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Gene Therapy in Parkinson's Disease: Rationale and Current Status

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Abstract

Neurodegenerative diseases pose a unique treatment challenge to clinicians due to the slow progression of disease, the profound neuron loss prior to clinical symptoms and the paucity of early diagnostic biomarkers and restorative therapies. Treatment options are further constrained by the post-mitotic nature of CNS neurons and restricted ability of these cells to regenerate. Lastly, because the blood brain barrier impedes peripheral access to the brain there are inherent limitations with respect to treatment especially protein and peptide-based therapeutics. Due to these intrinsic constraints, researchers are continuing to expand a therapeutic platform based on the delivery of genes engineered for efficient CNS expression. Gene therapeutic approaches were first tested almost 20 years ago and continue to evolve as a viable treatment for CNS neurodegenerative disorders. In this review we consider the current advances in human gene therapy for one common neurodegenerative disorder, Parkinson's disease (PD).

1. Parkinson's Disease

The second most common age-related progressive neurodegenerative disorder, PD, first described in 1817 by James Parkinson in his "An Essay on the Shaking Palsy", affects 1.5 million people in the United States and 4 million people worldwide.(1,2) Approximately 1–2% of the population over the age of 65 years suffers from PD and this number is expected to grow as the average age of the population increases.(3) The cardinal clinical features include: tremor, cogwheel rigidity, akinesia, bradykinesia and postural instability. However, there is a spectrum among PD patients in terms of clinical manifestations and disease progression. For example, while most patients exhibit the "typical" PD tremor, a rhythmic back-and-forth movement in the range of 4–6 Hz commonly present in the hands or limbs when the patient is at rest (i.e. resting tremor; hands supported against gravity), the severity of these motoric symptoms varies with a subset of PD patients classified as having a tremor-predominant PD phenotype. Although most recognized by the aforementioned debilitating motor dysfunctions, PD patients can also suffer from a variety of non-motor symptoms, including; depression, cognitive impairment, psychosis, hallucinations, compulsive behaviors, REM sleep behavior disorders, excessive daytime somnolence, orthostatic hypotension, gastrointestinal symptoms, constipation, urinary and sexual dysfunction, speech changes, skin problems, pain, difficulty swallowing and chewing.(4–8) Several of these non-motor complications such as those associated with cognition arise later in disease (Hoehn and Yahr Stages IV–V) while a subset (i.e. gastrointestinal system dysfunction) are prodromal, preceding the motor manifestations by as much as 10 years.(8–10)

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PD is characterized by the loss of substantia nigra pars compacta (SNpc) dopamine neurons, dystrophy of the associated projection fibers to the corpus striatum and diminution of the nigrostriatal neurotransmitter, dopamine. In fact, motoric symptoms are underappreciated until approximately 60% of the SNpc dopamine neurons are lost resulting in a greater than 80% loss of dopamine.(11,12) The pathological hallmark of PD is presence of intracytoplasmic proteinaceous inclusions called Lewy bodies, which are localized most notably to the few surviving SNpc dopaminergic neurons and were first described by Friederich Lewy in 1912. The function of Lewy bodies in PD pathobiology is not understood. Nonetheless, following immunohistochemical staining with antibodies against two abundant Lewy body proteins, ubiquitin and α -synuclein, the presence of these structures in the remaining SNpc neurons is used as a criteria for diagnosis of PD.(13,14) The spatial and temporal localization of Lewy bodies has recently been established as a marker for different neuropathological stages of PD. Data from this study demonstrate that Lewy body pathology in PD subjects does not begin in nor is it restricted to the SNpc which corresponds with the observation that non-motor symptoms are also present in this disease.(15) Another feature revealed in PD brains upon autopsy is the presence of activated microglia.(16–18) As the immune surveillance cells of the brain, it is not surprising that microglia are upregulated in many neurodegenerative disorders following substantial cell death. However, evidence from both tissue culture and animal models of PD suggest that microglial activation and the consequent increase in proinflammatory molecules may play an important role in the progression of PD, representing another potential therapeutic avenue.(19–23)

It has been nearly 200 years since PD was first described and yet the initiating mechanism for sporadic disease has not been fully elucidated. Risk factors including advancing age, exposure to environmental toxicants and drinking well water have all been linked to PD, yet the vast majority of cases are sporadic with no known etiology.(3,24–31) Genetic links to PD have been identified and form the basis for several animal models of this disease.(32–35) The first discovered PD-associated gene, *SNCA*, is localized to chromosome 4 and encodes a 16kDa protein called α -synuclein.(33,36) Mutations within this gene were linked with an early onset familial form of PD.(34,37) The importance of α -synuclein in both familial and sporadic PD etiology was further demonstrated following the discovery that duplication or triplication of *SNCA* was sufficient to cause PD suggesting that overexpression of α -synuclein alone leads to toxicity.(38,39) Other PD-related genes that have been identified include: parkin, ubiquitin carboxyl-terminal esterase L1 (UCHL1), Leucine-Rich Repeat Kinase 2 (LRRK2), PTEN (phosphatase and tensin homolog)-induced kinase 1 (PINK1) and DJ-1.(35,40–45) Of these genes, LRRK2 mutations are the most frequent and have been linked to both late-onset familial and sporadic PD.(46–48) Recent genome-wide association studies revealed several signals associated with PD, one in the gene coding α -synuclein, demonstrating a role for genetic variants in sporadic PD.(49,50) However, even when taken together, genetic mutations alone do not account for the majority of sporadic PD cases and it has now been generally accepted that a combination of genetic vulnerability and environmental insults lead to sporadic PD.(51) Current clinical diagnosis of PD does not distinguish between initiating factors and usually occurs only after the invariant loss of SNpc dopamine neurons has reached a disease threshold or tipping point. Consequently, existing pharmacotherapies have been focused on enhancing the dysfunctional dopaminergic system, a feature common to all PD patients, rather than halting the disparate initiating events.

2. Rationale for Gene Therapeutic Approaches

All commonly employed PD therapies are focused on the amelioration of symptoms and do not cure disease.(52–71) Most pharmaceutical approaches focus on augmentation of the diminished neurotransmitter, dopamine, by either increasing production, extending half-life and/or decreasing metabolism. Dopamine is normally synthesized from the amino acid tyrosine

in a series of enzymatic reactions initiated by the conversion of tyrosine to L-dopa by tyrosine hydroxylase and its co-factor tetrahydrobiopterin. L-dopa is then decarboxylated by L-amino acid decarboxylase (AADC) to form dopamine. Dopamine enhancement therapies are most effective when a portion of the nigrostriatal pathway is intact. Consequently, as the number of SNpc dopamine neurons and projection fibers decreases these treatments become less efficacious. The most common therapy for PD is the oral administration of the dopamine precursor, levodopa (L-dopa), usually taken in conjunction with an inhibitor of extracerebral dopa decarboxylase to prevent peripheral metabolism. Introduced more than 40 years ago, this treatment effectively quells the motoric symptoms of akinesia and bradykinesia.(52) However, many patients gradually develop levodopa-induced dyskinesias and motor fluctuations about 5–15 years after the initiation of L-dopa treatment.(72,73) The severity of the dyskinesias varies between patients and ongoing research efforts are focused on the development of new and more effective anti-dyskinetic medications.(74) A subset of these patients benefit from deep brain stimulation (DBS) which is used in conjunction with L-dopa treatment. Although the exact mechanism of action for DBS is not clear, it is proposed that the generated impulses suppress neural activity, which in the case of subthalamic nucleus DBS (STN-DBS), dampens the PD-increased STN abnormal activity resulting in an overall improvement of tremors, rigidity and akinesias.(54,57,60,62,⁶⁵,69–71,75–79) Often DBS-treated patients are able to reduce their L-dopa dose by ~50–60% resulting in a decrease in dyskinesias and report an improved quality of life.(71,76,77,79) Unfortunately, much like L-dopa therapy, the efficacy of DBS declines as PD progresses.

There is no debate regarding the need for novel disease modifying PD therapies. New PD treatments are currently in clinical trials and several are centered on gene therapeutic approaches to either compensate for the loss of dopamine or to protect SNpc dopamine neurons from further degeneration with the overall goal of restoring function (see <clinicaltrials.gov>; Table I; reviewed in (80,81)). There are several reasons for pursuing a viral vector-mediated gene therapeutic approach in the context of PD; 1) since the PD pathophysiology that subserves the motoric symptoms is largely confined to one brain region, the nigrostriatal pathway, a limited area will require treatment; 2) because of the physically restricted environment of the brain, repeated injections into the nigrostriatum are not desirable, making long-term gene expression following a single treatment appealing; 3) viral vectors are diffusible and theoretically capable of efficient transduction of the striatum; 4) genes have been identified that can either modulate the neuronal phenotype or act as neuroprotective agents; and 5) there is currently no cure for this debilitating disease.

3. Parkinson's Disease Gene Therapy Platforms

The earliest attempts at PD gene therapy utilized a variety of cell and tissue transplants including fetal and autologous adrenal medullary tissue grafts, xenografts, neurospheres, cell suspension grafts and embryonic stem cells with the overall goal of augmenting dopamine content.(82–88) Fetal nerve cell transplantation has been met with some success as a subset of treated patients experienced palliative relief for many years. The effects of long-term fetal implants have recently been evaluated and upon autopsy several subjects displayed PD pathology in the grafted tissue suggesting that the local “disease” environment within the brain brings about “de novo” PD.(89–92) However, since there have been only a small number of treated patients available for evaluation it is difficult to draw any definitive conclusions regarding the apparent “transfer of disease”. Regardless these patients demonstrate the potential of cell replacement as an option to stave off disease for several years. Currently one of the biggest obstacles for fetal implant therapy is the acquisition of tissue.

The advent of viral vector technology revolutionized the gene therapy field providing a method for the efficient delivery of genetic material (transgene) without the need for human transplant

tissue or cells. Although many different viral platforms have been developed, based on the criterion of safety, stability of gene expression and transduction of specific cellular targets only adeno-associated virus (AAV) and lentivirus vectors are currently in clinical trials for PD (see Table I; for review see (93)). These vectors share the common features of efficient transduction for both dividing and non-dividing cells and long-term transgene expression making them valuable for post-mitotic neuron-targeted therapies.

3.1 Adeno-associated virus vectors

Adeno-associated virus (AAV) is a member of the parvovirus family and a preferred gene therapy vector since this virus is apparently non-pathogenic; in fact, AAV has not been associated with any human disease.(94,95) Another benefit is that humans, of whom the vast majority have been exposed to AAV, exhibit a low immune response to this virus. Although circulating anti-AAV antibodies have been identified in the human population and innate immune responses to some AAV serotypes have been described, AAV has emerged as the vector platform of choice for gene therapy in humans.(96–99) Over 10 recombinant AAV serotypes (rAAV) have been engineered into vectors but rAAV2 is the most frequently employed serotype for gene therapy trials. Additional rAAV serotypes have been developed and tested in animal models that are more efficient at neuronal transduction but these are not yet in clinical trials (Reviewed in (93) and (100–103)). The combination of long-term expression, efficient transduction of neurons and diminished proinflammatory and immune responses in humans has thrust rAAV2 to the forefront of PD gene therapy clinical trials (Table 1).

3.2 Lentivirus vectors

Unlike rAAV, which has a restricted transgene size (~4.7 kb), lentivirus vectors can accommodate a larger transgene payload (~8 kb) and are the current vector of choice for multigene PD treatments.(104) Lentiviruses are RNA retroviruses capable of chromosomal integration and stable long-term expression. The most well studied and perhaps widely recognized wild-type lentivirus is the human immunodeficiency virus type I (HIV), the causative agent in acquired immune deficiency syndrome (AIDS). Because of the association of HIV with AIDS the use of lentivirus vectors for gene therapy has raised safety concerns. (105) In order to increase safety, recombinant non-replicating and self-inactivating lentiviruses have been engineered that display tropism for neurons, transduce with high efficiency, and display stable long-term transgene expression.(93,105,106) One non-human primate lentiviral vector system based on equine infectious anemia virus (EIAV) with the added capability of self-inactivation has been developed and is presently being used in PD clinical trials (Table I). (107)

4. Parkinson's Disease Gene Therapy Clinical Trials

Current PD gene therapy clinical trials employ either rAAV2 or lentivirus and are focused on three therapeutic approaches; augmentation of dopamine levels via increased neurotransmitter production, modulation of the neuronal phenotype, and neuroprotection. (106,108–113) One approach focuses on increasing dopamine production via direct delivery of genes involved in neurotransmitter synthesis while a second method is designed to change the neuronal phenotype bypassing the need for dopamine, both approaches should ameliorate symptoms associated with PD. These therapies are also intended to delay development of end-stage disease, an important accomplishment in a progressive age-related disorder such as PD. On the other hand, delivery of a neurotrophin gene such as glial cell-derived neurotrophic factor (GDNF) or neurturin (NTN), a GDNF-related protein, is projected to slow disease progression by enhancing neuronal survival. However in the long-term it is not known whether neurotrophins, while demonstrated to protect neurons in animal toxicant models of SNpc

dopamine neuronal death, will represent a cure for PD. One weakness, which applies to all current PD therapeutic approaches is the lack of a reliable clinical test affording early diagnosis of PD patients prior to the development of motoric symptoms and substantial SNpc dopamine neuron loss.

4.1 Augmentation of dopamine levels

The loss of SNpc dopamine neurons combined with dystrophy of the striatal projection fibers result in a loss of dopamine, which becomes more severe as PD progresses. While oral administration of L-dopa (levodopa) is currently the most effective therapy, ongoing neurodegeneration results in fewer healthy dopamine neurons available to convert this precursor to dopamine. Therefore considerable effort has been placed on the development of therapies aimed at increasing the activity of genes required for dopamine synthesis.(80,81) These strategies are being tested in Phase I and II clinical trials, as discussed below.

4.1.a. AAV-hAADC-2—Amino acid decarboxylase (AADC) is the enzyme responsible for the decarboxylation of L-dopa to dopamine and enzyme levels have been reported to be lower in PD brains compared with control individuals.(114,115) These observations led to preclinical studies designed to augment AADC activity. Non-human primate studies demonstrated that gene therapeutic delivery of human AADC via rAAV vector technology (AAV-hAADC-2) restored the ability of the striatum to convert L-dopa into dopamine and mitigated PD-like behavior in toxicant models of disease.(116–118) Presumably AAV-hAADC-2 was effective because of the direct transduction of nondegenerating striatal neurons providing a new source of dopamine in addition to increasing the efficiency of the remaining dopamine neurons. This treatment strategy is particularly appealing because it does not require healthy SNpc dopamine neurons, conversion to dopamine is regulated by oral administration of L-dopa and long-term robust gene expression is possible.(116)

The positive preclinical results led to a Phase I clinical trial sponsored by Genzyme Inc. employing the same AAV-hAADC-2 vector to transduce the putamen of ten PD patients (Table I; clinical trial identifier, NCT00229736). The entry criteria included the diagnosis of idiopathic PD with at least two of the four cardinal clinical features, a Hoehn and Yahr Stage III to IV off-medication, intractable motor fluctuations, positive response to L-dopa, a duration of L-dopa therapy ≥ 5 years, age ≤ 75 years and an age at diagnosis ≥ 40 years. Enrolled patients (5 subjects/treatment) received bilateral infusions into the putamen of either low-dose (9×10^{10} vector genomes) or high-dose (3×10^{11} vector genomes) rAAV-AADC-2 in conjunction with orally administered L-dopa to establish safety with a secondary outcome measure aimed at determining the effect of this treatment on clinical status. An initial analysis of 5 treated patients with moderate to advanced PD demonstrated that the rAAV2-hAADC delivery to the striatum was well tolerated and positron emission tomography (PET) using the AADC tracer, [^{18}F] fluoro-L-m-tyrosine (FMT), showed that AADC activity was detectable 6-months post-treatment.(119) Further evidence of safety and tolerability was recently reported for all 10 patients (5 male and 5 female) following 6-months of treatment.(120) Initially 12 patients were screened for this trial but 2 were excluded because they had elevated antibody titers to AAV, of the 10 remaining subjects all reported improvements in the total UPDRS off- and on-states at the 6-month time point. It is also encouraging that 8 of the 10 subjects, all of the high-dose cohort and 3 of the low-dose group, were able to reduce their levodopa dose, an expected outcome if AADC activity was enhanced. AADC activity was monitored with FMT PET and patients in the high dose group showed a greater putaminal uptake compared with the low-dose cohort (75% vs. 30%), which comports with the decrease in the required levodopa dose. Curiously, these subjects showed improvement both off- and on-medication suggesting that augmentation of AADC could increase the conversion efficiency of L-dopa to dopamine for endogenous L-dopa as well as for peripherally administered levodopa. The authors also

suggested that sprouting may occur due to glial reaction at the implant site as has been reported for 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys undergoing gene transfer and this event may also contribute to the clinical improvement.(120,121) Future studies will determine whether this is the case in AAV-hAADC-2 treated patients.

Although these studies are encouraging, this is a Phase I clinical trial that was not designed specifically to determine efficacy, another controlled trial is required with a placebo group before benefit directly related to AAV-hAADC-2 can be established. In addition, several adverse effects of treatment most related to the surgical procedure were reported. The most common effects included incisional tenderness, transient headache and in three subjects intracranial hemorrhage, all well described risks following stereotactic craniotomy. In addition, four subjects reported a transient increase in dyskinesias. Patient antibody AAV titers were monitored throughout treatment and increased in 4 of the 6 subjects tested. While no adverse effects were identified related to these increased titers the consequence of circulating AAV antibodies needs to be carefully monitored. Overall this Phase I trial demonstrated that intraputamenal infusion of AAV-hAADC-2 was well tolerated while efficacy needs to be further explored in a controlled Phase II trial. There are two important advantages of this AAV-hAADC-2 strategy; peripherally administered levodopa is used to regulate the amount of dopamine produced (i.e. a “pro-drug” gene transfer) and FMT PET can be used as an unbiased measure of AADC activity.

4.1.b. ProSavin—In a similar approach, Oxford BioMedica in affiliation with the French social security health care system is sponsoring a clinical trial that utilizes a multicistronic lentivirus vector (EIAV-SIN) to transfer 3 transgenes required for the synthesis of dopamine from tyrosine; tyrosine hydroxylase, GTP cyclohydrolase I (required for the synthesis of tetrahydrobiopterin an essential AADC cofactor) and AADC (ProSavin; Lenti-TH-AADC-CH1) into striatal neurons (Table I; clinical trial identifier, NCT00627588). In addition, the tyrosine hydroxylase gene is mutated to prevent the normal negative feedback inhibition of this enzyme by dopamine.(122,123) Intraputamenal infusions of ProSavin are given in conjunction with peripheral levodopa to modulate the transduced striatal neurons to produce dopamine from both levodopa and endogenous tyrosine making increased amounts of neurotransmitter available for release and binding to post-synaptic dopamine receptors.(107) Similar to the AAV-hAADC-2 treatment, Lenti-TH-AADC-CH1, bypasses the need for healthy SNpc dopamine neurons. In addition, because EIAV-SIN integrates and displays a natural tropism for neurons, long-term expression of the dopamine-modifying genes in striatal neurons can be achieved.

ProSavin therapy has had demonstrated success in animal models of PD and is currently in an open-label Phase I/II clinical trial (Table I) (106). The initial phase was a dose escalation study to evaluate 2 titers (1X and 2X) in a small cohort of patients (3 subjects/dose). Oxford BioMedica recently announced that both doses were well tolerated, improved motor function and quality of life with no evidence of immunotoxicity or reported adverse effects (16 October 2009). The high dose group showed further motor improvement (34% relative to base line) when evaluated at the 6 months time point using the UPDRS III ‘OFF’ score. Interim results are scheduled for presentation at the European Society of Gene & Cell Therapy Annual Congress in Hanover Germany on November 21–25, 2009. The second stage of the trial is designed to confirm the efficacy of the optimal dose however the preliminary dosage studies lend support for a continued dose escalation study. The Phase II portion is set to enroll 12 patients, 50 to 65 years old, diagnosed with bilateral idiopathic PD for greater than 5 years, with Hoehn and Yahr stage III and IV, motor fluctuations and positive response to dopaminergic therapy.

A potential caveat for this therapy, as well as for the AAV-hAADC-2 gene therapy described above, is that non-dopaminergic striatal neurons are transduced and co-opted to produce and release dopamine. These striatal neurons normally use γ -aminobutyric acid (GABA) as their neurotransmitter and it is unclear what effect producing cytoplasmic dopamine will have on these GABA neurons. For example, cytosolic dopamine is highly reactive and auto-oxidizes to form hydrogen peroxide and semiquinone which can lead to oxidative damage of proteins and lipids, a proposed mechanism for neurodegeneration.(124,125) Furthermore, it is not clear whether the transduced GABA neurons can behave like dopamine neurons and properly store, release and metabolize this neurotransmitter. In the case of AAV-hAADC-2 therapy these concerns are somewhat reduced since the gene therapy can be controlled by the peripheral administration of levodopa while Lenti-TH-AADC-CH1 therapy will continue to make dopamine from tyrosine in the absence of peripherally supplied levodopa. However, recent work suggests that long-term Lenti-TH-AADC-CH1 therapy may be safe as well as effective since in a non-human primate model of PD, Lenti-TH-AADC-CH1 treatment with an improved vector construct, provided long-term correction of motor deficits (~44 months for 1 animal; 5 additional animals demonstrated improvement up until the 12-month endpoint of the study) without evidence of L-dopa-induced dyskinesias.(126) These preclinical results are encouraging and support further clinical testing of this gene therapeutic approach.

4.2 Modulation of neuronal phenotype

One consequence of the loss of SNpc dopamine neurons is a change in the input to the internal globus pallidus (GPi) and substantia nigra pars reticulata via disinhibition of the subthalamic nucleus (STN). Both STN ablation and deep brain stimulation have been used in patients with advanced PD suggesting that phenotypic modulation of the STN may be beneficial for a subset of patients. An alternative approach to silence the overactive STN includes converting the excitatory neurons to an inhibitory phenotype. In an attempt to modulate the STN neuronal phenotype, one group used rAAV2 to deliver glutamic acid decarboxylase (GAD) to the STN of rats essentially converting glutamatergic neurons to GABA producing cells.(127) This conversion from an excitatory neuron to one capable of inhibitory neurotransmission suppressed the firing activity of the innervated substantia nigra and protected neurons from neurotoxicant-induced degeneration.(127) This neuronal phenotype modulation therapy has moved to Phase I and II clinical trials (Table I; clinical trial identifier, NCT00643890).(108, 127–129) The Phase I trial established safety and tolerability. A second randomized, double-blind, placebo controlled trial (Phase II) sponsored by Neurologix, Inc. is underway and enrolling patients that have idiopathic PD (for at least 5 years), UPDRS Part 3 score ≥ 25 in “off” state and demonstrated L-dopa responsiveness for at least 12 months.(129) This is a potentially powerful therapy because it bypasses the degenerating nigrostriatal system but with the caveat that the long-term consequence of “re-programming” glutamatergic/excitatory to GABA/inhibitory neurons is unknown.

4.3 Neuroprotection

Since PD motoric symptoms appear only after substantial loss of SNpc dopamine neurons protection of these cells is an obvious therapeutic goal. Neuroprotection has been achieved in animal models of dopaminergic neuron death following treatment with a prototypical neurotrophic factor, glial cell line-derived neurotrophic factor (GDNF).(130) Although initial clinical trials demonstrated effectiveness, the randomized placebo-controlled double-blinded trial of intraputamin delivery of the GDNF protein was not successful. Therefore GDNF clinical trials have been halted largely due to the failure of the neurotrophin to reach a large enough target area within the human striatum and more importantly, safety concerns.(55, 131,132) These concerns arose when neutralizing anti-GDNF antibodies were found in a subset of treated patients suggesting that an immune response might ensue following long-term treatment. In addition, further review of earlier non-human primate data revealed the presence

of cerebellar Purkinje cell degeneration suggesting that there was some GDNF peptide leakage outside of the injection site perhaps due to the use of an indwelling catheter.(133) These off-target effects of GDNF and failure to reach the entire striatum speak to the need for gene therapeutic approaches that promote efficient and regulated expression.

Another GDNF family member that supports dopaminergic neurons, neurturin (NTN), was shown to effectively protect dopaminergic neurons in rodent and non-human primate models of PD without the development of neutralizing anti-NTN antibodies or cerebellar degeneration. (55,134–142) In an attempt to enhance temporal and spatial expression of NTN, a viral vector-based platform was chosen to deliver this neurotrophin in human clinical trials rather than direct peptide delivery. Phase I and II trials were completed by Ceregene, Inc. and involved intraputamen injections of CERE-120, a rAAV2-NTN vector (Table I).(142,143) Preliminary evidence demonstrated safety and tolerability as well as an improvement in the off-medication motor subscore of the United Parkinson's Disease Rating Scale (UPDRS), however, other measures of motor function were not significantly improved.(144) Because the viral vector delivery was found to be safe, the Phase II double-blind, multicenter, sham surgery controlled trial was initiated in 58 patients with advanced bilateral idiopathic PD. Disappointingly, in November 2008 Ceregene reported that the Phase II trial did not demonstrate any significant differences in the protocol defined primary endpoint of UPDRS-motor off score at 12 months between patients treated with CERE-120 and control subjects (http://www.ceregene.com/press_112608.asp). However, the neurosurgery and gene therapy were well tolerated with no apparent adverse effects in these advanced-stage patients. In addition, thirty patients were clinically followed in a double-blind fashion for an additional 18 months at which time Ceregene officials reported a modest but statistically significant effect on the UPDRS-motor off score as well as on several secondary measures of motor function (Ceregene, Inc.; http://www.ceregene.com/press_052709.asp). It appears from these studies that over time there was a positive yet minor effect of rAAV2-NTN treatment. The small magnitude of the NTN effect was enigmatic since preclinical studies had indicated robust NTN expression from this vector. However the analysis of brains from two patients enrolled in the Phase II trial that died of causes unrelated to the gene therapy demonstrated NTN expression in the putamen but not within the substantia nigra pars compacta (SNpc). These data support a scenario whereby dystrophic striatal projections are compromised and inefficient in the retrograde transport of NTN to the damaged SNpc dopamine neuron cell bodies. Therefore rAAV2-NTN delivered to the SN where it would be available to directly transduce SN dopamine neurons may be more efficacious. In addition, these clinical trials enrolled patients with advanced PD and presumably significant dopamine neuron loss; neuroprotective therapies may be more effective in earlier stage patients.(81)

5. Conclusion

Recent therapeutic advances have been reported for another neurodegenerative disease, adrenoleukodystrophy (ALD), using hematopoietic stem cells transduced with a lentiviral vector expressing the adenosine triphosphate-binding cassette transporter (*ABCD1* gene). (145) These researchers report that ALD progression was halted following treatment. This work supports the concept that neurodegenerative diseases are amenable to gene therapeutic approaches. Although only incremental clinical advancements have been realized thus far for PD gene therapy there is hope that this technology will soon be an important addition to current therapeutic approaches. Gene therapy affords PD clinicians the opportunity to permanently alter dopamine production and neuronal phenotype. Even in the absence of a cure these types of therapies would represent significant therapeutic advancements.

There are critical issues which are currently being addressed that should facilitate the success of PD gene therapy.(133,142) For example, viral vectors are consistently undergoing improved

engineering to enhance delivery, diffusion, and regulated spatial and temporal transgene expression.(104,146,147) In addition, effective novel non-invasive imaging techniques to monitor transgene expression and diffusion of viral vectors are under development and will greatly aid the gene therapy field. (148,149) Furthermore, age-related neurodegenerative diseases such as PD are in need of specific easily accessible and accurate biomarkers that will allow for early diagnosis as well as monitoring of disease progression; these types of studies are currently underway.(112,133,150)

It is not clear why preclinical gene therapy studies are more successful than the ensuing clinical trials. Some of the discrepancies could arise from the many different animal models that used for preclinical studies. Researchers use both neurotoxicant and genetic models that do not faithfully recapitulate the progressive degeneration and behavioral aspects of PD. Current work in the field is focused on establishing improved robust animal models. Another important and sometimes overlooked concept is that sporadic PD may represent a syndrome of diseases and therefore therapies may need to be tailored to specific parkinsonian subtypes.(41,151) Both familial and sporadic PD arise from disparate initiating factors yet the final pathological hallmark is loss of SNpc dopamine neurons. Currently the path between initiation of disease and SNpc dopamine neuron death represents a continuum but with the identification of novel biomarkers clinicians may be able to separate PD patients into subtypes that will benefit from personalized therapies. Furthermore, consistent clinical trial criteria and outcome measurements would allow for reliable analysis of trial data. For example, different PD clinical trials using the same therapeutic molecule (i.e. GDNF) are difficult to interpret because of a lack of standard protocols.(133) Therefore establishing a uniform study design including the same viral vector serotype, injection paradigm, patient selection criteria and outcome measurements would allow for more consistent data interpretation. Finally, PD gene therapy may also benefit from multifaceted approaches, for example gene therapy cocktails such as rAAV2-NTN combined with rAAV2-hAADC and peripheral levodopa may improve both neuronal survival and function better than each treatment alone. In conclusion, as novel targets are identified and the hurdles mentioned above overcome, we anticipate that viral vector-mediated gene therapeutic approaches will have a profound impact on PD progression and outcome.

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Table I

Parkinson's Disease Gene Therapy Clinical Trials

Gene	Viral Vector	Study	Study Phase	Sponsor	Injection Site	ClinicalTrials.gov Identifier	Ref
Amino acid decarboxylase	rAAV	A Study of AAV-hAADC-2 In Subjects With Parkinson's Disease	Phase I	Genzyme	Striatum	NCT00229736	(116,118,119,121,152-156)
Amino acid decarboxylase; Tyrosine hydroxylase; GTP cyclohydrolase I	Lentivirus	Study of the Safety, Efficacy and Dose Evaluation of ProSavin for the Treatment of Bilateral Idiopathic Parkinson's Disease	Phase I/II	Oxford BioMedica	Striatum	NCT00627588	(107)
Glutamic Acid Decarboxylase (<i>GAD 65/ GAD 67</i>)	rAAV	Safety Study of Subthalamic Nucleus Gene Therapy for Parkinson's Disease	Phase I	Neurologix, Inc	Subthalamic nucleus	NCT00195143 Completed	(127-129)
Glutamic Acid Decarboxylase (<i>GAD 65/ GAD 67</i>)	rAAV	Study of AAV-GAD Gene Transfer into the Subthalamic Nucleus for Parkinson's Disease	Phase II	Neurologix, Inc	Subthalamic nucleus	NCT00643890	(127-129)
Neurturin	rAAV	Safety of CERE-120 (AAV2-NTN) in Subjects With Idiopathic Parkinson's Disease	Phase I	Ceregene	Putamen	NCT00252850 Completed	(144)
Neurturin	rAAV	Double-Blind, Multicenter, Sham Surgery Controlled Study of CERE-120 in Subjects With Idiopathic Parkinson's Disease	Phase II	Ceregene	Putamen	NCT00400634 Completed	(144)