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REGULATABLE PROMOTERS AND GENE THERAPY FOR PARKINSON'S DISEASE: IS THE ONLY THING TO FEAR, FEAR ITSELF?

Jeffrey H. Kordower¹ and C. Warren Olanow²

¹Department of Neurological Sciences, Rush University Medical Center, Chicago, Illinois, 60612

²Department of Neurology, Mt. Sinai School of Medicine, New York, N.Y.

Abstract

Gene therapy for Parkinson's disease has become a clinical reality with three different approaches currently being tested in patients. All three trials employ an adeno-associated virus with a type two serotype (AAV2). To date, no serious adverse events related to the injections of therapeutic vectors have been reported in any patient. This safety profile was predicted based upon, in some cases, exhaustive preclinical testing in both rodent and primate species. Still some argue that regulatable promoters are required so that expression of the transgene can be halted should untoward side effects arise. We argue that given the current empirical data base of AAV2, the lack of regulatable promoters that have been proven to be safe and effective, and the pressing clinical needs of PD patients, the mandatory use of regulatable vectors is not only unnecessary but, in some instances, misguided and potentially dangerous. This commentary will outline the issues related to the use of regulatable promoters for gene therapy for PD and express our opinion as to why mandating the use of such promoters might result in outcomes that are unsafe, unproductive, and counter to the progress of scientifically sound, clinical research.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder with over 1,000,000 Americans suffering from this disease. A small percentage of cases have a purely genetic etiology (Farrer, 2006), but the vast majority occur sporadically and are of unknown origin. It has recently become better appreciated that in addition to the characteristic motor features of the disease (tremor, rigidity, bradykinesia), PD is associated with potentially troubling non-motor symptoms including depression, pain, autonomic dysfunction, sleep disorders, and dementia (Langston, 2006). Pathologically, PD is characterized by degeneration of nigrostriatal neurons coupled with proteinaceous inclusions known as Lewy bodies. PD pathology is also known to be widespread and to involve more than just dopaminergic regions. Indeed, alpha synuclein pathology has been described in multiple areas of the central neuraxis (Braak et al., 2006) as well as the spinal cord and peripheral autonomic nervous system (see Langston, 2006). Still, the hallmark symptoms that are used to make a clinical diagnosis of PD are two of bradykinesia, cogwheel rigidity, and resting

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Address all correspondence to: Jeffrey H. Kordower, Ph.D. Department of Neurological Sciences Rush University Medical Center 1735 West Harrison Street Chicago Illinois 60612 312-563-3570 312-563-3571 (fax) jkordowe@rush.edu.

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tremor. The presence of postural instability, asymmetry and a good response to levodopa increase the accuracy of diagnosis (see reviews: Jancovic 2006; Hughes et al, Brain 2002). Motor deficits are largely if not completely mediated by a reduction in striatal dopamine secondary to degeneration of dopaminergic nigral neurons, and modern therapy is still based on a dopamine replacement strategy. Even more than fifty years following its discovery, levodopa remains the pharmacological “gold-standard” for treatment, and no therapies, including deep brain stimulation, have been able to provide benefits that cannot be achieved with levodopa. Levodopa therapy is based on trying to restore dopaminergic neurotransmission. However, levodopa does not restore dopamine in a physiological manner, and chronic treatment is associated with the development of motor complications (wearing off, dyskinesias) in the majority of patients (Olanow et al, 2006). While there are several other medical therapies available for the treatment of PD, they all primarily aim to reduce the risk or diminish established motor complications. Surgical therapies similarly primarily affect motor disability and motor complications and have not been shown to provide benefits beyond those that can be achieved with levodopa. Thus most currently available therapeutic strategies for treating PD are still directed toward treating the dopaminergic motor features of PD that result from underlying nigrostriatal dysfunction or reducing motor complications associated with the non-physiologic administration of levodopa. Alternate therapies that can provide the benefits of levodopa without motor complications, as well as neuroprotective therapies that might slow or stop disease progression remain important unmet medical needs.

Gene Therapy for Parkinson's disease

Gene therapy offers the potential to restore dopaminergic function in a more physiological manner than can be attained with current medical therapies, and thereby to reduce the risk of motor complications as well as to potentially influence the non-dopaminergic features of the disease. This once futuristic concept of treating patients with PD using gene therapy has now become a reality. In 2006, three different approaches have begun Phase I or Phase II clinical testing (Starr et al., 2006, Neurologix, 2006, Christine et al., 2006; Eberling et al., 2006, Starr et al., 2006). Two approaches are directed towards symptomatic benefit while one is modeled for both disease modifying and symptomatic effects. Based upon the preclinical work of Krystof Bankiewicz (2006), first Avigen Inc, and now Genzyme, are testing the hypothesis that gene delivery of the dopamine synthesizing enzyme aromatic amino acid decarboxylase (AADC) to the striatum of PD patients using an adenoassociated virus (serotype 2; AAV2) is safe and well tolerated in patients with PD (Christine et al., 2006, Eberling et al., 2006). The rationale for this approach is that in the PD brain, levodopa is inefficiently and intermittently converted to dopamine by residual dopaminergic terminals resulting in non-physiologic activation of dopamine receptors. It is proposed that by over-expressing AADC using viral vectors, more constant and physiologic striatal dopamine levels can be achieved, possibly even with a lower dose of levodopa without inducing levodopa-induced dyskinesias. A second approach sponsored by Neurologix Inc. uses the same AAV2 vector system to deliver the enzyme glutamic acid decarboxylase (GAD) to the subthalamic nucleus (STN). The rationale behind this approach is that delivery of the GAD gene to the STN will decrease the well-established over-activity of this nucleus in a manner analogous to what is done with lesions, and with deep brain stimulation. This approach has also reached Phase 1 clinical trials (Kaplitz et al, 2007) in spite of negligible efficacy in parkinsonian monkeys (Emborg et al, 2007). Both of these gene therapy approaches are symptomatic in nature and are not anticipated to affect the underlying disease process. At the time of this writing, Ceregene Inc had recently completed a modest Phase 1 trial testing the safety, tolerability, and efficacy of AAV2-neurturin (also known as Cere-120; a trophic factor) delivered to the putamen (Starr et al., 2006) and has launched a multi-center, double-blind, sham surgery controlled phase II trial. The goals of this approach are to: (1) improve

symptoms by restoring the function of degenerating nigrostriatal dopamine neurons by augmenting dopamine biosynthesis and (2) to help protect these neurons, and perhaps downstream and upstream neuronal systems, from further neurodegeneration, thus slowing or perhaps halting further disease progression. This approach has the advantage that it facilitates the production of endogenous dopamine without disruption of the basal ganglia system. Towards this end, AAV2-neurturin has been shown to augment dopamine biosynthesis as well induce neuroanatomical and functional neuroprotection in rodent (Gasmi et al., 2007) and nonhuman primate (Kordower et al., 2006) models of PD. AAV2-neurturin has also been demonstrated to provide robust regenerative effects in aged nonhuman primates (Herzog et al., 2007). All of these studies have demonstrated efficacy at doses well below those shown to be safe and well tolerated. Indeed, all three gene therapy approaches (AADC, GAD, and neurturin) have generated preclinical data supporting an acceptable safety profile while employing constitutive (non-regulatable) vectors. Data from Phase 1 clinical trials performed without regulatable promoters similarly suggest that each is safe, though this excellent safety profile has been generated in a relative small patient population. Nonetheless, there has been concern that unregulated virus and protein expression could lead to intolerable side effects, and some have argued for the concomitant use of regulatable promoters that permit down-regulation of the virus/gene/protein in such instances.

Do gene therapy approaches need regulatable vectors?

“First do no harm”; this well known medical axiom clearly applies to gene therapy approaches. Unfortunately, harm has come to some who participated in early (non-PD) gene therapy trials employing pioneering but problematic approaches. In one case, a patient suffered a severe immune reaction to an adenovirus vector (known to be highly inflammatory) and died five days after treatment (Raper et al., 2003). The patient suffered from ornithine transcarbamylase (OTC) deficiency and was infused into the right hepatic artery with an adenovirus encoding the human OTC gene. The fatal outcome may have occurred because the patient had been previously exposed to adenovirus, and this may have facilitated and supranormal autoimmune response to the vector. In another case, a gene therapy trial using a retrovirus for patients with severe combined immune deficiency was halted after three of eleven children developed leukemia (Hacein-Bey-Abina et al., 2003). Retroviruses notoriously integrate their DNA into the chromosome of the transduced cell and DNA analysis revealed that the retrovirus had indeed integrated into the host genome at 40 different sites. Unfortunately, in these patients, the integration included LMO-2, which is related to oncogenesis and contributed to the abnormal growth of a T-cell. Insertional mutagenesis is therefore a major concern with the use of integrating viral vectors, especially those such as AAV/5 or lentivirus, which are capable of infecting glial cells. While the AAV2 vectors preferentially infect neurons in animals, and appears from preclinical and clinical studies to be safe, its specificity for neurons in the parkinsonian brain is unknown.

A major advance in the gene therapy field has been the substantial development of adeno-associated virus, serotype 2 (AAV2) as an effective viral vector for therapeutic purposes. Importantly, AAV2 does not integrate into the host chromosome, but rather forms stable episomes, thus making insertional mutagenesis extremely unlikely. Another recent innovation in all current approaches for PD is the use of relatively small vector titers, targeted to a specific region within the relatively immunologically privileged human brain. These two advances, thus avoid many of the key elements of prior gene therapy approaches where serious problems were noted when systemic exposure of high titers of highly immunogenic vectors were employed.

Defining the concerns

When considering whether gene therapy approaches for PD require regulatable vectors, it is important to define first what the concerns might be. Are there reasonable concerns that the vector or the transgene will induce general toxicity? Are their specific functional side effects that are inherently more risky for PD patients? The answers to these questions are yes and yes. It is possible that injecting any vector into the brain could cause generalized toxicity. We know that adenovirus (Choi-Lundberg et al., 1997) and herpes simplex virus (During et al., 1994) are particularly immunogenic and often causes inflammatory reactions. These effects occur soon after injections. If the toxic effects are due to the vector, the use of a regulatable promoter would not be effective in halting these toxic effects and thus would be without benefit to this gene therapy approach. EMPIRICALLY, however, the choice of AAV2 as the vector for the clinical trials was due to the fact that in multiple species, across multiple time points, and multiple doses, this vector has not been shown to cause significant inflammatory or immune responses in the brain (e.g. Kaplitt et al., 1994; Kordower et al., 2006; Herzog et al., 2007). Indeed, AAV2 has been shown to have an excellent safety/toxicology profile in preclinical PD studies. If potential immune or inflammatory effects are due to the expression of the transgene, THEORETICALLY shutting off this transgene might reduce or halt the inflammation. However, to date, there is no EMPIRICAL evidence for any inflammatory reaction in any of the approaches currently in trials in PD patients. Nor is there any other EMPIRICAL data for any other approach that is currently being tested preclinically and might realistically be in the pipeline for future clinical testing for PD.

A second THEORETICAL cause for concern is the potential that the transgene delivered via gene therapy approach would induce severe neurotoxicity (manifested as clinically serious side-effects). Much of this concern was generated following newly discovered side effects that were seen in clinical trials evaluating fetal transplant in patients with Parkinson's disease. Fahn and colleagues were the first to report (Freed et al. 2001), followed independently by Olanow (2003) and Hagell (2002) and their colleagues, that a percentage of PD patients receiving fetal transplants experienced a previously undescribed form of dyskinesia that was associated with the fetal transplant procedures. While all patients pre-operatively displayed typical levodopa-induced dyskinesias during "on" period, significant numbers of these patients experienced severe dyskinesias postoperatively in the practically defined "off" state when the levodopa dose had been lowered or even stopped. It remains unclear whether these abnormal movements are due to the transplant causing extended "on" period. It has also been proposed that they might represent a prolonged form of diphasic dyskinesia associated due to continued delivery of suboptimal levels of dopamine or to non-physiologic delivery of dopamine. Nor is it clear whether these "off-period" dyskinesias are the same across studies. In the Olanow et al trial, (2003) the "off" medication dyskinesias were predominantly seen in the lower extremities and the abnormal movements were stereotypic, both feature analogous to diphasic dyskinesias. In contrast, the dyskinesias reported by Fahn and coworkers (Freed et al., 2001) were typically seen in the upper extremities as well as the head and neck and were non-stereotypic, more analogous to peak-dose dyskinesias. Still in each case, these treatment-related side effects were real and often severe; to the point where many of these patients had to undergo a second neurosurgical procedure, deep brain stimulation, to treat these graft-induced symptoms. One can make the argument that since dopamine replacement by fetal nigral transplants caused these abnormal involuntary movements, gene therapy induced augmentation of dopaminergic systems could as well, and thus regulatable promoters should be required for gene therapy trials. However, the logical and empirical links between the fetal tissue transplantation approach and those using gene therapy approaches in current clinical trials seem obscure, at best. First, fetal transplants are fundamentally different than any of the gene therapy trials currently under clinical investigation. Fetal transplants employ embryonic neurons and graft them in an

ectopic location without their normal regulatory afferents. They potentially cause focal increases in dopamine or “hot-spots” which have been shown to augment dyskinesias in rats (Maries et al., 2006) and monkeys (Bankiewicz et al., 2006) and have been reported in patients with “off medication” dyskinesias following grafting (Ma et al., 2002). Gene therapy trials, in contrast, are designed to try and normalize, enhance and/or strengthen the endogenous dopaminergic systems within the host parkinsonian basal ganglia. With respect to AAV2-neurturin, the enhanced dopamine production seen in rodents (Gasmi et al., 2007) and monkeys (Kordower et al., 2006; Herzog et al., 2007) did not occur as hot-spots. In fact the dopaminergic enhancement was remarkably homogeneous throughout the target region an effect associated with diminished dyskinesias in animal models (Bankiewicz et al., 2006; Carlsson et al., 2005) and likely in PD patients (Olanow et al., 2006). So why did “off-medication” dyskinesias occur in the fetal transplant trials? One likely explanation is that in spite of the plethora of fetal transplant experiments that led up to clinical trials, none ever placed dopaminergic grafts into levodopa primed dyskinetic monkeys. This was a failure to model the universal fact that all patients receiving fetal transplants in clinical trials had already been primed with levodopa and experienced peak dose dyskinesias prior to receiving the graft. In contrast, we have tested the hypothesis that gene delivery of GDNF to levodopa-treated dyskinetic parkinsonian monkeys would not induce “off-medication” dyskinesias, and they do not (Soderstrom et al., 2007). Indeed, gene delivery of novel trophic factors such as GDNF has, in general, been shown to reduce dyskinesias in animal models of PD (Miyoshi et al., 1997). Similarly, in the recently completed Phase 1 trial with AAV2-neurturin, “off” dyskinesia were not encountered and indeed there was even a reduction in troubling dyskinesias during “on” time. (Starr et al., 2006). This is likely because AAV2-neurturin exerts its effect on the endogenous dopamine system and induces a homogeneous restoration of dopaminergic function which does not induce “hot-spots” of focal dopaminergic neurotransmission. In the AAV2-GAD trial, there is similarly a theoretical risk that increased inhibition of STN neurons could result in hemiballismus, although here too this was not identified in any patient in open label clinical trials (Kaplitt et al., 2007).

Thus there are no EMPIRICAL data to date that would indicate that current gene therapy approaches induce off dyskinesias or other serious side effects that might warrant the use of regulatable promoters. Furthermore, there are no EMPIRICAL data to indicate that even if such unwanted side effects did occur, that preventing gene expression with the use of regulatable promoters would reverse the development of this unwanted side effect.

What are the down-sides to regulatable promoters?

One can easily imagine that in the coming decades, many gene therapy approaches for PD will employ regulatable vectors. However, the current status is that no regulatable vector system has yet been proven to be either safe or effective. While several different approaches to developing systems to regulate gene expression are being pursued, the regulatable promoter that has been most widely tested is the tetracycline system. This system inserts a tetracycline trans-activator into the vector construct so that administration of tetracycline, or its analogue doxycycline, can turn on (tet-on system) or turn off (tet-off system) transgene expression, thus theoretically halting any gene-related unwanted side-effects. Using the tet-on system, patients would need to administer one of these antibiotics (orally) for as long as required to maintain the expression of the transgene. If unwanted side effects did occur, they THEORETICALLY could stop taking the antibiotic, thus halting therapeutic transgene expression. This approach might be practical since tetracycline has been given chronically to adolescents for the treatment of acne without serious side effects, although the potential of side effects with very long term therapy in an elderly population can not be excluded. Using the tet-off system, patients would only take tetracycline or doxycycline if serious unwanted

side effects did occur, thus THEORETICALLY shutting off the transgene. Under these circumstances, they would have to take tetracycline for the rest of their lives to keep the transgene turned off with the potential risk of drug side effects. Further, while laudable, these approaches are problematic for clinical use at present since the tet-transactivator can be toxic when utilized in primates (Latta-Mahieu et al., 2002; Farve et al, 2002). Multiple studies have shown that the use of the tet-regulatory systems in monkeys causes immune and inflammatory responses. Indeed, all currently developed regulatable gene transfer systems require expression of a 'regulator protein', which ironically, is itself expressed in an unregulated fashion, and for which current safety and tolerability data remain woefully inadequate.

Secondly, for optimal use, these systems would have to be engineered to be on the same vector backbone; otherwise success would depend upon all of the same cells being infected with both the therapeutic transgene and the tet-transactivator gene. This is a significant challenge. Even if the toxicity issue could be mitigated, and even if the tet-transactivator and the therapeutic transgene could be fit on the same vector backbone, the tet system is inefficient. It is clear that they are "leaky". While reporter genes can often be well controlled, EMPIRICAL data demonstrate that the therapeutic transgenes are still expressed *in vivo* under the THEORETICAL control of the tet-regulatable systems (Georgievska et al., 2004). Indeed, we have preliminary EMPIRICAL data demonstrating the failure of the tet-on or tet-off systems to appreciably turn on or turn off GDNF in nonhuman primates (Kordower and Aebischer, unpublished). Thus at present, the tet-on and tet-off systems have not been established to be either safe or effective for clinical use and other (less well-developed) approaches suffer similar limitations and liabilities.

Non-tetracycline based regulatable systems are currently in development and significant effort has been put into using steroid hormones to promote regulation of therapeutically delivered genes. Most prominently, the use of progesterone has been advanced for this purpose. As reviewed by Goverdhana and coworkers (2006), the progesterone system contains a gene that encodes the progesterone receptor with a C-terminal truncation, which prevents binding with progesterone. However, the truncated receptor retains the ability to bind with the progesterone antagonist mifepristone (RU 486). In addition, mifepristone acts as an agonist, promoting the transcription of reporter genes containing progesterone-responsive elements (Baulieu et al., 1989). However, the concentration of mifepristone needed to induce this effect is similar to the level of estrogen replacement therapies used by women during menopause (Wang et al., 1994). Even though steroid hormone receptor-based regulatable systems have the advantage that the majority of the system comprises modified humans proteins and should not be a potent activator of the immune system, inducers of the steroid hormone receptor system are generally able to activate endogenous steroid hormone receptors in cells in addition to regulating transgene expression and thus may have significant side effects. Thus they are likely not to be useful clinically (Goverdhana et al., 2006). In addition, at present, there is no EMPIRICAL data using this system in the models of PD and it would be inappropriate to use this approach in PD patients until it is well tested for safety and efficacy in both rodent and nonhuman primate model. This will take many years to achieve.

The Ecdysone system is being developed to overcome the limitations of the steroid hormone receptor system. This regulatory system utilizes the nonmammalian steroid hormone Ecdysone receptor which is involved in initiating metamorphosis in *Drosophila*. This system employs ecdysteroid ligands to bind to the Ecdysone receptor and a unique ecdysone-responsive element. There are a number of positive aspects of this regulatory approach including the use of short half-life ligands to potently activate the system. However, the safety and tolerability following the expression of insect proteins in the primate brain is

virtually unknown. Indeed, like other steroid based regulator systems, it will take many years to determine whether the expression of insect proteins will cause an immune response in the primate brain. Furthermore, like the progesterone-based regulatable system, there are no data for effective gene regulation using the Ecdysone system in any animal model of PD and no EMPIRICAL data to support its effectiveness in any of the gene therapy approaches currently being tested clinically. Thus this approach is also not nearly ready for clinical testing. We believe that it would be inappropriate to deny seriously ill PD patients the opportunity to participate in gene therapy clinical trials that otherwise have an impressive safety profile and might benefit many thousands of patients.

Another interesting gene therapy approach is the use of encapsulated genetically modified cells that express the trophic factor GDNF. The rationale for this approach is that xenografted genetically modified cells could be employed since the pores of the capsule are large enough for the trophic factor to be secreted but too small for immune cells to enter the capsule and destroy the graft. From a safety perspective, this approach has THEORETICAL interest since it is possible to retrieve the capsules should unwanted side effects occur. The retrievability of the capsule has been viewed as a safety valve that has value in comparison to constitutive gene therapy approaches and represents a different form of regulation than previously described for *in vivo* gene therapy. Still, it merits discussion in this forum. This approach has the potential to be seriously flawed from two perspectives. First, numerous studies have shown that the secretion and distribution of therapeutic molecules from capsules using *ex vivo* gene therapy is very limited (Kordower et al., 1994; Kordower et al., 1996; Emerich et al., 1997; Emerich et al., 1998). This approach could theoretically cause “hot-spots” of dopamine production around the capsule, surrounded by areas lacking in therapeutic protein. EMPIRICAL data from gene therapy (Bankiewicz et al., 2006) and fetal transplant (Ma et al., 2002; Maries et al., 2005; Carlsson et al., 2006) studies suggest that the creation of “hot-spots” of enhanced dopamine production could **exacerbate** levodopa-induced “on” dyskinesias and potentially contribute to “off” dyskinesias. Furthermore, there is NO EMPIRICAL evidence that unwanted side effects once established, could be obviated if the capsules are removed. Thus this approach has EMPIRICAL data to indicate that it may be unsafe and NO EMPIRICAL data to demonstrate that the proposed “safety valve” (removing the capsules) would be of value.

So what do we do?

There are millions of PD patients in the world with severe disability who have exhausted standard antiparkinsonian therapies and require novel therapeutic strategies to improve their symptoms. More importantly, these patients desperately need therapies that could retard or halt their disease progression. These therapies need to avoid the severe-side effects characteristic of current approaches such as wearing off and dyskinesias. Gene therapy strategies offer the potential to address some of these problems, and have demonstrated safety and efficacy in preclinical studies. Should such trials wait for 5-10 years (or longer) in the hope that a regulatable promoter system can be established that is safe and effective? We believe not. All currently available EMPIRICAL data from rodents, nonhuman primates, and clinical trials in PD patients suggests that current gene therapy approaches are safe and are not likely to do harm. Secondly, regulatable promoters may not offer the level of control that is hoped for, and indeed may themselves induce side effects that are more disabling and serious than the study intervention being tested.

We recognize that the current gene therapy trials are experiments, and the courageous patients that agree to participate are doing so with the a priori knowledge that these experiments have inherent risks. This is why these trials require federal and institutional oversight with IRB approval and full informed consent prior to subjects' participation.

Before commencing such studies, it is imperative that sufficient preclinical data and toxicity studies exist to provide some expectation of safety and some hope for efficacy. In the case of AAV2-neurturin, prior to initiating clinical Phase I trials, Ceregene, Inc. performed 17 safety and efficacy studies in rodents and nonhuman primates, testing doses hundreds of times higher than those demonstrated to provide efficacy in a range of animal models.

So where do we go from here?

There is great value in the continued development and characterization of safe and effective regulatable systems in spite of the fact that as presently employed, gene therapy trials currently under clinical investigation have engendered only THEORETICAL concerns that have NO EMPIRICAL BASIS. Further, it will be many years before any regulatable system is ready for clinical use and no assurance that they in turn will be safe or effective. Nonetheless, only small numbers of patients have been tested to date, and it is too early to exclude the possibility that regulatory systems may prove to be invaluable at some time in the future. This may be a particular issue in PD as current therapies focus largely on restoration of the dopamine system even though it is now evident that neurodegeneration is more widespread and associated with potentially disabling clinical features. The development of gene therapies targeted at these non-dopaminergic systems may involve different levels of risk which might be modulated by regulator promoters. Our point is that to date, no EMPIRICAL data support the use of regulatable vectors because of either genuine safety concerns or a sub-optimal efficacy profile with current gene therapy approaches and Millions of patients with PD and other degenerative diseases suffer disability that can not be controlled with current therapies and that might benefit from gene delivery approaches. They are in need of effective therapies today. We argue that they should not be denied access to experimental treatments that have been EMPIRICALLY tested for safety and efficacy in an exhaustive and effective manner based upon fears that are founded solely on THEORETICAL concepts.

As in any study, patients participating in a gene therapy trial must sign an IRB-approved informed consent before participation in the study. As part of this process, they must be fully informed of all theoretical risks, the possibility that unanticipated adverse events might occur, and what potential treatments might be available. Interestingly, despite the lack of a regulatable vector, patients have not been reluctant to participate in gene therapy trials, as evidenced by the accelerated recruitment for both the open label and double blind trials of AAV2-neurturin. This reflects the terrible disability that PD can inflict on a patient and their willingness to take risks. In turn, investigators have an obligation to take all reasonable steps to ensure that the procedure has been adequately tested for safety in the laboratory, that there is a reasonable possibility of efficacy, and that the study is conducted in such a manner as to yield meaningful clinical results.

Significant scientific progress is often made by taking bold steps. These bold steps should be guided by a strong EMPIRICAL data base evidencing reasonable prospects for safety and efficacy, cognizant of theoretical concerns. We recognize fully our responsibility to the patients that, to the best of our ability, before novel therapies are tested in human volunteers, they are determined to be as safe as possible with reasonable expectations for efficacy. We also recognize that there is a responsibility to ensure that all steps are taken to avoid tragedies that might harm the field in such a way that it might prevent the development of disease modifying or symptomatic therapies in the future. Indeed, we owe it to the patients not to destroy their possibility to receive future cures or more effective therapies. However, we also owe it to patients to pursue new therapies with responsible vigor and give them the opportunity to avail themselves of such therapies at a time when there is sufficient EMPIRICAL scientific data supporting safety and efficacy. We believe that this is the case for current gene therapy approaches using constitutive gene delivery. As stated above, there

are no EMPIRICAL data indicating that any of the current gene approaches are unsafe. Just the opposite. There is an extensive EMPIRICAL data base supporting the concept that these approaches are safe. In contrast, there are NO EMPIRICAL data to indicate that regulatable promoters are either safe or effective. Just the opposite. The call for the use of regulatable promoters at this time is based upon THEORETICAL concerns that are not supported in preclinical studies. Give the current state of science, patients with disabling disease that can not be adequately controlled with existing therapies deserve to have the right to make an informed decision as to whether they wish to be participants in clinical experiments that have the opportunity to enhance their quality of life in a substantive way and where the potential adverse events have been extensively studied in preclinical trials and thoroughly explained to the patient.

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