Published in final edited form as:
Mol Ther. 2008 April ; 16(4): 640–646. doi:10.1038/mt.2008.42.

Gene Delivery to the Nervous System:

NINDS Workshop on Gene Delivery to the Nervous System

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The National Institute of Neurological Disorders and Stroke (NINDS) sponsored a workshop on gene delivery to the nervous system, which took place on 12–13 November 2007 in Washington, DC. The purpose of the workshop was to convene neuroscientists, molecular virologists/vectorologists, and surgical neurologists to assess the state of the art of gene therapy for neurologic diseases and brain tumors and to address the challenges for advancing promising preclinical studies to the clinic.

Since the last NINDS workshop on gene therapy, in 2000 (ref. 1), which focused on Parkinson's disease (PD) and lysosomal storage diseases (LSDs)—perceived to be the lowest-hanging neural gene therapy fruits—much progress has been achieved on several fronts. Several phase I and II clinical trials have been completed or are currently in progress, including trials for PD, LSDs, Alzheimer's disease (AD), amyotrophic lateral sclerosis (ALS), epilepsy, peripheral neuropathy and pain, multiple sclerosis, and muscular dystrophy. Since the first clinical gene therapy trial, in 1989, more than 1,300 clinical trials have been carried out or are in progress worldwide. Of these, approximately 100 trials were directed either against the neurologic diseases listed above (http://www.wiley.co.uk/genetherapy/clinical)2 or brain tumors.3 These encouraging numbers are evidence of a growing and maturing field. The shared vision of permanently correcting a neurologic defect by the delivery of a gene attracted most of the workshop participants to this research. This quest requires a detailed understanding of the dynamics of the disease, the therapeutic alternatives and strategies and their potential side effects, and the mechanisms and routes for safe *in vivo* gene delivery, all of which represent enormous and unprecedented challenges.

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Publisher's Disclaimer: This NINDS workshop was co-organized and co-chaired by Drs. Manfred Schubert and Pedro Lowenstein. Formal presentations were by invitation only. Attendance was open to the public and only limited by available seating. Drs. Breakefield, Federoff and Frederickson summarized the discussions by three separate breakout discussion groups followed by a full-panel discussion. The views, conclusions, and recommendations from these discussions are included in this report but are not necessarily endorsed by NINDS.

HIGHLIGHTS

During the workshop there was great excitement about the promise for gene delivery to the nervous system on several counts. First, there was a collective realization that a variety of gene delivery methods now provide strong scientific underpinnings for the design and implementation of powerful therapeutic strategies that can tackle focal as well as global disease; further, as a result of the field's maturity, the enthusiasm is now combined with a realistic assessment of the challenges to be overcome. Second, based on many gene therapy trials for a wide range of diseases, it appears that the nervous system may offer practical advantages for intervention, including a reduced immune response to vectors and transgenes in the brain parenchyma as compared with other peripheral sites of injection, focal targets that require limited gene delivery within the brain (e.g., in PD), the presence of only a few normal replicating cells that might be transformed by vector integration into their genome, and the reduced ability of vectors injected into the brain to access germline tissues as compared with peripheral injections. Third, multiple strategies and clinical trials are being implemented for several diseases of the adult nervous system, including PD4 and glioblastoma multiforme,3 with the hope that at least one will provide therapeutic benefit in these high-profile and relatively common conditions.

Routes of gene delivery: the long and winding road

Many studies have been published regarding delivery of genes to the nervous system. Strength of transgene expression and distribution throughout the brain are dependent on vector backbone, promoters used, and site, volume, and rate of injection.5,6 Different vectors, as well as delivery in various stages of brain development, have been used to achieve either focal or diffuse delivery throughout larger areas of the brain. However, diffuse vector delivery covering large areas of the brain remains an important challenge. This will be especially important in the treatment of diseases such as AD, which affects the structure of most of the neocortex and hippocampus.

One approach that has been proposed to homogeneously cover larger areas of the brain is convection-enhanced delivery.7 This method, using programmable pumps and specifically designed injection catheters, has been used to make adeno-associated virus type 2 (AAV-2) vector delivery to the nonhuman primate striatum much more even, efficient, and reproducible than single injections of smaller volumes (as reported at the workshop by Drs. Bankiewicz, Starr, and Lonser—see accompanying list of participants). The convection-enhanced-delivery approach can achieve comprehensive delivery to the striatum/globus pallidus/subthalamic nuclei using AAV-2 vectors. Gene delivery can also be imaged in real time using co-injections of liposomes containing gadolinium (Bankiewicz) or gadolinium bound to albumin (Lonser).

An alternative approach to accomplishing widespread delivery throughout the nervous system was described by Dr. Wolfe, who achieved essentially global gene product delivery expressed from AAV vectors in animal models of LSDs. Upon viral entry, the vector cores are retrogradely transported within neurons through their extensive processes depending on the vector serotype and the injection site. This delivery strategy consists of injecting vectors into nuclei from which originate long diffuse neuronal tracts throughout much of the forebrain (e.g., the locus coeruleus). In addition, the vector-encoded gene product moves within neurons through axonal transport, is released at synaptic terminals, and is then taken up by cells located at the target areas. In this way the replacement enzyme ends up being transferred from cell to cell. One microliter of vector suspension was sufficient for nearly global delivery to mouse brain, representing 1–2 ml on the scale of the human brain. This strategy replaces the invasiveness of multiple vector injections by using the neuronal network itself for delivery of the transgene and its encoded product.8

Direct delivery of virus vectors into the brain ventricles has also been attempted in order to achieve diffuse distribution of transgene products. Although this may play a role during *in utero* gene delivery or very early postnatally (when the immune system is still immature), once the immune system has developed, this is unlikely to be of clinical utility. Data presented by Dr. Lowenstein indicate that intraventricular injection of adenoviral vectors can elicit a strong T-cell response, similar to those stimulated by direct injection of adenoviral vectors systemically. Such responses can block transgene expression in the central nervous system (CNS); some of the loss of transgene expression is due to the immune-mediated killing of vector-transduced brain cells following local cell recruitment induced by inflammatory responses. In contrast, intraparenchymal injections of viral vectors do not stimulate a systemic immune response, most likely because of the absence of viral vector diffusion and a lack of immune-cell recruitment. This finding has important implications for the selection of target sites for vector injection because it can affect the safety and efficiency of gene delivery to the CNS.9

Delivery of therapeutic transgenes or even vectors from peripheral tissues into neurons remains an important step toward delivery to areas of the brain such as the spinal cord.8 Some success was reported using monoclonal antibodies to the transfer-rin receptor or insulin receptor conjugated to liposomes to cross the blood—brain barrier (Boado), via uptake through the neuromuscular junctions using the tetanus toxin C (TTC) fragment conjugated to either superoxide dismutase (SOD) (TTC:SOD; Fishman) or β-glucuronidase (GUSB:TTC; Wolfe), and by intraventricular injection of glial cell line—derived neurotrophic factor (GDNF:TTC; Fishman). Nevertheless, there are serious immunologic considerations to take into account when using TTC, because tetanus toxin is highly immunogenic and the majority of the US population has been immunized against it. To increase targeted delivery from the periphery using viral vectors, Dr. Boulis finds increased neuronal uptake of AAV-1 by inserting into the viral capsid protein a short tet1 peptide, specifically selected from a phage display library to recognize the tetanus toxin receptor. Peptide insertion resulted in retrograde transport, but peptide presentation still needs to be optimized. Neurotropic lentiviral vectors—equine infectious anemia virus (EIAV) and HIV-1—pseudotyped with rabies glycoprotein were considered potential alternatives for targeting neurons,10,11 including motor neurons following intramuscular injections12,13 (Mazarakis and Schubert). AAV vectors expressing insulin growth factor-1 for the potential treatment of ALS have also been delivered to the spinal cord via muscles, presumably through transport by way of motor and sensory neurons.14

Synthetic vectors

Perceived limitations in the use of viral vectors in terms of potential toxicity have stimulated the development of “synthetic” or “nonviral” vectors. Nonviral vectors have come of age, with various clinical trials either finished or in preparation.15 However, none of these has yet been used in the CNS or for the clinical treatment of brain diseases. Data presented by Dr. Wolff support the advantage of rational design in generating synthetic vectors for nucleic acid delivery. Chemical components (polyethylene glycol) were used to achieve serum stability and receptor targeting, joined to a polymer with pH-cleavable bonds, which allow release from the endosomal compartment for delivery of genetic information—e.g., short interfering RNAs—to the cytoplasm. This strategy is currently very effective at delivery to the liver; studies in the brain are ongoing. Dr. Pun described ways to increase gene delivery to neurons, including targeting using the tet1 peptide (developed by Dr. Boulis), which enters via the tetanus toxin receptor. Her strategy combines peptides that target retrograde motors with peptides that are lytic so as to achieve nucleic acid release from endosomes into the cytoplasm, which is critical for activity. An issue not directly addressed at the workshop was that, as compared with viral vectors, the relatively short duration of transgene expression by most synthetic vectors must be improved if they are to be useful for many therapeutic applications—especially if they

require invasive delivery procedures. For clinical indications that require only short-term expression (e.g., during early post-stroke interventions), transitory gene expression may be less of a limitation.

Brain tumors: gene therapy clinical trials moving into phase III

Relative to other diseases, brain tumors are rapidly fatal, killing patients in less than 2 years after diagnosis. Although the main tumor mass is usually localized at diagnosis, even complete resection of adult glioblastoma multiforme fails to cure affected patients because of invasive microfoci. Thus, novel alternatives are being discussed. Despite recent improvement in the treatment of such tumors using novel chemotherapeutic approaches, none of these is likely to work in all patients. At the workshop, three very promising and potentially complementary strategies for gene therapy of brain tumors were outlined. Dr. Castro described an enhancement of immune rejection of tumors by using the adenovirus—thymidine kinase/ganciclovir (Ad-TK/GCV) vector system for delivery, combined with Ad-Flt3L. Flt3L has been shown to attract dendritic cells to the brain; importantly, those immune cells stimulate a systemic, long-term, $CD8^+$ T-cell-mediated immune response directly from the brain itself.16 The idea is that this strategy may prime the immune system to recognize glioma cells that have undergone new mutations, one of the main challenges in the treatment of glioblastoma multiforme. In another strategy, oncolytic adenoviral vectors with delta-24 mutation in E1A were used because they proliferate preferentially in Rb(–) tumors. Infection can be targeted to integrins, preferentially found on glioma cells via the RGD tripep-tide. Subsequent death of the tumor cells apparently occurs through an autophagic pathway17 (Fueyo). Dr. Chiocca also demonstrated that innate immune responses can reduce the efficiency of viral vectors in the brain. The therapeutic effect of oncolytic herpes simplex virus (HSV) vectors can be enhanced by co-administration of cyclophosphamide, which decreases on-site production of γ-interferon that would otherwise stimulate $CD68^+$ and $CD163^+$ lymphocyte infiltration, result in a curtailment of the spread of the oncolytic virus, and cause an enhanced toxic inflammatory response in the brain.18 Importantly, in the United States there have been phase I trials of Ad-TK and replication-competent adenoviruses, and others are expected to begin soon; moreover, the results of a large multicenter double-blind phase III trial recently completed in Europe are expected this summer. A phase I trial combining Ad-TK and Ad-Flt3L is expected to commence in late 2008 or early 2009.

Other neurologic diseases

Late infantile neuronal ceroid lipofuscinosis (Batten disease)—Treatment for the LSD known as late infantile neuronal ceroid lipofuscinosis using AAV-2-mediated gene delivery is in early-phase clinical stages; the field will need to await the results of longer follow-up of patients and larger clinical trials to deduce indices of therapeutic benefit. Current protocol modifications include switches to AAV-10 for increased time of transgene expression and earlier intervention in the course of the disease (Crystal).19

Pain—Chronic neuropathic pain is the consequence of microglia and astrocyte activation in the spinal cord with release of proinflammatory cytokines. This response can be alleviated by the delivery of interleukin 10 using adenovirus, AAV, and nonviral vectors. The duration of interleukin 10 expression should be extended (Milligan). The groups of David Fink (University of Michigan) and Joe Glorioso (University of Pittsburgh) are also very close to implementing clinical trials of gene therapy for pain using HSV-1 vectors20 to deliver the endogenous morphine-like peptide precursor proenkephalin and/or other proteins such as interleukin 4, glutamic acid decarboxylase, tumor necrosis factor-α, or GDNF, which can reduce pain.

Neurodegenerations: ALS, AD, PD, and prion diseases—Delivery of insulin growth factor-1 in a mutant SOD model of motor neuron degeneration acts by reducing activation of

astrocytes and microglia and prevents death of motor neurons in mouse models of ALS through activation of the Akt pathway (Kaspar).14 Initial clinical trials using fibroblasts and AAV to deliver nerve growth factor in AD brains showed no adverse events with positive signs of increased glucose uptake and neuronal sprouting, which are signs of reversal of the metabolic decrease after gene delivery. A phase II clinical trial is being planned on AAV-mediated gene delivery of nerve growth factor in AD (Tuszynski).21 Another strategic approach for AD is active vaccination against the amyloid-β (Aβ) peptide, which accumulates in amyloid plaques, initially using HSV-derived amplicon vectors. This approach allows for immune shaping in which a T-helper 2 (Th2) response driving therapeutic anti-Aβ antibodies was achieved instead of the Th1-type response, which results in cytotoxic T-cell response; the latter is believed to have caused aseptic meningoencephalitis in the Elan Pharmaceuticals AD peptide vaccination trial. This approach was followed by a prion model of neuronal degeneration using another immunologic strategy involving intracranial (thalamus/striatum) injections of AAV-2 vectors expressing a single-chain antibody, which blocks the conversion of the cellular prion protein (PrP) to scrapie PrP^{Sc} amyloids. This strategy conferred increased survival, reduced disease burden, and improved neurobehavioral functions in mice that were challenged with PrP^{Sc} (Federoff).22

Several strategies are being tested in phase I trials for PD. In one trial an effort was directed toward reducing neuronal loss by AAV delivery of the neurotrophic factor neurturin, which reacts with the same receptors that GDNF does and leads to an increase in ^{18}F-dopa uptake in the brains of both aged monkeys and monkeys treated with MPTP (Kordower). There is no evidence of toxicity at the maximal tolerable dose in Different animal models in phase II trials being conducted by Ceregene Inc. (Bartus). Another promising trophic effort is the delivery of the heat shock protein HSP104 and the PD gene protein parkin in mice overexpressing α-synuclein using a lentivirus vector (Zufferey). Interestingly, GDNF does not prevent α-synuclein toxicity. In some cases human PD is caused by elevated expression of α-synuclein. This indicates the need for multiple animal models to parallel the multiple known causes of PD in humans. Given that l-dopa is the common therapy for PD but loses its effectiveness over time, efforts are also underway to increase l-dopa conversion to dopamine by AAV delivery of aromatic amino acid decarboxylase to facilitate the conversion of l-dopa to dopamine (Bankiewicz)23 or to increase *de novo* synthesis of dopamine using a multicistronic lentivirus vector based on EIAV that encodes the biosynthetic enzymes tyrosine hydroxylase, aromatic amino acid decarboxylase, and guanosine triphosphate cyclohydrolase (Mazarakis).11 Phase I–II trials have been approved and are commencing in France. Another promising PD therapeutic strategy is AAV-2-mediated delivery of glutamic acid decarboxylase in the subthalamic nucleus to increase levels of limited γ-aminobutyric acid production and thereby inhibit excessive firing of neurons in the substantia nigra, which contributes to compromised motor function in PD. A phase I safety trial was completed without adverse effects. The clinical protocol approved by the FDA limited the treatment to one hemisphere. A phase II trial is planned to verify the potential benefits that were recorded on the patients' contralateral side, as had been anticipated (Kaplitt and During).4,24

RNAi: turning off the gene tap

For many years, gene therapy's strength was in the overexpression of therapeutic genes. In a number of applications—foremost the dominantly inherited neurologic disorders, as exemplified by Huntington's disease—inhibiting the expression of the disease-causing alleles would be the ideal therapeutic goal. The use of RNA interference is now fulfilling this promise, and a number of experimental approaches have achieved major breakthroughs in this area. It is likely that clinical trials using RNAi for the treatment of dominant diseases will be implemented in the very near future.25

FUTURE CHALLENGES

Vectors: what works now, what will work in the future, what works in experimental models—can we predict what will work in human patients?

Vectors that seem most promising for clinical applications in neurologic diseases are nonreplicating AAV and gutless adenovirus vectors for neurodegenerative disorders and replication-defective and conditionally replicating (oncolytic) adenovirus and HSV vectors for brain tumors. The first clinical trial with HIV-1-derived lentiviral vectors targeted $CD4^+$ T lymphocytes in AIDS patients. The cells were stably transduced by the vector and expanded *ex vivo*. The autologous HIV-1-resistant cells were reinfused into the patient with no apparent adverse effects (Dropulic).26 Integrating lentiviral vectors are currently used in clinical trials to target hematopoietic cells to treat mucopolysaccharidosis type VII. The facility with which lentiviral vectors transduce bone marrow *ex vivo* is likely to be harnessed to treat several diseases, including metachromatic leukodystrophy.27 Safety and efficacy studies of lentiviral vectors in the nervous system have been carried out in nonhuman primate models of PD (Mazarakis). There is a lack of evidence that HIV-1 integration can cause tumors in AIDS patients, and long-term follow-up will be necessary to evaluate the potential risk of integration by HIV-1-based vectors. The potential use of these vectors for the nervous system should be further explored. Results of the recently approved phase I–II clinical trials of an EIAV-based lentiviral vector against PD will be very important, although the vector safety may not be known for many years. In the case of AAV, cataloguing of the many new serotypes derived from humans and rhesus macaque monkeys with respect to infection of cells in the nervous system must be undertaken. Differences in vector-uptake efficiency among the various neural cell types, along with differences in the cellular transport mechanisms for the vector core, could greatly help in the targeting of transgene expression to specific regions within the nervous system (Mandel). For all vector types, standards will need to be established. Ideally, this should be done in concert with the US Food and Drug Administration (FDA). Resources will need to be made available for the construction of Good Manufacturing Practice—grade vectors and for carrying out clinical trials.

A systematic functional mapping of vector distribution and expression might highlight new vector delivery routes that could potentially reduce the total number of injections and thereby increase vector efficacy and safety. Vector loss during purification and injections (e.g., binding to tubes) must be minimized to lower the cost of highly purified vector preparations and reduce toxic contaminants.

The need for transgene regulation continues to be debated within the community. There was disagreement about whether transgene regulation should be required for clinical trials. For some therapeutic purposes, drug regulation of transgene expression may prove important, but the current regulatory strategies have complications of leakiness, added side effects of the drugs used to induce or inhibit transgene expression, and the potentially unknown immunogenicity of the regulatory proteins, most of which are derived from prokaryotic organisms. Further, from a regulatory standpoint, the use of currently available inducible systems in clinical trials substantially raises the financial bar in testing the safety and efficacy of the inducible systems themselves. Innovative and potentially simpler approaches to transgene regulation should be explored and are likely to have a major impact on the future of gene therapy. Gene therapy researchers have worked for decades to achieve long-term expression vectors. Added to the particular immune privilege of the brain, the long-term effects of expressing powerful growth factors in the brains of patients over decades must be approached with caution.

Vector repository: making viral vectors readily available to the neuroscience community

Although the use of viral vectors has increased exponentially over the past 10 years, most laboratories that wish to use state-of-the-art vectors and expression cassettes still need to construct and produce the vectors in their own laboratories. The need is thus felt for a repository of vector constructs that would be shared within the neural gene therapy field. This would greatly facilitate the exchange of vector constructs and inserts that are useful for the nervous system (Bohn). The availability of small amounts of preclinical vector preparations for *in vivo* testing could become a valuable national resource for the neuroscience community, including investigators not engaged in translational research. Small amounts of *in vivo*—grade vectors could be made available and serve as tools for hypothesis-driven research. Investigators would be able to explore fresh ideas that might lead to unexpected and potentially valuable findings. A repository would significantly reduce the time it takes to develop an initial idea into the actual vector injection into an animal model. Sharing vectors and DNA constructs would greatly help advance the field by reducing redundancy in the evaluation of new DNA constructs. Preferably, this should be accomplished in highly interactive collaborations among investigators in neuroscience, molecular virology, and surgical neurology, which will enhance the safety of clinical studies while efforts are being made to achieve therapeutic efficacy.

Animal models: is small always beautiful…enough?

A goal in translational gene therapy is to perform experiments that will facilitate the future implementation of successful clinical trials. Using the most relevant models for each disease is a challenge that, given the accelerated translation of many gene therapy approaches, is coming more and more to the fore. The relevance of some animal models for neurologic diseases has been questioned—that is, whether these models are equivalent to the human disease. Often the cause of human disease is unknown, and there may be multiple causes, as with PD. Therefore, therapeutic strategies often focus on increasing neuronal survival without directly counteracting the cause of the disease; this has been the case for many effective drugs. Immune responses to vector antigens or encoded transgene products can greatly differ between humans and the animal model, which could potentially invalidate the relevance of preclinical animal studies. Differences in the sizes of the human brain and the brains of animal models—2,000-fold for mice and 14-fold for rhesus macaque monkeys—pose enormous challenges for scaling up vector delivery.

The use of somatic nuclear transport would enable larger animal models of human genetic diseases to be cloned at high efficiency for preclinical studies. For example, efficient cloning of mini-pigs with a brain the same size as that of a rhesus macaque monkey may be more cost-effective for testing. Most importantly, it would allow the assembly of cohorts of large, genetically identical animals, thereby limiting variability during efficacy studies of therapeutic gene delivery. Mini-pigs that express the Aβ precursor protein (β-APPsw), which includes the Swedish mutation for early disease onset of AD (Jørgensen), were recently cloned. According to Dr. Jørgensen, some diagnostic cognitive tests with pigs can be made similar to tests with humans. Despite these advantages, it seems unlikely that such larger, cloned animals would be readily adopted as models because few groups are using mini-pigs. The pharmaceutical industry has traditionally used canines for toxicological studies. Dogs and cats have slightly smaller brains than rhesus macaque monkeys do, but they suffer several diseases that gene therapy researchers wish to target, such as brain tumors and LSDs.28 Although performing studies in larger animals has its own challenges, being able to treat natural rather than induced diseases may provide a more accurate perspective on the treatment of some human diseases. On the other hand, when a disease has a known genetic cause, and a larger animal model can be created with the identical genetic etiology in a congenic background, this can provide a platform for reduced variability in quantitative assessment of therapeutic efficacy. The lack of large animal models for most human neurologic diseases may necessitate separate studies on

the efficacy of gene delivery vs. the therapeutic efficacy of the transgene in animal species of Different sizes. Whether this scenario will meet approval by the FDA and the Recombinant DNA Advisory Committee (RAC) depends on the disease and the animal models used, and it will require special review.

FDA and RAC regulation

The FDA's Center for Biologics Evaluation and Research (CBER) presented at the workshop a list of criteria for evaluating the safety of proposed trials (Chen) before and during the trial and its follow-up period (Maher). Investigators continue to look to the CBER for guidance in the planning of clinical trials with respect to such aspects as safety/toxicity, dosing, and vector design/production. The FDA is responding to these needs and is willing to help (Chen and Maher), although there is a feeling on both sides that insufficient resources are available to provide all the expertise needed for standardization and rapid compliance. Increased funds could help ease frustrations on both sides and might more quickly advance Investigational New Drug applications for new vectors to the clinic. The RAC is viewed in two lights. To some, it is a hurdle to regulatory aspects already carried out by the CBER, which nevertheless impinges on conclusions reached by the local institutional biosafety committee (for example, although not strictly mandated by the FDA, a local institutional biosafety committee will not consider gene therapy clinical protocols that have not been reviewed by the RAC). Others see the RAC as a source of helpful input and advice from peers on the improvement of trial design and the minimization of untoward effects, while maintaining an open-door policy for the general public. There have been calls for multiple review processes to be harmonized, and it is clear that the FDA and the RAC now collaborate more closely than in the past to facilitate review and recommendations.

New venues for funding translational neurologic gene therapy

Collectively there is a sense of the huge promise of gene therapy for neurologic disease as well as an increasing awareness of the time it will take to achieve this potential. Investigators are encouraged to assist one another in standardizing vectors and sharing Investigational New Drug information to help promising strategies reach the clinical trial level because the success of any one clinical trial will surely serve to promote the field and help realize its long-term promises. For example, it would be helpful to have a Cold Spring Harbor course at Woods Hole, Massachusetts, on such topics as vector generation, animal models, and biodistribution, as well as a shared repository of vector backbones and packaging cells/plasmids. The most critical basic science topics are immunogenicity, transgene regulation, establishing the veracity of animal models, understanding the cause and process of disease, vector delivery, cell targeting, gene silencing, and accessing the brain across the blood—brain barrier. In the clinical arena, the pressing issues are standardization of trials across studies, the use of biomarkers, the understanding of optimal patient cohorts, and the parameters to be monitored to evaluate efficacy and safety.

The National Institutes of Health (NIH) supports hypothesis-driven research primarily through the R01 grant mechanism. Translational research that includes gene therapy is supported by either a single-component U01, a multicomponent U54, or the U24 resources funding mechanisms (Koroshetz and Porter). Various review panels are aware of the translational or resource goals of U proposals. Although many efforts in gene therapy development are hypothesis-driven, there needs to be a transition of hypotheses that appear valid into a milestone-driven mode and toward potential clinical trials. Gene delivery thus serves many modalities and can be used to discover the cause of neurologic disease. As such, it is more closely related to basic neurobiology, while at the same time it can highlight new therapeutic opportunities. *In vivo* DNA/RNA delivery for protein or siRNA expression will undoubtedly be used in modalities for an increasing number of applications and has great therapeutic

potential. It was acknowledged at the workshop that there is a clear need for close collaboration and new and creative partnerships among academic institutions, clinical research centers, and biotechnology companies to negotiate the hurdles to clinical trials as well as to find innovative funding mechanisms to pay for clinical- and Good Manufacturing Practice—grade vectors, biodistribution and toxicity studies, and clinical trials. An external consulting facility could facilitate movement of strategies into early stages for clinical trials.

Establishing a gene therapy resource program for basic and translational gene delivery to the nervous system could greatly help the entire field; such a project was recently

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implemented by the National Heart, Lung, and Blood Institute (http://www.gtrp.org). Another possibility is a program similar to the NIH's Rapid Access to Interventional Development pilot project but designed specifically for gene therapy for diseases of the nervous system. The benefits would include cost sharing and avoidance of redundancy. Such concerted efforts may help in the discovery of new vector tropism that could lead to more efficient delivery routes for the vectors and their encoded gene products in humans, important routes that might not have been anticipated.

Although in past years the public's perception of gene therapy has not been favorable following some early sad outcomes, the tide has recently been turning back to enthusiastic support. Early expectations for miraculous cures were not fulfilled, and even now the importance of the exciting success of the X-linked severe combined immunodeficiency trial is not fully appreciated. Because of the complexity of gene therapy approaches, the risks of failure remain, but they have been greatly reduced by the growing experience gained from numerous preclinical studies and clinical trials. For neurologic diseases, a breakthrough success for a prevalent disease such as PD might change the public's perception very quickly. The etiology of PD is complex, and careful selection of patients will be important for demonstrating therapeutic success. At the workshop the question was raised as to whether there should be a project for PD or brain tumors similar to the project on spinal muscular atrophy—that is, one focused on drug development and medicinal chemistry for a single, carefully selected disease. To achieve more broadly applicable and effective gene delivery is much more challenging. Special focus on a particular neurologic disease such as PD or brain tumors potentially could accelerate efforts to attain the clinical breakthroughs that seem within our grasp and that the field needs. Success would restore the initial confidence of the public in gene therapy and attract financial support from the private sector in the future.

CONCLUSION

There is much optimism and confidence in the potential to develop an effective treatment for neurologic diseases in the near future. Tremendous progress has been made in the past decade, and clinical trials are beginning to include more efficacy studies. The complexity of achieving effective gene delivery in the nervous system is enormous; it cannot be compared to drug development. Because gene therapy efforts can overwhelm small laboratories, collaboration among multiple expert laboratories is necessary. The many challenges were well identified

during this workshop, and future needs and directions were discussed. The neural gene therapy community hopes to attract broad or targeted support for the field as outlined above, which could bolster the infrastructure and help achieve the much-needed success in a clinical trial for a prevalent neurologic disease. There was agreement that a breakthrough demonstrated in clinical trials would stimulate support by the public, which in turn would increase the willingness of the private sector to invest in this technology for the future benefit of many patients suffering from neurologic diseases and brain tumors.

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