company could sustain. Decisions regarding trial design should be based on the specific indications planned for the labeling in the new drug application. If a biomarker is already available, or discovered en route, that finding could define an enriched group of potential responders. If a biomarker is available, the Phase III trial might focus on such special patient subsets, weighing the hoped-for increase in success probability against the smaller potential market of the initially approved drug. A fairly wide dose range is advisable.

In all of the above activities, close contact with the FDA is essential. The FDA is usually very helpful, particularly in giving advice to small companies with novel compounds or concepts. It is not unusual to see all of the key development people from a tiny company come a very long distance (e.g., from Europe or Australia) for a one-hour meeting with its respective FDA group and be very happy with the service and advice they receive.

Costs of Drug Development

The success/failure rates and costs of drug development are of great public interest and debate. There is even some debate regarding the actual rate (percentage) of ultimate approval for new drugs that enter clinical testing; the figures currently range from approximately 12% to 23%, depending on the therapeutic area (data from the FDA and Tufts Center for the Study of Drug Development).

The cost to develop a new drug is a topic of much controversy, although the basis for the high costs is obvious to those who work in the process of drug development. The average out-of-pocket cost of developing a drug during the approximately 12 years that it usually takes is estimated to be greater than $400 million (Tufts Center for the Study of Drug Development data). This average cost includes the costs of developing drugs that fail along the way, and, if those are removed from the calculation, the average out-of-pocket cost is currently thought to be approximately $200 million. However, the $400 million figure is more valid, because one cannot predict the failures in advance. Because the $400 million

is expended during such a long period, the costs of capital become very substantial. The most recent Tufts Center for the Study of Drug Development study indicates that the interest cost is almost the same as the original expenditure (i.e., the average cost per successful drug, including interest, is double the out-of-pocket cost). This is the basis of the current figure of $802 million (fully capitalized) per new drug approved. Many segments of the public, including interest groups, insist that this number is greatly exaggerated, but an informed look at the data, and at the process of development from inside a pharmaceutical company, would show the skeptics the huge costs and high failure rate of the development process.

Small companies work on the premise that they can develop drugs for much less money. Some orphan drugs have achieved approval with only 10 clinical studies or even fewer; but some of these drugs had already been approved for other indications, and the new use may have entailed only changing the dose, the target, or the type of patient. It is now an established strategy, and quite feasible, to develop a drug for a very small (orphan) indication with the hope that it will subsequently find other indications and a larger market. The lure of obtaining a highly successful drug via this route is an important stimulus to the welcome flow of investigational drugs for orphan indications.

Attracting Pharmaceutical Companies as Partners in Drug Development

LESTER J. KAPLAN, PHD

The criteria used by pharmaceutical companies to evaluate potential partnerships for developing new drugs and inventions are similar to those used by venture capitalists. In short, pharmaceutical companies look at every opportunity in terms of two things: risk and reward. Because we review many opportunities every week, we need quick and accurate ways to efficiently review as much data as possible to assess risks and rewards. The two types of data that are most important are the scientific data, to understand the basis for the therapeutic opportunity; and the commercial data, to evaluate the potential reward.

The hierarchy of scientific data, with the most exciting type of data listed first, includes human efficacy trials, human pharmacokinetic data, human safety data, preclinical safety data, and preclinical efficacy data. Proof of efficacy in humans, obviously, would garner the most attention from a pharmaceutical company, but that is rarely available at the presentation stage. Basic studies are more likely to be the initial basis for consideration.

In evaluating preclinical efficacy data, we ask a number of questions. For example, are there quality data in relevant animal models, and can it be reproduced in a number of relevant animal models? Has the work been performed by qualified investigators? Has it been peer reviewed?

Preclinical safety data would, ideally, include the results of safety studies in animals. At the preclinical stage, it is also important to consider whether the product can be manufactured according to good manufacturing practice. What is known regarding the pharmacokinetics, the distribution of the product, the metabolism of the product, and the elimination of the product? These are all important aspects of both preclinical and clinical studies. Preclinical studies should demonstrate adequate evidence that the drug can be delivered to the target. A common reason for failure in Phase II trials of drugs that succeed in animal models is that adequate concentrations are not reaching the target tissue.

Next in the hierarchy is the human safety data. These Phase I data are crucial for understanding whether the animal models of toxicology predicted safety in humans. If so, this reduces the risks of failure and the length of time that will be required to bring the drug to market. Even more important is proof of principle in humans, which comes in early Phase II studies. Companies considering a drug development partnership are very interested in clinical pharmacologic data—for example, biomarkers or drug levels in target tissues in small patient populations. Objective data that confirm that the drug is acting in humans as predicted by animal studies create confidence in the potential partner, even if the data are from a very few patients.

Human pharmacokinetic data represent an area that is often overlooked in presentation packages. If the drug is safe, we have to know how much of it reaches the target in a concentration adequate to produce the desired effect. These data are necessary to proceed to the human clinical efficacy trials, Phase II and, ultimately, Phase III. The message here is to acquire efficacy data in humans even if they are for endpoints that will not be used to support registration. This can be tremendously powerful in attracting partners. Data from as few as three to five patients can be very powerful in augmenting the animal data.

Member of the Board of Directors of the National Neurovision Research Institute, and former Executive Vice President for Research and Development at Allergan, Inc., Irvine, CA.

An Overview: Attracting Partners in the Pharmaceutical Industry

GEORGE LASEZKAY, PHARMD, JD

Successful clinical trials are costly and contain inherent risks. Small companies or laboratories may seek to attract a pharmaceutical company as a partner to maximize the chance that their compound will reach its target market. Potential partners evaluate certain risks and types of scientific and commercial data when they consider investing in or forming a partnership with a small company.

It takes four to six years to move from target identification to clinical trial, and another four to six years for clinical development. Even at the clinical trial stage, when the product is well on its way toward approval, there are still inherent difficulties and risks. Problems may require backtracking and redoing steps performed at earlier stages. A carefully considered preclinical package is important for avoiding problems in clinical trials.

The difference between studies in humans and animals must be carefully considered. In 2002, 30% of Phase II trials failed to meet their primary endpoints, and the number is approximately twice as high now as it was in 1998. A number of factors contribute to the increasing failure rate in clinical trials, and because the clinical trial process is long and expensive, every possible effort should be made to minimize the possibility of failure. Estimates of the costs of bringing a drug to market range from approximately $150 million to $800 million.

Contract research organizations can be helpful in estimating the costs of clinical trials in specific diseases. Costs vary considerably, depending on the number of patients needed to achieve approval. This is an important factor in all phases of the trials, but it is particularly important in Phase III trials. In addition to a thorough product development plan to minimize or mitigate the inherent risks of clinical trials, it is important to maintain ongoing communication with the Food and Drug Administration to assure that the trial design will meet their ultimate requirements.

In planning a clinical trial, it is critical to understand the nature of the disease itself—its natural history and its progression—to avoid problems with active controls or active placebos.

The type of data that is important to potential partners, whether they are venture capitalists or other pharmaceutical company partners, comprises two groups: scientific and commercial. Proof of human efficacy is the most persuasive scientific data. Small companies often try to interest partners based on very early animal data, but these attempts are seldom successful at that stage.

Preclinical indications of efficacy are acceptable under certain circumstances, depending on the quality of the process and the expertise of the people involved. If outstanding and well-recognized researchers are involved and the process is high quality and well run, then partnering or venture capital funding might be possible, based on preclinical indications of efficacy in a relevant, well-established animal model.

Clinical pharmacology data can be used to demonstrate that the drug can be delivered to the target tissue in humans. This is a new area that corporate partners, especially, are looking at to make sure that the effect being seen is caused by the drug rather than something else that may be confounding the situation. Thus, pharmacology studies should be described in the clinical development plan.

In summary, potential venture capitalist and corporate partners want to see high-quality clinical data together with full analysis of the target population, the competitive products, and the project development costs, as well as a plan for a regulatory pathway that has been clarified with the Food and Drug Administration. In all of these areas, clarity is of the essence.

Principal in Turning Point, LLC, a consulting group advising life science companies on strategy and business development. He is also Director of Acuity Pharmaceuticals, an early stage ophthalmic product company.

COMMERCIALIZATION PROCESS FOR THERAPEUTIC AGENTS OF HEREDITARY AND ORPHAN RETINAL DISEASES

Hurdles and Opportunities for a Venture Capitalist Investing in Therapies for Orphan Retinal Diseases

JAMES C. BLAIR, PhD

The future opportunities for novel therapeutic approaches for retinal and neurologic diseases are exciting, and the venture capital funding process will play a role in advancing such promising science in the clinic.

During 25 years, Domain Associates, LLC has developed a process for evaluating any given opportunity according to a set of criteria that have characterized our most successful investments. Next, we will look at the most obvious retinal diseases that would merit our consideration. Retinal disease seems to be an area that will attract funding from my colleagues in the venture capital area.

At present, Domain Associates is making investments in life science product companies from a $500 million fund. These funds are provided to us for approximately 10 years by various institutional investors, and most are US pension funds and college endowment funds. These are not grant funds. Although grant funds are equally demanding of good science, their so-called *return on investment* consists largely of improving the welfare of patients. In contrast, the return on venture capital investment should be primarily financial. We invest our money in companies that plan to develop and market specific products (i.e., biopharmaceuticals, 50%; medical devices, 30%; and diagnostic/medical instruments, 20%). Recently, we have become very interested in medical devices with a therapeutic effect.

General Partner of Domain Associates, LLC, with offices in New Jersey and California, and Principal Partner in 3i Bioscience Investment Trust.

We devote a great deal of time to working with the management of companies we invest in to help them develop their plans and organization. Because of the need for our active involvement, we invest solely in US-based companies.

The characteristics of investment opportunities, which have worked well for us, are summarized below in 4 main points: focus on big ideas; have clinical evidence that the product will work; focus on poorly served needs; and avoid the 800-pound gorilla.

Focus on Big Ideas

Obviously, cancer and heart disease are two major diseases that represent big ideas. Can any orphan indication have a chance of being characterized as a big idea? One of our first investments, and the most successful, was in Amgen, Inc., whose initial product, erythropoietin for treatment of anemia associated with chronic renal failure, was developed and protected under the orphan drug rules. Other companies that dominate the biotechnology industry also have had orphan drugs as key elements of their initial corporate strategies. Orphan products can represent critical platforms for establishing effective, well-functioning clinical and regulatory organizations in a young company.

A big idea is not defined solely by the incidence and prevalence of disease, but also by the societal cost of the disorder—what the cost has been and is likely to be without medical intervention. We are also interested in the trends of incidence and prevalence, and the influence of the aging population on these numbers.

Clinical Evidence that the Product Will Work

It is well known that the odds are very low that a new chemical entity will eventually become an approved drug. Even after all of the preclinical tests are completed, the odds are greater than 10:1 that a compound starting in Phase I clinical trials will not prove to be safe and efficacious and subsequently receive Food and Drug Administration approval.

It should not be surprising that we are reluctant to invest in a product that has not been tested in human subjects. In the early days of biotechnology, when the products being tested were well known, such as human growth hormone or insulin, the odds were better. We knew what the product would do. The challenge was in producing it. Although we have, in the last few years, decreased our allocations to preclinical opportunities, they still represent approximately 40% of our currently investments. There need to be clear clinical endpoints that lead to a regulatory path to approval.

Focus on Poorly Served Needs

In our experience, we have found that the shortest paths through the clinic and regulatory agencies are well correlated with poorly met clinical needs. The Food and Drug Administration does not just drop everything to evaluate the next new allergy drug. It is difficult to convince most cardiologists that they should enroll their patients in the next trial of a new blood-pressure-lowering medication. However, there is much more enthusiasm for testing a product that promises to retard or prevent macular degeneration, or a drug that will combat obesity, because these address a poorly served need. This is true of retinal diseases, and it is the principal reason we are enthusiastic about getting involved in this area.

Avoid the 800-pound Gorilla

Over the past 25 years, the biotechnology industry has come into its own. There has been remarkable cooperation from these smaller emerging companies and the larger pharmaceutical companies (what we call the *800-pound gorillas*). There are no signs that this spirit of cooperation is lessening.

Venture capitalists are interested in products with annual sales potential of approximately $50 million to $100 million—a return too modest to attract the larger pharmaceutical companies. We are finding that our natural alliance partners are more likely to be the Genzymes and Amgens than the Mercks and Novartis's for many of the products we are developing. For every small company that has been swallowed up in a mega-merger during the past 10 years, there have been two or three newly profitable biotechnology companies arriving on the scene.

In the development of therapies for retinal and neurologic disease, the large pharmaceutical companies are more likely to be the Allergans and Alcons—maybe we can call them 600-pound gorillas. We have found the doors to these companies to be wide open for collaborative and cooperative efforts. The key to dealing with any of the gorillas—small, medium and large—has always been a strong intellectual property platform.

Need for New Therapies for Eye Disease

The projections for prevalence of major eye disorders are astounding. According to the National Eye Institute, approximately 1 in 28 Americans older than the age of 40 years are affected by blindness or low vision. More frightening is the likelihood that by the year 2020, our aging population will have a major increase in the number of affected individuals. It is not surprising that the major diseases of the eye (i.e., age-related macular degeneration [AMD], glaucoma, diabetic retinopathy, and cataract) receive a high level of attention.

Some retinal diseases in need of therapies include AMD, retinitis pigmentosa (RP), Usher syndrome, Oguchi disease, choroideremia, retinoschisis, blue-cone monochromacy, juvenile macular degeneration, Stargardt disease, Best disease, malattia leventinese, cone–rod and rod–cone dystrophies, and Leber congenital amaurosis. With the exception of AMD, these disorders receive dramatically less attention than glaucoma, diabetic retinopathy, and cataract. Regarding mechanisms in these diseases and approaches to slowing the progress of these mechanisms, we are aware of two common themes. One, all of the diseases seem to be degenerative in nature. Two, genetic mechanisms are involved, therefore, nucleoproteins are likely to represent the best potential for treatment.

Given the prevalence of AMD, juvenile macular degeneration, and RP, most of the venture investors will concentrate in these areas. During the last year, approximately six new companies have been formed to attack AMD, and, in particular, the less prevalent and more debilitating wet form of the disease. The success of Eyetech's recent initial public offering has garnered much interest. A number of clinicians have expressed skepticism regarding the long-term clinical value of the Eyetech product, Macugen. However, because approximately 50% of patients respond with an effect that seems to last or improve over time, the drug should receive a great response from the market. The real significance of Eyetech's success is that there is heavy financing of this opportunity. Other new drugs offer the promise of easier forms of delivery and better response rates. Some of

these drugs will be directed toward the more prevalent dry form of the disease. The prospects for many AMD patients at this time are promising.

We are unable to find any real prevalence information regarding all forms of juvenile macular degeneration; this term seems to be a general descriptor for several clinical manifestations of macular dystrophies affecting younger people. The most common form of juvenile macular degeneration is Stargardt disease. Whereas a common gene exists in approximately 20% of patients with Stargardt disease, there seems to be no genetic pattern in more than 60% of the patient population. In the case of Best disease, we were unable to determine prevalence, but its hereditary aspects are clear.

Three points are worth noting: 1. The number of retinal diseases for which specific genes can be implicated is accelerating rapidly. 2. Several venture-backed companies now exist and are developing so-called RNA chemistry that promises to be highly selective and effective in treating disorders characterized by an overexpression of unwanted proteins. Such protein overexpression seems to be at the heart of several retinal disorders. 3. Advanced gene therapy techniques—second- and third-generation approaches—make experimentally treated dogs healthier. This provides the basis for moving these trials into humans.

Soon there will be specific mechanistic information available regarding many retinal diseases, and the translational process is poised to swing into very high gear.

Approximately 1 in 3,500 individuals in the United States have RP, and approximately 100,000 people are affected. Retinitis pigmentosa is clearly an orphan disease, even by the tightest new standards. It is a clearly progressive disease with strong genetic characteristics. Very few good drug targets exist today for RP. The disease mechanisms are such that the best therapies expected during the next several years are drugs that might slow the progress of this disease. This alone would be extremely well received by patients, and this therapeutic approach represents an opportunity that would attract substantial investment.

In the past, we have evaluated some radical approaches, which include devices to implant photosensors in the eye—an artificial retina. Although these suggested great promise, we think the era of nanotechnology will be vital to their success. The work being done by Second Sight, Inc. is exciting, not only because of its actual clinical use, but because of its multidisciplinary nature.

Domain's "big idea" criterion is clearly met by AMD and diabetic retinopathy because of their high prevalence. The societal costs of diseases such as RP are clearly large, and so are the market opportunities. The societal costs and the potential market value justify the consideration of drugs for these diseases as a big idea, even though they do not qualify by the criterion of patient population size alone.

The specific mechanisms for all types of RP may not be well understood, but as soon as they are better defined, it will be possible to move existing drugs rapidly into the clinical setting. Whether they succeed or not will require a trial-and-error process for the next several years. The first wave of treatments will not be with novel compounds or new chemical entities; rather, they will be based on identifying what the targets are, and using on-the-shelf chemicals that have a very good safety profile, and that can be confidently administered to the patient population. Improved delivery mechanisms will be important.

Clearly, the needs of these markets are poorly met. Rather than avoid the 800-pound gorillas, they may well be engaged in collaborations of the magnitude of the Pfizer/Eyetech relationship. It is easier to collaborate than to compete.

Facilitating Progress

I serve on the Board of the Prostate Cancer Foundation, formerly known as *Camp Cure*. I see some similarity in the mission being undertaken by the National Neuroscience Research Institute now and the mission that was defined by the Prostate Cancer Foundation when it was formed in the early 1990s. Their mission then, as the mission of the National Neuroscience Research Institute is today, was to stimulate a process for translational research to move rapidly and take research concepts into the clinic. Molecular biologists and geneticists alike are finding that their special skills will not get the job done in a vacuum. The buzzword today is "systems biology," which I interpret to mean an integrated, multidisciplined approach to attacking the problem at hand. The disease mechanisms we demand to know about when considering investments are multifactorial. It ultimately comes down to attracting a nucleus of committed scientists who want to work together for years until these mechanisms are both understood and validated in the clinic. In the early 1990s, very few researchers wanted to apply their skills in the area of prostate cancer research. It was deemed academic suicide. The National Neuroscience Research Institute needs to stimulate scientists to commit their careers to these efforts in the area of rare retinal disease and to stimulate our federal funding agencies to join the cause. The importance of organizing a large common effort to build economic leverage cannot be overemphasized. It is a very important aspect of the mission of this organization.

Finally, innovative investigators cannot be afraid to

experience failure in the clinic, despite the obvious disappointment that such failures generate.

Conclusion

- Venture capitalists will definitely support trials for orphan retinal diseases.
- The approaches that have early appeal will be those that test the proposed mechanism with currently available drugs, to quickly get the compounds into the clinic.
- We are confident that many of the newer chemistries will be more likely to provide longer-term solutions.
- Finally, keep cheering for the Eyetechs of this world. Their success will keep the rest of the venture capital companies moving in the right direction.

Crucial Factors in Commercial Success for Ophthalmic Drugs

VICENTE ANIDO, JR., PHD

ISTA, Inc. was founded in 1992 as a one-product, development-stage company. It is now a full-fledged specialty pharmaceutical company with a focus on ophthalmology.

A number of factors are crucial to ISTA's commercial success and to assuring that, when the company proceeds to a clinical trial, the process will be successful. One factor is that we require that we do something for the patient that has never been done before, or that we do it better. We also look at certain product characteristics. For example, is the product a new class of compound? Is there an established market? Considering Food and Drug Administration guidelines, we ask whether there have been other drug studies in this area; i.e., whether there are established guidelines. In calculating how much we should invest in clinical trials, we ask whether the market is large enough to sustain the costs of the development process.

We think that the current market for ophthalmic drugs in the United States is approximately $2.8 billion. We expect that it will grow to more than $4 billion during the next 3 to 4 years, driven principally by 2 therapeutic areas that have not existed in the past: dry eye treatments and macular degeneration drugs. Allergan, Inc. has developed the drug Restasis in the dry eye field, and this will be followed by many other dry eye products. A number of new entries into the area of macular degeneration will produce growth in that market, from roughly $300 million to more than $600 million in the upcoming years. One of the interesting facts is that, in 2003, only 8 ophthalmic drugs in the United States sold more than $100 million per year, and 18 sold more than $25 million.

In considering the cost of developing compounds and comparing those costs to the potential returns, in many cases it is very difficult to substantiate the extensive and expensive development process for many ophthalmic drugs. For our product portfolio, we look at all of the parameters and try to calculate a risk-to-reward relationship (i.e., the cost of developing compounds relative to what ultimately we may earn in the market place). Two current ISTA drugs provide interesting case studies. They are Vitrase, for vitreous hemorrhage, and Xibrom, a nonsteroidal antiinflammatory drug for the treatment of ocular inflammation. Vitrase was developed for a condition that had no other treatment, and Xibrom was developed for a well-understood and studied disease.

ISTA investors clearly want the risk associated with clinical trials to be well-managed. They prefer products that are differentiated and have a less risky development pathway, but they understand that it must be balanced with the potential market-expansion opportunities that breakthrough therapies provide. There is always a challenge in working with the Food and Drug Administration to find acceptable ways of studying new therapies. At ISTA, we have decided to pursue a balanced strategy, whereby we have products such as Xibrom, which has a straightforward development pathway, and selectively pursue riskier programs if the market potential is there. In either case, we work closely with the Food and Drug Administration to understand the regulatory path and use the regulations such as the Special Protocol Assessment to achieve closure on how best to study our new drugs.

President and Chief Executive Officer of ISTA Pharmaceuticals, Inc., Irvine, CA.

Key Considerations for Seeking Product Development Funding

WILLIAM M. WARDELL, MD, PHD

Regardless of whom you approach to obtain funding for the development of a new product, the following recommended actions can greatly facilitate the process, and increase the probability of obtaining financial support.

Have a One-Sentence Description

For focus and clarity, have a one-sentence description of your discovery and potential product. Consider the following example: "We have discovered inhibitors of the new receptor X, and the first product we intend to develop is a potential treatment for disease A."

Write a Product Development Plan

Write the outline of a product development plan. It should include the medical need that will be met by your product, and the steps that you think will be required to develop your product. The initial outline does not need to be more than approximately five pages long.

It would be useful to work with technical consultants who can critique and verify various aspects of the product development plan.

Many small companies do not have a plan at the start. No small company begins with a perfect development plan, but having a well thought-out draft to present to potential partners and funding sources is very helpful. The product development plan includes outlining the anticipated time and cost required to proceed, at least as far as filing an investigational new drug through Phase IIa (proof of concept). The product development plan should include meeting the manufacturing requirements for the quantity and purity needed for performing the clinical program (the microgram production quantities used for research will not suffice when 180,000 tablets of various sizes may be needed for a Phase II study).

Describe in some detail the preclinical/clinical program, to demonstrate that you know what is required.

Know Your Competitors

Use all available resources to find out about competing products. Read the press releases of competing companies, and talk to analysts who may have evaluated them. What is the design of their program? At what stage of development are they? Have they met with the Food and Drug Administration? What did the Food and Drug Administration say would be required to have their product approved? What is their clinical and regulatory plan? A large amount of information is freely available.

Anticipate Questions and Criticisms

Know the weaknesses of your product and be prepared to answer questions calmly in this regard. Be prepared for tough questions and be objective, not defensive, in your answers.

Plan Your Staffing Needs Realistically

Make sure you have (or will have) the right people to develop the product and get to the investigational new drug filing, and then to new drug application approval. At a minimum, show that you have a plan for recruiting the people you need. Will you staff at the beginning with all of the people you will need to complete the program? Or will you start with consultants or a contract research organization to help you get started while you make some well-thought out decisions regarding hiring? Do not make the mistake of hiring too early; be sure you know what your needs are before selecting the people to fulfill them. Do not hire an experienced industry person, and put her/him in the wrong function in your company.

Make Sure You are able to Manufacture Your Product Properly, Early on

Failure to ensure manufacturing quality can result in long delays that can sink a tiny company.

Head of Wardell Associates International, LLC, Princeton, NJ. Wardell Associates International is a consulting firm in drug development, regulatory approval, and postmarketing.

Business Modeling

DANIEL C. LUBIN

Venture capital is a critical ingredient in the financing of preclinical and clinical studies in the field of retinal diseases. We are a necessary financial partner in bringing science to reality and to achieving the dreams.

The success of Eyetech's Macugen and the clinical progress of other very exciting compounds have stimulated a great deal of interest from professional investors to study opportunities in ophthalmology and to invest aggressively. It is important to realize that venture capital is not a uniform field, particularly as related to the life sciences. There are venture capitalists who specialize in different sectors of this industry; e.g., drugs, devices, services, etc. Moreover, they focus on different therapeutic categories. My firm focuses on four areas—ophthalmology, oncology, cardiology, and orthopedics. We have a rationale and a strategy based around those therapeutic categories. Other venture capitalists are interested in the central nervous system or infectious disease, or, perhaps, genomic tools.

There are also venture capitalists who focus on different stages of the company life cycle; e.g., the seed stage, "series A" investments, late preclinical studies, or clinical trials. Within the category of "clinical," some investors are interested in the early clinical phase, and will commit funds with data on fewer than 20 patients. Others require data from more than 20 patients, whereas others will fund work with 20 patients' worth of data all the way to Phase III. It is important for the inventor/entrepreneur to study the portfolios of different venture capitalists and learn about their interests, their investment histories, their reputation, and their advisors. Choosing the venture capitalist most appropriate for the type and stage of the product increases the probability of a successful outcome. Even if a meeting does not conclude with the writing of a check, the participants should be able to come out feeling that they had an intelligent dialog and that both sides learned something.

I cannot overemphasize the importance of making the correct decisions early on. This includes decisions regarding selecting the securities law firm, the intellectual property counsel, the scientific advisors, and a consultant to help design the clinical development plan. If every effort is made to create an A+ team, probabilities of success are greatly increased. If the entrepreneur takes the path of least resistance, selecting people who are readily available and provide a "comfort level," but who are not necessarily the leaders in their respective fields, this can interfere with the clinical development and establishment of value. It is very important to be methodical and patient in choosing the people who will comprise the development team.

Data need to be packaged in a way that serves to accelerate a collaborative dialog with potential investors and to facilitate assessment of the potential product and the market opportunity. Understand the clinical and competitive factors that will affect how a particular compound will ultimately be established in the marketplace. The entrepreneurs know better than the venture capitalists what is on the market and in the clinic. They can accelerate our review by providing such information. A realistic and well-engineered preclinical and clinical development plan is very valuable for our assessment process.

We need to see a well-articulated assessment of the intellectual property. We need to know where the problems are and how they might constrain the freedom to operate. The venture capitalist has resources to help work out problems, but if problems are not disclosed initially, this breeds skepticism that impedes the gathering of momentum internally to focus on the project.

Finally, understand the milestones. Milestones drive value, but not all milestones drive return. The biggest milestones are filing the investigational new drug application and completion of the Phase I study.

We have to recognize that only a small percentage of presented projects will successfully attract the classic venture capital paradigm, therefore, we ought to be realistic regarding this. The projects that succeed will create enormous societal benefit and deliver very high returns to investors. That is what this is all about—curing disease and making money at the same time.

Principal in Radius Ventures, LLC, New York City, NY.

Role of Venture Capitalists in Funding Clinical Trials

DANIEL C. LUBIN

Venture capitalists are a necessary component of the process of commercializing promising science. The venture capitalist represents investors from around the world, including insurance companies, universities, pension funds, and families and individuals who have committed their capital with the hope that the venture capitalist can generate a very high return for them.

Venture capital is an asset class that fits into a category called *alternative assets*. Within this category, which also includes hedge funds and private equity funds, venture capital investment is the riskiest type of investment. The goal of the venture capitalist is to generate incremental returns of 20% to 40% versus the much lower percent return expected in other parts of the investor's portfolio. The typical investor in my fund anticipates making four to five times the capital that was invested. The venture capitalist, in addition to earning a modest salary and management fee, receives 20% of the fund's profits. Thus, the generation of a high profit is in the interest of the venture capitalist as well as the investors. Venture capitalists are constantly under pressure to demonstrate good performance to their clients, because the holdings of a fund are not all spent at once, and if clients are dissatisfied, they may want to withdraw unspent portions of their investment.

The Application: High-Priority Factors

When we evaluate applications for support, several factors have particularly high priority in our considerations: people, data, and the intellectual property (IP) package.

All of the people involved in the project are of extreme importance—the person who makes the initial presentation, the scientists, the management team, their partners, their lawyers, and their clinical development partners. It is very important for the scientists to realize that the people they select as their partners in developing and commercializing a compound can have significant ramifications for how they are perceived by the venture capitalist.

Data are important, not only in their substance but also in their presentation. We review a very large number of proposals, and we have developed a methodology for studying data rapidly and efficiently. The effort and quality of the science presented, whether preclinical or clinical, are crucial to capturing the interest of venture capitalists. The data need to create a story that conveys the essence and validity of the project. We often are presented with ideas that are promising, but the data are not packaged in a way that we can evaluate. Then, we have to decide whether to expend the time and effort required to figure it out. Often, we decide not to. The scientist and entrepreneur should appreciate that the first impression is of utmost importance, and they should endeavor to do an exquisite job of packaging their information.

The quality of the IP package is also crucial. When a proposal is of interest to us, we immediately refer it to our IP attorneys for evaluation. It is important that the law firm that has prepared the IP package has a reputation for doing extremely high-quality IP work. Before approaching a professional venture capitalist, the scientist/entrepreneur should have taken steps necessary to ensure that the IP estate will withstand a scrupulous review.

If, after evaluating the initial proposal, we are interested in investing, we need to determine whether the proposed product presents an opportunity for us to make a proper return on the capital that we are risking. This entails understanding the markets. We have companies in our portfolio that address markets of $300 million to $5 billion. It is critical to understand what the market is and how to segment that market, and to determine that a compound can find a market place big enough to justify the millions of dollars necessary to bring it ultimately to a commercial state.

With some proposals, the scientist/entrepreneur has taken the time, perhaps working with a strategy consulting firm or market research group, to realistically evaluate the size and segmentability of the market. They have done a competitive analysis, identifying competing compounds and describing the clinical superiority of the new compound under consideration. That kind of analysis can be extremely beneficial, because it allows us to accelerate our due diligence process and provide a faster response.

Orphan drugs, by definition, will not have a large market. However, they may involve a technology

Principal in Radius Ventures, LLC, New York City, NY.

whose initial indication is for an orphan disease, but also has potential applications for a much broader platform. In this case, the orphan indication becomes a strategy to expedite getting the product into the market, after which, the larger platform can be developed. I would encourage anyone who is working on development for an orphan indication to investigate the extent to which it might apply to a larger platform. If a larger platform potentially exists, we can work together collaboratively to understand the scale of the opportunity and the scale of the market.

Stages of Development of a Compound

The life cycle of a compound begins with discovery and is followed by early preclinical, late preclinical, early clinical, and late clinical stages, and the investigational new drug application. The cycle ends with the new drug application. Filing an investigational new drug application is one of the most important and valuable milestones that a company can achieve.

Within the venture capital community, there are investors who specialize in each stage of the compound's life cycle. It is important to direct a proposal to a company that typically finances projects at the current stage of the life cycle and to be relatively realistic regarding the perceived valuations of compounds at these various stages.

The milestones that mean the most to me as a venture investor, the ones I think create the most value for all the constituents of a project, are the investigational new drug application, Phase I clinical trials, and, ultimately, the new drug application. Radius Ventures is a venture fund that tries to focus on funding first-demand studies. We think that tremendous value can be created by picking the right compound and developing it effectively. Achieving milestones drives value, but not all milestones drive return. The scientist/entrepreneur needs to understand what the return milestones are and to help the venture capitalist calculate the timetable and the cost of getting to those milestones. From the information we have, we try to estimate how long it will take to reach the next major return-driving milestone, the risks and probabilities associated with reaching that milestone, and the value of that milestone. Some milestones are worth $5 million, and some milestones are worth $50 million. Obviously, we try to fund the milestones that can be worth as much as $50 million. These considerations all contribute to the valuation that we place on companies.

Radius Ventures is interested in orphan drugs, although not all venture capitalists are. One advantage of an orphan drug designation is that it provides a monopoly; thus, even a small market could be lucrative. Drugs related to preventing and curing blindness are good candidates for orphan designation, because of the high societal costs of blindness. Therefore, payers and the government would probably be willing to allow a very high price for a drug that could treat an orphan disease in ophthalmology.

Some venture capital funds now specialize within life sciences, and it would be a mistake to take a proposal for a pharmaceutical compound to a venture capital fund that does not specialize in life sciences. These companies know the healthcare field and have teams of advisors with MDs and PhDs who understand the science and the drug development process. When we invest in a company, our experts are available to help guide scientist/entrepreneurs as they try to build their company. In enlisting the support of a venture capitalist, entrepreneurs should consider not only the issues of money, trust, and integrity, but also what benefits the venture capitalist will add to the company to increase the chances of a successful outcome.