

Harvesting the Neural Gene Therapy Fruit

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INTRODUCTION

This is a critical time for the field of gene therapy. There have been spectacular recent successes (e.g., in the treatment of SCID and hemophilia B), but public wariness and government scrutiny have escalated following Jesse Gelsinger's death in the University of Pennsylvania OTC trial in late 1999. Although it is too early to predict the final effects of these events on government oversight of gene therapy, there has been a notable slowdown in the movement of gene therapy applications from the bench to the clinic. Gene therapy-related Investigational New Drug (IND) applications to the U.S. Food and Drug Administration (FDA) have decreased by half during the past year, and amendments to current INDs have almost tripled. Advances in the basic science underlying gene therapy are occurring at a breathtaking rate, but there appears to be an ever-thickening barrier to translating these advances to the clinic.

One area in which gene therapy holds great promise is in the treatment of neurological disease. There is a wide range of neurodegenerative disorders, including Parkinson's disease and Alzheimer's disease, for which no entirely effective therapies currently exist. Successes in treating a variety of neurological diseases in animal models are spurring investigators to consider clinical trials in humans with these diseases. However, recent events have led these investigators to hesitate. Where should the line between appropriate caution and the urgent need for developing treatments be drawn?

To address this and other questions, the National Institute of Neurological Disease and Stroke (NINDS) sponsored a two-day workshop on October 23 and 24, 2000, in Rockville, Maryland. The goal of the meeting was to assess the state of preclinical gene therapy science for both Parkinson's disease (PD) and the group of diseases collectively known as lysosomal storage disorders (LSDs). The meeting was organized by Robert Finkelstein and Robert Baughman of NINDS in conjunction with Inder Verma and Fred H. Gage from the Salk Institute for Biological Studies (see Table 1 for the complete list of participants). Experts in PD or LSD from basic and clinical science in academia and industry were brought together to assess the state of their respective area of study and to outline the remaining questions and/or needs before moving into clinical trials. In addition, regulatory officials from the NIH and the FDA were present, as well as representatives from several voluntary patient advocacy groups. Another goal of the meeting was to encourage new collaborations and information exchange that could help push

neurological gene therapy farther down the translational pathway. The following is both a report on the meeting discussions and a review of the many difficult issues that surfaced in the course of what all agreed to be a most productive and interesting gathering.

NEURODEGENERATIVE DISEASES AND GENE THERAPY: COMMON PROBLEMS

In many respects, the unresolved issues holding back trials of gene therapy for neurological disorders are the same as those facing any gene therapy application: What gene is appropriate for transfer? Which vector will be most effective? What route of administration is best? How do we monitor the expression of a transferred gene? Which animal model is most appropriate to the human disease we want to treat? And what are the outcome measures that tell us this intervention is working? These questions are fundamental to any translational gene therapy undertaking.

However, the nervous system, particularly the CNS, poses additional challenges. Gerald Fischbach, NINDS Director, pointed out in his opening remarks that "in the brain, geography is everything," and that it is therefore critical to get any gene introduced into exactly the right place. Furthermore, although many of the morphological and chemical effects of neurodegenerative diseases are known, the precise mechanisms underlying those defects are not yet well understood. Although advances are being made, the precise immune status of the brain remains uncertain. Finally, because of its complexity, the CNS can react in unanticipated ways to any kind of invasive procedure.

Nevertheless, tremendous strides in developing possible treatments have been made in animal models of human neurodegenerative diseases. The critical question is how to determine, for a specific disorder, when sufficient animal data have been generated to justify moving into the clinic. The two disorders considered at the meeting, PD and LSDs, highlight the problems involved in making this decision.

PARKINSON'S DISEASE: THE RIPEST FRUIT?

Current Status

A devastating and common disease, PD is linked to the gradual loss of dopaminergic neurons in the brain stem and their terminals in the striatum, although other subpopulations of neurons may also be involved. In his overview of the disease, Un Kang of the University of Chicago outlined the clinical features that lead to diagnosis of PD and the current treatment options aimed at slowing dopaminergic loss. Of all the treatments tried to date, oral

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TABLE 1
Gene Therapy for Neurological Disorders

Invited Participants

Krys S. Bankiewicz <i>National Institute of Neurological Disorders and Stroke Bethesda, Maryland</i>	Eugene M. Johnson, Jr. <i>Washington University St. Louis, Missouri</i>
John A. Barranger <i>University of Pittsburgh Pittsburgh, Pennsylvania</i>	Un Jung Kang <i>University of Chicago Chicago, Illinois</i>
Robert W. Baughman <i>National Institute of Neurological Disorders and Stroke Rockville, Maryland</i>	Jeffrey H. Kordower <i>Rush Presbyterian–St. Luke’s Medical Center Chicago, Illinois</i>
Martha C. Bohn <i>Northwestern University Chicago, Illinois</i>	Pedro R. Lowenstein <i>University of Manchester School of Medicine Manchester, United Kingdom</i>
Roscoe O. Brady <i>National Institute of Neurological Disorders and Stroke Bethesda, Maryland</i>	Ronald Mandel <i>University of Florida College of Medicine Gainesville, Florida</i>
Ennio A. Chiocca <i>Massachusetts General Hospital Boston, Massachusetts</i>	Anne Messer <i>David Axelrod Institute of Public Health Albany, New York</i>
Beverly L. Davidson <i>University of Iowa Iowa City, Iowa</i>	William C. Mobley <i>Stanford University Stanford, California</i>
Matthew J. During <i>Thomas Jefferson University Hospital Philadelphia, Pennsylvania</i>	Diane Murphy <i>National Institute of Neurological Disorders and Stroke Bethesda, Maryland</i>
Howard J. Federoff <i>University of Rochester Medical Center Rochester, New York</i>	Philip D. Noguchi <i>U.S. Food and Drug Administration Bethesda, Maryland</i>
Robert Finkelstein <i>National Institute of Neurological Disorders and Stroke Rockville, Maryland</i>	Amy Patterson <i>Office of Biotechnology Activities/NIH Rockville, Maryland</i>
Gerald Fischbach <i>National Institute of Neurological Disorders and Stroke Bethesda, Maryland</i>	Mark S. Sands <i>Washington University School of Medicine St. Louis, Missouri</i>
Fred H. Gage <i>Salk Institute for Biological Studies La Jolla, California</i>	Giovanna Spinella <i>National Institute of Neurological Disorders and Stroke Bethesda, Maryland</i>
Sandra L. Hofmann <i>University of Texas Southwestern Dallas, Texas</i>	Greg Stewart <i>Genzyme Corp. Framingham, Massachusetts</i>
Ole Isacson <i>McLean Hospital Belmont, Massachusetts</i>	Mark H. Tuszynski <i>University of California at San Diego La Jolla, California</i>
	Inder M. Verma <i>The Salk Institute for Biological Studies La Jolla, California</i>

L-dopa (levodopa) is still the most effective in a majority of patients that subsequently show PD pathology, despite the side effects and eventual “wearing-off” of this therapy in as many as half the cases. New chemical therapeutic agents currently in trials have the same goal, i.e., to stim-

ulate dopamine receptors in a more prolonged and sustained fashion or to restore dopaminergic function.

Because systemic delivery of L-dopa can lead to side effects such as hallucination and disorientation, there is great interest in developing a therapy that targets only the

area of the brain affected. There has been a great deal of publicity about one of these approaches, fetal tissue transplantation. Currently two double-blind, placebo-controlled trials are under way. Although there is some amelioration in clinical symptoms, a significant number of patients have developed runaway dyskinesias, indicating that there may be excessive dopaminergic stimulation from the transplant (a concern with some gene therapy approaches as well—see below). Even should this treatment ultimately prove effective, serious issues about tissue availability will probably prevent it from being widely used. Other approaches being studied include treatment with nerve growth factors (see below), the nondopaminergic suppression of “downstream” neurons that respond to the lack of dopaminergic stimulation, a variety of NMDA antagonists, pallidotomy, and direct stimulation of subthalamic nuclei.

The variety of approaches is encouraging, but it also reflects the uncertainty about the ultimate cause of the disease. The majority of cases are spontaneous, although genetic susceptibility has been linked to the genes encoding parkin, α -synuclein, and UCH-L1, all of which play a role in ubiquitin-mediated protein degradation. But most cases may arise as a result of oxidative stress, resulting finally in mitochondrial dysfunction.

Gene Therapy for PD: Two Approaches

There are several different gene therapy studies using animal models that hold promise for the clinic. These can be categorized into two approaches: (1) providing localized growth factors to sustain dopaminergic neurons and (2) replacing/supplementing critical enzymes in the dopamine pathway.

Jeffery Kordower (Rush Presbyterian–St. Luke’s Medical Center, Chicago) described his group’s recent work in which the gene encoding glial-derived nerve growth factor (GDNF) was introduced into both MPTP-treated and aging monkeys (1). Using a lentiviral vector that gave “amazing” expression, they showed that gene transfer gave a restoration of levodopa uptake, cell recovery, and even axon sprouting, as well as an increase in cell number. Little, if any, immune reaction was detected.

Enthusiasm for these results is tempered by questions about the relevance of the monkey models to the human disease, as the monkeys do not show the same neuronal loss pattern seen in humans. However, it may be a good model for early stages of the disease. It is also uncertain whether the injections of GDNF-bearing vectors would be more effective in the striatum or in the substantia nigra, based on rat studies presented by Martha Bohn (Northwestern University, Chicago, IL). Her results suggest that early disease may respond well to striatal injection, but later disease will not. Furthermore, it is clear that GDNF expression will need to be more tightly regulated before moving into human trials.

A second gene therapy approach, dopamine replacement, was described by Un Kang in a rat model and Krys Bankiewicz (NINDS) in primates. Issues include identifying

the optimal set of genes necessary to restore dopamine production and determining the most appropriate assays for evaluating the treated animals. The three most relevant enzymes are tyrosine hydroxylase (converts L-tyrosine to TH), GTP cyclohydrazase (produces the BH-4 cofactor), and aromatic amino acid decarboxylase (AADC, converts L-dopa to dopamine). However, simply transferring these genes without providing regulatory control could easily lead to the kinds of side effects seen with continual dopamine stimulation in other treatment approaches.

Bankiewicz described some success in a monkey model in replacing either the dopa decarboxylase or the AADC gene using convection-enhanced delivery of an AAV vector in conjunction with heparin. This prodrug-regulated strategy relies on subsequent treatment of the monkeys with oral L-dopa. Although the targeted cells were able to convert and release dopamine, they failed to store it. Kang has tried to address this issue by transferring not only the therapeutic gene, but also the gene for vesicle monoamine transporter, in order to produce more vesicle formation and, it is hoped, more sustained release. This approach does, indeed, enhance the effect of L-dopa treatment in a rat model. However, as Kang pointed out, “we know how to replace dopamine, but now we need to use an assay where there needs to be good longer smooth response without fluctuations and disruptive behaviors: we need models for efficacy assessment in animals that mimic this.”

What Blocks Movement to the Clinic?

Three issues emerged from discussions that appear to be obstacles to initiating clinical trials for PD: (1) determining the relevance of the current animal models to the human disease, (2) assessing the suitability/regulatability of any vector–gene combination, and (3) deciding whether the dopamine replacement or GDNF strategy is most promising.

There is significant concern about the appropriateness of current animal models of PD to the human disease. Howard Federoff (University of Rochester, Rochester, NY) pointed out that there are a variety of “causes” for PD that lead to the final shared pathway of dopamine depletion, and thus there are likely several different mechanisms at stake, all of which could be the appropriate target for intervention in any given animal/patient. This diversity of causes raises the question of whether *any* of the current animal models are truly appropriate, at least for a majority of human PD patients. Underlying the imperfection of the current animal models is, as pointed out by William Mobley (Stanford University, Stanford, CA), the fact that the basic biology of the pathways involved in PD is still unknown. How dopaminergic axons interact with their targets, how to determine when they are injured, and how the circuit works are all important unresolved issues, particularly in terms of possible use of growth factors. In addition, Kang argued that perhaps more important than finding animal models that show analogous neuropathophysiology is identifying good behavioral models by which researchers can assay the efficacy of experimental treatments. Finally, many participants pointed out that

long-term safety data, particularly in nonhuman primates, is still lacking (e.g., overexpressing GDNF for several years to see what effects it has).

What vector or construct is most appropriate for neural applications in human gene therapy? This is an even more difficult question to answer. As Matthew During (Thomas Jefferson University) said, everyone has their “flavor of the month.” However, it is clear that AAV, gutless adenovirus, and lentivirus vectors are farther along in development than others. Indeed, based on safety profiles alone, AAV has the clear lead, although lentivirus is rapidly gaining. One problem, particularly in the CNS, is that it remains unclear what cell types are being regularly infected and which are more likely to express the genes carried by the vector.

A more difficult problem is that the tools available for controlling the expression of introduced genes are currently very limited. The best of the existing crop, the tetracycline regulatory system, presents major concerns about the long-term use of tetracycline. There is also significant concern, particular in treating PD, that the inability to turn off the introduced genes will result in unacceptable side effects. Furthermore, if gene introduction causes unanticipated problems, it would be almost impossible to reverse the effects given the current constructs. Thus, many participants argued that it is critical to find better systems for regulatable gene expression before moving into clinical trials with either growth factors or enzymes in the dopamine pathway.

Which, if any, approach should move first into the clinic? This question is a difficult one to resolve. It is clear that growth factor (GDNF) intervention has had significant success in primate models, but that rodent models and our current understanding of PD pathogenesis seem to favor replacing enzymes in the dopaminergic pathway. Both strategies hold great promise, and it was clear to the participants that each should be pursued. In both cases, however, it will be important to consider these approaches in the light of currently available treatment strategies.

An interesting side note to this deliberation emerged at the workshop. The U Penn incident revealed just how extensive and expensive the necessary regulatory infrastructure is, a fact that many academics believe effectively prevents them from undertaking phase I clinical trials (not all NIH institutes provide money to adequately meet this need). Nevertheless, Fischbach told participants that there is “no funding limitation to phase I trials” and that NINDS is interested in moving neural gene therapy applications forward to the point at which safety can be demonstrated in phase I trials.

LYSOSOMAL STORAGE DISEASE: THE LOWEST-HANGING FRUIT?

Current Status

The second part of the meeting shifted attention from PD, a highly localized, late-onset, and relatively common dis-

order, to the family of LSDs, which affect every cell in the body very early in life, but are fortunately very rare. As Sandra Hofmann (University of Texas Southwestern Medical Center) outlined in her overview of the LSDs, the genetic deficiencies in these disorders affect critical enzymes normally located in the lysosome of the cell, resulting in excessive storage of undegraded molecules and, ultimately, cell death. Mutations affecting at least 40 different gene products that can lead to LSDs have been identified, including proteases, glycosidases, phosphatases, sulfatases, and several other classes of enzymes. In addition, defects in enzyme cofactors, chaperone proteins, enzyme recognition signals, and lysosomal movement and biogenesis proteins can lead to LSDs.

This complex situation is further obscured by nomenclature: an LSD can be named for its discoverer, its histology, the chemical nature of the undigested material in the lysosome, the underlying enzyme deficiency, or all of the above. Add in the vast variety of clinical features and the genetic heterogeneity associated with any given LSD, and it becomes almost impossible to keep track, with approximately 150 different names for the 40 known disorders.

The precise prevalence of LSDs is unknown, although a recent Australian study suggests that any given LSD has a prevalence of 1 in 50,000 to 1 in 1 million. As a group of diseases, LSDs have a relatively high prevalence of 1 in 5000. The relative rarity of individual disorders may be the biggest obstacle to gene therapy, as discussed below.

Current treatment for lysosomal disorders includes enzyme replacement (e.g., for Gaucher’s disease, and in trials for MPS1, Fabry’s, and Pompe) and bone marrow transplantation (for Krabbe and metachromatic leukodystrophy). Stem cell transplantation has been suggested as an alternative to bone marrow transplantation, but the response in those tested to date has been incomplete. Enzyme replacement therapy runs into the problem of crossing the blood-brain barrier, limiting the number of successful treatments.

Gene Therapy of LSD

Despite their complexity, several aspects of the LSDs make them attractive candidates for gene therapy clinical trials. Unlike PD, in which the precise defect is unknown, most of the LSDs have been associated with a specific gene target. Further, unlike most cases of PD, there is a relative lack of environmental and outside factors on the phenotype. Since mannose 6-phosphate receptors can take up excreted enzyme from adjacent cells, there is a strong potential for “cross-correction” in the brain. More importantly, because of the pleiotropic effects of these disorders, highly spatially localized regulation of the transgene is probably not essential. Finally, well-characterized biochemical markers and intermediate endpoints have been worked out in animal models.

One of the best-characterized animal models of a human LSD to date is the naturally occurring mucopolysaccharidosis (MPS) type VII (aka “Sly’s disease”) rodent. MPS type VII animals and humans are deficient in the enzyme β -glucuronidase, which breaks down glycosaminogly-

cans. This enzyme deficiency causes a variety of clinical and behavioral abnormalities. The human form of the disease is marked by progressive mental retardation from an early age, while the mouse model shows significant defects in spatial learning and memory. In both cases, the hippocampal region appears to be most significantly affected by the disease.

Gene therapy treatment with the β -glucuronidase gene has been successful in animals using either an AAV (Mark Sands, Washington University School of Medicine) or a feline immunodeficiency vector (Beverly Davidson, University of Iowa). "Success" here is defined as correcting both the behavioral defects and the gross pathology. Interestingly, the defect appears to be correctable even after the onset of the disease, although the mechanism of this correction is unclear. This is an important consideration for possible human trials, as the pathology for most of the LSDs begins to develop well before birth.

What Prevents Movement to the Clinic?

Despite successes in animal models and the knowledge of the genetic lesions underlying most forms of LSD, there are still significant obstacles to proceeding to clinical trials. The largest, and most intractable, is the relative rarity of any given LSD. For the best-characterized animal model, MPS type VII, very few patients have been identified. Indeed, as one participant pointed out, there are probably 1000 cured mice for every human patient! Most LSDs are not this uncommon; patients typically number in the low hundreds for a given disorder. Even so, establishing a clinical trial that will give significant results may be difficult for purely methodological reasons, not to mention the difficulty in funding such a venture.

Getting neural expression of a gene throughout a rodent brain is relatively easy: a bigger brain is another question. A vector that can infect larger areas of the brain may be necessary. In addition, although regulation is not as big a concern as it is in PD, the gene should be regulatable in order to "turn it off" should unforeseen adverse reactions arise.

A third issue involves the goal of gene therapy for LSDs. For example, although the pathology of MPS VII mutant mice greatly resembles that of patients with the disease, clinical/behavioral symptoms are quite different. It will be important to determine exactly what outcome measures will be used to determine the effectiveness of gene therapy before initiating clinical trials.

Finally, as is true of PD, the biological processes underlying the LSDs is poorly understood. Stated differently, it is unclear how aberrant storage leads to cell death and ultimately to the pathological features of these diseases. If the pathways leading to each of the LSDs were better understood, gene therapy interventions aimed at replacing elements of these pathways in addition to the affected enzymes might prove effective.

Despite these obstacles, Greg Stewart (Genzyme Corporation) gave an overview of the company's decision to

aim for gene therapy trials for at least one of the neural forms of the LSDs. Genzyme is currently considering Batten disease, Niemann-Pick A, and Krabbe's disease as candidates for further development. Stewart's optimistic presentation, and the active encouragement by meeting participants, suggest that even with few patients the probable success of such a trial will not only give a needed boost in the arm to gene therapy as a field, but also provide valuable information for other neurological applications of gene therapy.

WHERE DO WE GO FROM HERE?

Despite the important issues that must be resolved before undertaking clinical trials for PD or the LSDs, participants in the meeting were very positive about moving ahead. Identified problems that they agreed must be addressed include:

1. The need for better regulatable vector systems.
2. The need to streamline the manufacturing and testing of vectors.
3. The development of appropriate animal models (e.g., larger animals for LSD and models more closely resembling the human disease for PD).
4. Overcoming the extremely high costs of developing regulatory infrastructure to support clinical trials in an academic setting.
5. Our incomplete understanding of the basic scientific mechanisms that underlie both PD and the LSDs.
6. The need to facilitate the resolution of basic safety and toxicology considerations prior to any intervention.
7. Determining the appropriate clinical markers and endpoints for clinical trials (PD and LSD).

How can the goal of translating recent successes into the clinic best be achieved? Because this can be a daunting objective for isolated investigators or even institutions, Philip Noguchi from the FDA suggested that NINDS create a "task force" to facilitate the process. Such a group, which would include representatives from academia, NIH, FDA, industry, and patient advocacy groups, could work with investigators to help make gene therapy clinical trials a reality. The participants at the meeting were enthusiastic about pursuing this course.

Perhaps the final unifying theme that emerged from this workshop was the need to promote translational research. The participants agreed that further basic research is essential, but that NINDS should promote specific research that addresses the translational issues that were raised in the course of the meeting. Recent treatment successes in animal models suggest that gene therapy clinical trials for PD and the LSDs may be closer than is now thought; what is needed are the tools and labor for ensuring the success of the harvest.

REFERENCE

- ¹ Kordower, J., *et al.* (2000). Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. *Science* 290: 767-773.