

Gene-based therapy of Parkinson's Disease: Translation from animal model to human clinical trial employing convection enhanced delivery

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ABSTRACT

The existing treatment of Parkinson's disease (PD) is directed towards substituting dopamine loss with either dopamine replacement therapy or pharmacological therapies aimed at increasing dopamine at the synapse level. Emerging viable alternatives include the use of cell-based and gene-based therapeutics. In this review, we discuss efforts in developing *in vitro* and *in vivo* models and their translation to human clinical trials for gene-based therapy of this distressing and prevalent neurodegenerative disorder. Given the mismatch between expectations from preclinical data and results of human pivotal trials, drug delivery has been identified as the key emerging area for translational research due to limitation of limited efficacy. The chief highlights of the current topic include use of improved delivery methods of gene-based therapeutic agents. Convection-enhanced delivery (CED), an advanced infusion technique with demonstrated utility in *ex vivo* and *in vivo* animal models has recently been adopted for PD gene-based therapy trials. Several preclinical studies suggest that magnetic resonance imaging (MRI)-guided navigation for accurately targeting and real time monitoring viral vector delivery (rCED) in future clinical trials involving detection of gene expression and restoration of dopaminergic function loss using pro-drug approach will greatly enhance these PD treatments.

KEYWORDS: Parkinson's disease, Vector, Gene-based therapy

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Introduction

Parkinson's disease (PD), a common neurodegenerative disorder with worldwide prevalence, is characterized by progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SN_{pc}) and is evidenced by apoptotic neuronal cell death involving key inducers, excitotoxicity, oxidative stress and mitochondrial respiratory failure.¹⁻³ It is estimated that PD affects as many as 1% of patients over 60 years old⁴ and incidence increases with age.⁵ PD prevalence in India (40 to 328 cases/hundred thousand people) and annual incidence (5 to 7 cases per hundred thousand people) shows the significant rise of this disease worldwide.³

Recently employed pharmacological and surgical approaches offer symptomatic relief in early in the disease but are relatively ineffective at preventing disease progression. These gaps have led to a necessity for *in situ* gene delivery to develop novel viral vector-based treatments with the promise of neuroprotection or neuroregeneration.¹ Several viral vectors mediating stable gene expression in the central nervous system were employed for administration of neurotrophic factors, apoptosis inhibitors, and anti-oxidative agents. Unfortunately, the blood-brain barrier precludes systemic delivery of these factors and often poses problems in the treatment of the disease and the administering agents and consequently direct cranial delivery of therapy

has been advocated.¹ In 2003, Kordower⁴ reported delivery of glial cell-line derived neurotrophic factor (GDNF) via lentivirus (LV) as a safe neuroprotective strategy in rodent and non-human primate (NHP) models. Furthermore, they stated it would be inappropriate if we identify the right molecular targets but do not use the right trophic factor and/or effective delivery method to ensure the distribution of the factor in the neuronal populations. Deep brain stimulation (DBS) offers a treatable supernumerary but only a small number of PD patients meet the stern requirements for surgery.⁵

In present review, we have highlighted contributions of researchers globally working with suitable strategies and approaches to develop disease modifying treatments for this neurodegenerative disease. The multiple angle approach comprises of translation of emerging animal models to human clinical trials, embracing the most reliable, safe and established convection enhanced delivery (CED), and considers key milestones: the theory of intervention, target validation, gene expression/location/durability, efficacy of therapeutic agents, and complications (Fig. 1).

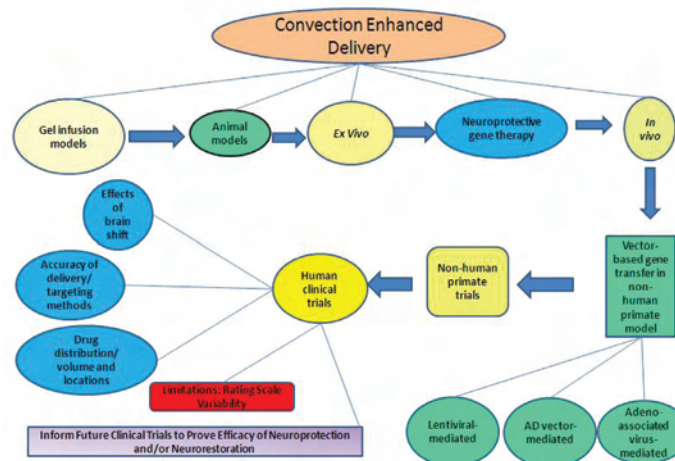


Fig. 1: Approach to gene-based therapy in Parkinson's disease-translation of emerging animal models to human clinical trials employing CED.

Rationale for use of gene-based therapy in Parkinson's disease

Potential roles for therapy

Treatment for PD is moving from palliative to neurorestorative. Understanding the movement in this direction provides a rich backdrop of neurorestoration offered through gene based therapy. Palliative treatments for Parkinson's disease early have involved surgical lesioning within the pyramidal system. After the development of stereotaxic and radiofrequency lesioning, destructive treatments within the basal ganglia were initiated in the form of thalamotomies and pallidotomies. Later, with the development of carbidopa-levodopa, non-surgical treatment was offered.⁶ Surgical treatments were left in the background until it became clear that disease progression and loss of efficacy of medication treatments left patients with medication induced side effects in the setting of a progressive disease.⁷ Concern has been voiced that levodopa therapy may actually worsen certain aspects of PD over time. Oral medication trials have shown neuroprotection in the sense that patients started on a dopamine agonist were less likely to develop levodopa induced dyskinesias within a specified time period than the trial counterparts given levodopa.

In an effort to minimize the development of levodopa induced dyskinesias potentially related to fluctuating levels of dopamine presented to the brain, continuous dopamine delivery has been advocated. A pump delivers continuous liquid dopamine replacement therapy to the jejunum, allowing for improved control over blood levels of medication. This method of delivery can improve systemic dyskinesia management with higher levels of medication; however, it has not been shown to be neuroprotective.⁸

Given limitations of dopamine replacement therapy, treatment turned to neuromodulation to improve symptoms. The theory of neuromodulation within the basal ganglia to alter basal ganglia function was extensively explored in animal models. Early theories of the method of action of deep brain stimulation intervening within the subthalamic nucleus (STN) or within the internal segment of the medial globuspallidus (GPI) are summarized in the much discussed rate model of PD.^{9,10} More recent work is focusing on disrupting transmission of abnormal oscillatory activity within the cortical-basal ganglia-thalamocortical circuit.¹¹

Deep brain stimulation (DBS) has become the gold standard in the surgical treatment of medication refractory PD. Over 80,000 cranial implants have been performed for neurostimulation. Although the methods of neurostimulation are still not fully clarified, DBS has been shown to improve standard clinical rating scales and allow a decrease in levodopa equivalents in a randomized controlled trial.¹² Longevity of therapy has been documented, although success of long-term control of symptoms is variable with speech, posture, and gait progressing over time versus relative retention of tremor control.¹³ Those patients with degenerative diseases such as PD, are hoping for much more in regard to neuroprotection than has been demonstrated with neuromodulation,¹⁴ continuous medication administration, or substitution of dopamine agonists as a first line treatment.

While DBS significantly improves the cardinal symptoms of PD, gene-based therapy offers the possibility of neuroprotection not afforded by DBS.^{13,15} Direct brain infusion gene-based therapy via CED, has been shown efficacious in previous studies. While gene-based therapy controlled trial clinical outcomes have fallen short of the high hopes of the clinical and scientific community, new methods of real time MRI guided CED (rCED) with combination gene-based therapy cocktails, metabolic markers, and objective measures monitoring of the outcomes are becoming a reality to further guide development. The promise of a treatment to alter or reverse the course of PD has driven significant preclinical research towards achievement. Understanding the role of animal models in the translation of new therapies to human clinical trials is timely as investigators are moving forward with the goal of translating more of these preclinical findings into the clinic.

History of vectors used in Gene-based therapy

In gene-based therapy, commonly used viral based vectors allow introduction of therapeutic agent directly to the brain, bypassing the blood-brain barrier and avoiding the use of a cell mediator. Commonly used viral vectors include adenoviruses (AV), adeno-associated virus (AAV), lentivirus (LV) and herpes simplex virus (HSV). However, AAV and LV vectors have shown promise in animal models¹⁶ and being considered as potential tools in the delivery of neuroprotective therapies in patients with PD and other neurodegen-

erative disorders. Considerations when selecting an optimal potential vector: i) ability to be delivered in high concentration; ii) affluence in reproducible production; iii) adaptability in host chromosome; iv) lacking immunity causing components; v) competency in regulating transcription and vi) proficiency in targeting anticipated cell type.^{4,17-20} Several studies have reported gene transfer of GDNF using viral-based vectors AV, AAV, HSV and LV some with analysis of morphological and behavioral outcome, striatal biochemistry, transduction percentage, expression duration and toxicity in rats.^{18,21-29}

Ad-mediated gene transfer

Connor²⁴ examined whether injection of recombinant adenovirus encoding GDNF (AV-GDNF) could protect the older rat nigrostriatal DA system from progressive neuronal degeneration. Injection of GDNF vector into either the striatum or substantia nigra (SN) offered significant cell protection against 6-OHDA lesion. However, only striatal injection of Ad GDNF protected against the development of certain behavioral and neurochemical changes that occur in the DA-depleted brain. Perinigrally injected rats with recombinant adenovirus GDNF (AV-GDNF), adenovirus LacZ (Ad-LacZ) and a week later injected intrastrially with 6-hydroxydopamine (6-OHDA), AV-GDNF gene transfer rats showed protection of dopaminergic neurons.³⁰ GDNF transgene operating under the glial fibrillary acidic protein (GFAP) promoter injected into the striatum of rats one week prior to provoking striatal 6-OHDA lesion demonstrated a high level of GDNF in the striatum and prolonged GDNF expression after injection of AV.³¹ While comparing the efficacy of AAV and AV vectors,³² the AAV showed promising results over AV vectors. However, Ad vectors overcome limitations in payload size and targeting. Furthermore, the cellular tropism of AV5-based vectors is regulated by the Ad attachment protein binding to its primary cellular receptor, the coxsackie and adenovirus receptor (CAR). Many clinically relevant tissues are intractable to AV5 infection due to insignificant CAR levels but can be targeted by tropism-modified, CAR-independent forms of AV.

Adeno-associated virus (AAV)-mediated gene transfer

Recombinant AAV (rAAV) vectors were used to transfer genes to produce 3,4-

dihydroxy-L-phenylalanine (L-dopa) directly into the striatum of PD patients.¹⁸ This study demonstrated that L-dopa levels are sufficient to affect behavior in a dopamine-deficient animal models, produce long-lasting expression, regulate tyrosine hydroxylase (TH) and guanosine triphosphate cyclohydrolase (GTPCH) through transcription, and do not activate the immune response in brain. In rat models, GDNF vectors injection in the striatum is not only effective in rescuing the cell bodies in the SN, but also in preserving nigrostriatal projections and functional striatal dopamine innervation.¹⁶ Long-term studies employing AAV-GDNF and LV-GDNF vectors demonstrated sustained GDNF delivery over 3 to 6 months can promote regeneration and significantly improve functional recovery in both 6-OHDA-lesioned rats and MPTP-lesioned monkeys.¹⁶

AAV-mediated delivery of GDNF provides neuroprotection in aged parkinsonian rats and long-term delivery of these factors to specific regions of the CNS can prove as a new strategy for treatment of neurodegenerative disorders.²⁴ The significance of *ex vivo* and *in vivo* genetic modifications via viral-based or non-viral based vectors, efforts to influence detrimental cell signaling pathways and inhibiting overactive basal ganglia structures have led towards clinical studies using animal models.²⁵

A clinical device designed for human use was evaluated in regard to the delivery of rAAV2 into brains of Rhesus monkeys.³³ AAV2 encoding the human aromatic L-amino acid decarboxylase gene (AAV-hAADC-2) was infused into the putamen of normal rhesus monkeys as a supportive study for a clinical trial in PD patients. Clinical observations included transgene expression five and a half weeks after vector infusions revealed no behavioral abnormalities, no differences in gross pathology, comparable putamen histopathology, and minimal localized inflammatory tissue reaction. Ulusoy *et al*³⁴ reviewed *in vivo* gene delivery for the development of mammalian models, emphasizing on overexpression of PD-associated genes using recombinant viral vectors. Recombinant AAV vectors, in particular, have been very useful in targeting the nigral dopamine neurons both in the rodent and the primate brain. Gene transfer of glutamic acid decarboxylase (GAD) and other methods that modulate production of GABA in the subthalamic nucleus improve basal ganglia function and Parkinsonism in animal models.³⁵

Lentivirus mediated gene transfer

Olson³⁶ discussed that motor deficits experienced by many Parkinson's patients are the result of the degeneration of dopamine neurons. Olson referred to a study by Kordower and his associates³⁷ revealing administration of a LV vector containing the gene encoding GDNF into the nigrostriatal pathway of parkinsonian monkeys precludes neuronal loss and reverses some of the motor deficits. Björklund *et al*.¹⁶ reported administration of LV-GDNF vector resulted in sustained GDNF delivery over 3-6 months promoting regeneration and significant functional recovery when employed in both 6-OHDA-lesioned rats and MPTP-lesioned monkeys. Zurn *et al*.³⁸ used a LV vector encoding the GDNF gene allowed protection of nigral dopaminergic neurons against lesion-induced cell death in rodent and monkey models of PD. Furthermore, enhanced graft survival and differentiation from co-transplantation of embryonic dopaminergic neuronal grafts and a GDNF-releasing capsule resulted in improved animal behavior symptoms. Kordower³⁷ established the efficacy of LV vector as it prohibits further loss and also restored preceding degeneration. Additionally, these injections revealed long-term gene expression and may prove to be a useful technique in the treatment of PD.

The potential of LV vectors lies in i) its ability to infect non-dividing cells that allows stable gene transfer in post-mitotic cells such as mature neurons; ii) to provide a unique tool to integrate siRNA expression constructs with the aim to locally knockdown expression of a specific gene, enabling functional assessment of a gene in a very specific neuronal pathway. The use of LV for stable expression of siRNA in the brain is a powerful aid to probe gene functions *in vivo* and for gene-based therapy of diseases of the central nervous system. Recently, LV gene transfer has been an invaluable tool for evaluation of gene function in behavioral disorders such as drug addiction and attention-deficit hyperactivity disorder or in learning and cognition.³⁹

HIV-1 based vector gene transfer

Cockrell and Kafri⁴⁰ explained the ability of LV vectors to transduce and stably integrate their genomes into non-dividing cells was the reason for developing HIV-1 based vector gene delivery system. The fruitful use of the advanced HIV-1 based vector system opened new opportunities in establishing transgenic animal mod-

els for basic research. Additionally, they described the achievements using HIV-1 based vectors to precise pathological courses of incurable diseases in preclinical animal models including PD.

Methods of gene delivery and rationale for the use of Convection Enhanced Delivery

Convection-enhanced delivery (CED) is an advanced infusion technique used to deliver therapeutic agents into the brain⁴¹ and has demonstrated promise in recent clinical trials.⁴² The infusion process involves mechanically controlled pumps as opposed to hand injection,⁴³ which has proven to be both more efficient and clinically useful, producing a larger and more precise volume of delivered agent.⁴² CED allows for direct intracranial administration of drugs and facilitates the introduction of macromolecules that could not otherwise penetrate the blood-brain barrier^{41,44} have stated about the introduction of CED by the NIH researchers in early 1990s. The technique was adopted for delivery of drugs that could not cross through blood brain barrier and are large enough to diffuse over desired distances into the parenchyma. Several key features of CED, its modeling and speculations on optimization are discussed. CED methods have been standardized following its testing in various models ranging from basic gel infusion to *in vivo* human clinical trials. (Fig. 2)

Physics of infusion

Raghavan and Brady⁴⁵ have underlined direct infusions of biological therapeutic agents into brain parenchyma. However, variation in discrete brain areas and cytoarchitectural precincts within the brain is conducive for uncertain response to fluid flow and pressure. Two main inquiries of significance remained to consider were i) infusion-induced interstitial expansion and the backflow and ii) up-graded elucidation of the diffusion tensor of a particle limited to the interstitial spaces. Raghavan and associates⁴⁶ used a porcine model to accomplish infusions of a saline solution of the magnetic resonance marker gadodiamide into brain parenchyma and paved an efficient way to monitor infusate distribution. These findings, added to the MRI data, facilitated the quantification of the spreading of the volume fraction of the interstitium primarily in white matter of the brain exhibiting infusion edema.

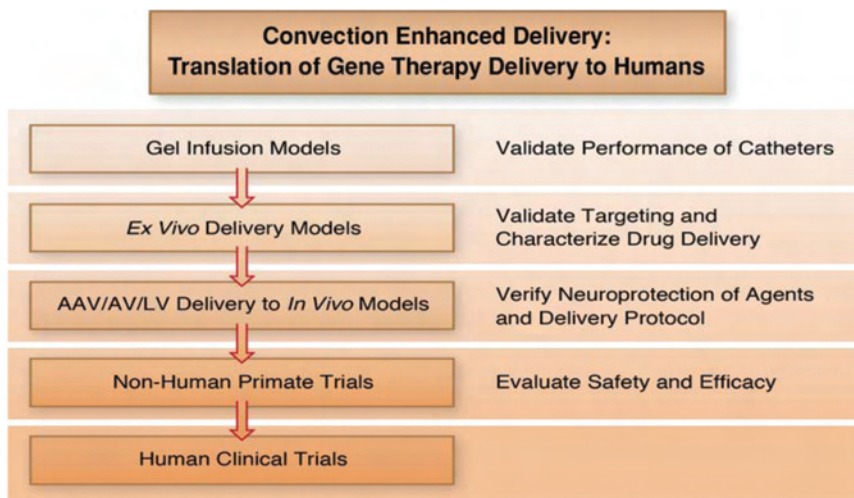


Fig. 2: Convection enhanced delivery: A method of choice for gene-based therapy trials.

Modeling CED therapy delivery and translation to the human

Gel Infusion Models

CED is an emerging and promising infusion tool employed for clinical trials to facilitate delivery of macromolecules that could not otherwise penetrate the blood brain barrier (BBB) or delivering more precise volume of therapeutic agents into the brain via mechanically controlled pumps.^{41–43,47,48} Panse and associates considered CED to be a valid alternative for systemic administration of agents by intravenous or oral routes, which may minimize side effects.⁴⁹ Infusion protocols and catheter design have an important impact on delivery. The agarose gel model is helpful in benchmarking first principles of catheter based CED.⁵⁰ Methods of delivery of multiple spherical payloads to fill a non-spherical target may influence treatment.⁵¹ Recent published ramped infusion protocols^{52,53} using an open endpoint infusion catheter were replicated in gels and found to be viable. These results in an agarose gel model of brain suggest that specific performance characteristics of an infusion catheter proposed for CED were in line with benchmark data when backflow, infusion cloud morphology, and volume of distribution were compared, thus providing confirmation that proposed CED techniques appear promising for eventual clinical application. Our published paper⁵⁰ comprises of detailed specific methods including preparing agarose gel, infusate tracker, tubing, computer control, catheter calibration, and specifications of two different catheters viz. the

ERG valve-tip and the Smart Flow™ used for experiments. Results include measurements of visible indicator dye at the time of pressure stabilization (backflow distance) and at the end of the infusion, proximal infusate distance (backflow at the end of the infusion), distal infusate distance (backflow forward of the catheter tip), and morphology of infusion cloud. Independent confirmation of proposed protocols is important to establish optimal devices and protocols designed for human clinical trials employing CED and the agarose gel model can help refine first principles and prepare for tissue-based testing.

Drug Distribution, volume and location

The implications of MRI is suggestive in determining key alterations in tissue properties of varying patient populations and correlation between the infused volume and distribution, and distribution characteristics similar to the co-infused surrogate tracer involved in studies. To recuperate the inclusive effectiveness of CED and conception of the delivery process through *in vivo* experiments, practical alterations were suggested by using nanoliposomes.¹⁵ Panoliposomes were proposed for dual purpose: to standardize delivery of vehicle for the convection of drugs into the target tissue and as an MRI contrast agent. These developments were considered for further improvement in efficacy of the treatment-volume delivery. Gadolinium Diethylenetriaminepenta acetic Acid (Gd-DTPA) was recommended as potential way to monitor the distribution of large molecules.⁵⁴ Furthermore, CED combined with Gd-DTPA has the

potential of demonstrating the anatomic and volumetric distribution of large molecules and leaks into the cerebrospinal fluid spaces and resection cavities. In an animal model of PD, six months effect of GAD injection while modulating GABA production in the STN revealed 8.1 and 4.7 points improvement in surgery and sham surgery, respectively.³⁵ The accuracy and efficiency of CT/MRI scans of 58 patients receiving either MRI, or a CT scan, or both facilitated characterization of cystic pancreatic masses.⁵⁵ The comparison of patient and scan-based diagnosis revealed relatively accurate information whether a mass was malignant or benign. Rainov *et al*⁵⁶ assert that targeted toxins currently being employed specifically bind to surface receptors overexpressed in tumor cells and also extremely effective against these types of cells that are resistant to many other types of treatment. The progress of phase 3 trials, efficacy of therapeutic agent, acceptable levels of toxicity and safety for patients has been discussed.

Ex Vivo whole brain animal models

Two noteworthy contributions documented high flow microinfusion and its impact on tissue penetration and related pharmacodynamics.^{43,57} A published NIH infusion model was utilized to measure uniform medium validating the role of flow rate, catheter diameter, targeted tissue, and resulting backflow during focal delivery and direct infusion to brain. Hadaczek *et al*⁵⁸ infused AAV2, fluorescent liposomes, or bovine serum albumin into the rat striatum via CED and report i) the high BP/heart rate rats exhibited significantly larger distribution of the infused molecules in target site with extensive transport to the globus pallidus; ii) AAV2 distribution was 16.5-fold greater in the rats with high BP/heart rate compared with no heart beat. The results from liposomes distribution were consistent with viral capsids. However, the distribution of infused molecules was confined to the space around brain blood vessels in all the rats. They have stated that fluid circulation within the CNS through the perivascular space is the primary mechanism of distribution of administered therapeutic agents by CED. Furthermore, they observed widespread distribution of viral agents within rodent and monkey brain tissue. Future investigation of the use of *ex vivo* NHP and human cadaveric tissue is warranted. -04007; No. of pages: 10; 4C.

In Vivo animal models

In vivo animal models have been used for investigating both the progression of PD and the treatment efficacy. When gel and *ex vivo* models are not sufficient modeling the disease in animals allows researchers to better understand the underlying mechanisms of neuron degeneration helpful in finding novel approach to control and reverse the progression. The introduction of gene vectors into animal models, determining the risks, benefits, and efficacy of therapeutic agent will pave a way to enter the human clinical trial phase. The knowhow obtained from insect models, mouse models, rat models, and non-human primate models will facilitate advancement of the field of innovative treatments for PD.

Insect model

Perhaps the most basic animal model has been the insect though it cannot offer a direct connection to human anatomy but is nevertheless crucial in investigating gene mutations. Considering several previous studies, a *Drosophila* model was proposed to determine the role of single-gene mutations in rare heritable forms of PD.^{59,60} These studies revealed new molecular mechanisms in terms of the role of genetic risk factors in disease occurrence and pathogenesis of PD, a step towards finding the potential efficacy of neuroprotective complexes.

Mouse model

The mouse models have the potential to provide further insight into the disease pathogenesis. The role of the LAP2 gene in the expression of latency-associated transcripts (LATs) in the HSV,¹⁷ the mice showed protection after six months of injection. Adding more to the pathogenesis of PD the vector-mediated overexpression of the antioxidant enzyme glutathione peroxidase (GPX) was investigated by employing LV vector carrying the GPX1 gene.⁶¹ A two-fold increase in GPX activity when compared to cells infected with the control vector demonstrated the small but significant protection of nigral dopaminergic neurons against drug-induced toxicity.

Rat models

In the past decade the rat models have shown much prominence than the mice models in regard to the efficacy of treatment employed. The role of aromatic L-amino acid decarboxylase (AADC) in

gene-based therapy with TH by adding the gene for AADC to the paradigm using primary fibroblasts transduced with both TH and GTP cyclohydrolase was investigated.^{62,63} Results from both studies showed that adequate AADC near striatal grafts produced L-DOPA and the close proximity of the enzyme to TH is detrimental for optimal dopamine production. In a progressive lesion PD model, the efficacy of Ad-mediated GDNF gene transfer in striatum demonstrated protection of neurons.²³ A safe, non-pathogenic purified viral vector rAAV was able to mediate long-term striatal hTH transgene expression in neurons which could effectively deliver L-Dopa to the striatum.²⁶

Non-human Primate (NHP) models

The primates including *Homo sapiens* and their close neuroanatomical resemblance make NHP models of considerable significance for gaining knowhow of mechanisms of disease pathophysiology and search of potential therapeutic treatment. Injection of LV-GDNF into the striatum and substantia nigra (SN) of non lesioned aged rhesus monkeys or young adult rhesus monkeys treated one week prior with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) resulted in: i) extensive GDNF expression with anterograde and retrograde transport in all animals; ii) LV-GDNF augmented dopaminergic function in aged monkeys; iii) LV-GDNF reversed functional deficits and completely prevented nigrostriatal degeneration in MPTP-challenged monkeys; iv) LV-GDNF injections revealed eight months of gene expression in intact rhesus monkeys; v) LV-GDNF treatment reversed motor deficits in a hand-reach task in MPTP-challenged monkeys. Thus efficacy of LV-GDNF system preventing nigrostriatal degeneration and inducing regeneration in primate models of PD might be a viable therapeutic strategy.³⁷ Based on the stable phase of the dopamine-depleted state in three age groups of female rhesus monkeys showed increase in combined striatal trophic activity in the contralateral hemisphere proportionally with age⁶⁴ where younger but not the middle and old age monkey showed an increase. The role of a common receptor tyrosine kinase (RET) in GDNF and neuroturin (NTN) was studied where neither RET-immunoreactive cells nor RET protein intensity exhibited any change with age of monkeys.⁶⁵ As trophic factors associated signaling remain intact with the age the administration of GDNF

and NTN can enhance the dopaminergic function of neurons in the nigrostriatal system irrespective of age group.

Functional analysis revealed that the LV-GDNF but not LV-LacZ treated monkeys displayed behavioral improvements that were associated with increased fluoro-dopa uptake in the striatum ipsilateral to LV-GDNF treatment.⁶⁶ Furthermore, the aged MPTP-challenged primate brain has the potential to respond to trophic factor delivery and that the degree of neuroprotection depends on GDNF levels.⁶⁶ The efficacy of AAV2 vector encoding human NTN (CERE-120) delivery to the caudate and putamen of monkeys monitored for one year evident by long-term expression and bioactivity with no adverse effect supports the ongoing clinical testing in PD patients.⁶⁷

Human Clinical Trials

Applications of CED in neuro-oncology

In this section we review the key features of CED as well as modeling of the procedure and indulge in informed speculation on optimizing the direct delivery of therapeutic agents into brain tissue. Hall and Sherr⁶⁸ discussed targeted toxin trials employing CED focusing on various parameters of delivery and infusion rate, treatment period, and drug dosing. The results supported their ongoing investigation towards glioma treatments. Kunwar *et al.*⁶⁹ employed CED for infusing in *Cintredekinbesudotox* in parenchyma (a recombinant chimeric cytotoxin comprising of IL-13 and a truncated exotoxin produced by the *Pseudomonas aeruginosa* targeting malignant glioma cells). They proposed the usefulness of this study and recommended the use of CED technique in any clinical trials. Sampson *et al.*⁷⁰ tested the ability of a software algorithm using MR diffusion tensor imaging to predict patient-specific cytotoxin, *Cintredekinbesudotox* co-infused with iodine 123-labeled human serum albumin (123I-HSA), in patients with recurrent malignant gliomas. The use of this simulation algorithm was considered clinically useful in 84.6% of catheters. Routine use of this algorithm should improve prospective selection of catheter trajectories and thereby improve the efficacy of drugs delivered by this promising technique.

Optimizing safety and efficacy of CED in the clinic

A safety profile supported by data such as optimization of cannula placement for

infusion in the target tissue is must to practically advance in clinical studies.

In order to further our understanding of this novel technique, a safety and toxicity profile of *Cintredekinbesudotox* administration via intra-parenchymal CED after the resection of supratentorial recurrent malignant glioma was reviewed in 53 patients.⁶⁹ This information provided crucial information pertaining to the prevention of adverse effects and the minimization of their severity. Ratliff and Oldfield⁷¹ who are pioneers in CED technique, demonstrated the convection capacity of ferry macromolecules across sites of nerve injury. Several aspects studied include i) functional effects of convection; ii) a safe and effective macromolecule delivery to the spinal cord; iii) a safe and effective means to deliver macromolecules; iv) under limitation of convection block in lacerated and suture-repaired nerves. Convection can be used for delivery to spinal cord gray matter via peripheral retrograde infusion.

Human clinical trials for gene-based therapy voice imperative safety issues that confuse the design being considered and necessitate all-embracing preclinical testing. Development of *ex vivo* delivery of gene, CED protocols modifications prior to use in human showed exceptional safety profile. AAV2-based gene delivery vector encoding NTN (CERE-120) has shown potential for PD⁷² evident by long-lasting efficacy protecting substantia nigra cells, preserving fiber innervation of the striatum and behavioral recovery for six months,⁷² safe with no side effects or toxicological responses. The findings⁷³ suggest that genetically β -nerve growth factor (NGF)-modified bone marrow stromal cells (BMSC) can be effective for PD treatment.⁷³

Many considerations must be made in regard to efficiency, coverage, and catheters utilization when considering a human clinical trial with multiple stacked infusions.^{35,44-46} In phase I of clinical trial, employing CED of AAV2 vector into the putamen resulted consistently as in NHP experiments.⁷⁴ MRI scans confirmed the precise target of CED cannula tracts, the colocalization of T2 hyperintensities and increased PET uptake around distal cannula tracts, and the relationship between T2 hyperintensity and hAADC immunohistochemistry. Bartus⁷⁵ followed this study with a 2011 phase II human clinical trial focusing on direct infusions of therapeutics into the SN. This therapeutic

agent contained a neurotrophic factor to impede cell death and delivery strategies of four trajectories produced insignificant results. This is unusual considering the LeWitt³⁵ study, which did produce significant results in regard to AAV2-GAD gene delivery into the subthalamic nucleus. Later analysis determined only a 15 percent coverage of the target region in the Bartus⁷⁵ study.

Limitations

Gene-based therapy is uniquely poised for delivering therapeutic molecules to site-specific regions of the central nervous system. Gene-based therapy facilitates segments of DNA placed into a carrying vector encoding for a gene of interest and can be introduced into specific cells.³² Sampson *et al.*⁵⁴ considered that CED is currently limited by suboptimal methodologies for monitoring the delivery of therapeutic agents that would permit technical optimization and enhanced therapeutic efficacy. Disease progression in clinical trials is limited to ranges in clinical scores of cognitive and motor tasks as determined by the researcher. Various scales used for PD include i) Hamilton rating scale for depression (HAM-D-17; Williams *et al.*);⁷⁶ ii) UPRDC (Mittermeyer *et al.*);⁷⁷ iii) Parkinson's disease questionnaire (PDQ39, Harris *et al.*);⁷⁸ iv) Core Assessment Program for Surgical Interventional Therapies in Parkinson's Disease (CAPSIT-PD, Antonini *et al.*);⁷⁹ v) abnormal involuntary movement scale (AIMS; Wolz *et al.*);⁸⁰ Non motor symptom questionnaire (NMSQ) where NMS of PD were recognized by James Parkinson himself (Parkinson J.,⁸¹ c.f. Chaudhury *et al.*);⁸² and are modulated by medication intake stays in modulating another source of result variance. Neuroimaging is a possible surrogate marker of PD progression that may aid in differentiating pharmacological and non-pharmacological neuroprotective effects.^{4,20,37,83,84} For example, performing a bore hole craniotomy, air immediately rushes into the subdural space between brain and skull causing movement of the tissue relative to the skull. This movement, called brain shift, occurs suddenly and typically resolves in approximately one week with the resorption of retained pneumocephalus. Van den Munckhof *et al.*⁸⁵ investigated this complication. As subdural air enters the skull during surgery, it causes the brain to shift as the electrode is implanted into the shifted brain. Then, when the brain shifts back to its original position,

the previously implanted electrode shifts with it, creating displacement. 14 patients were retrospectively reviewed, and their CT scans confirmed that brain shift causes post-operative electrode curving. A similar study reviewing 12 patients experiencing brain shift while considering effects on other treatments and future treatments was also performed by our team.⁴⁷ The conclusion of this study was that brain shift and subsequent electrode displacement and deformation may occur in all patients undergoing DBS as a treatment option. Intraoperative brain shift may complicate surgical treatment and lead to electroderetraction. Brain shift may have greater detrimental effects on future treatments, especially those performed in the supine position and those administered with a rigid catheter instead of a flexible electrode with proposed infusion times measured in hours. Intra-operative brain shift is now being as operative suites allow certain surgeries to occur while documenting dynamic brain movement over time (University of Wisconsin unpublished data). Future engineering adaptations may prevent tissue damage that occurs as a result of brain shift,⁴⁷ such as incorporation flexibility in the proximal catheter after the tip has reached its target.

Future treatments and directions

There are several necessary improvements that may speed further progress in gene-based direct infusion therapies. One of these advances involves improved understanding of diffusion within the brain after completion of CED infusions. While CED has become a possible future treatment alternative as a method of delivery and distribution of small dosages of therapeutic agents targeting specific tissues directly,⁷⁴ it does not effectively account for the fluid flow and pressure responses that vary in different regions of the brain. The dynamic responses of the tissue that require a nonlinear treatment based on poroelasticity may better comply with the fluid flow and drug transport to the interstitial space of the brain.⁴⁵ Another necessary improvement includes the composition of the therapeutics themselves. *In vivo* biochemical assays using microdialysis may effectively target the major genes responsible for dopamine synthesis and processing. Another area of research would be to explore other options for gene delivery.³² The characteristics of the AAV vector system, currently used in several gene-based therapy trials,

will ultimately create barriers to progress in clinical therapy. Several studies conclude that MRI-guided navigation for targeting CED of AAV vector in parkinsonian non-human primates (NHP) involving detection of gene expression and restoration of dopaminergic function loss using pro-drug approach will pave a way for promising treatment for CNS diseases.

Contributors to key milestones

The review would remain incomplete without introducing the noteworthy contribution of the researchers approaching

this disease from different angles. Recent development in the field of neuroprotective therapeutic interventions using gene-based therapy to modify the progression of PD is hope in future. The researchers are engaged in developing new strategies by employing emerging animal models of transgene viral vectors, target validation of CED and testing efficacy of novel therapeutic agents using newer designs of clinical trials.

Target validation employing MRI became a significant tool for monitoring precise

volume and distribution of administered therapeutic agents. Novel platform for MRI-guided CED of therapeutics with special reference to validation in NHP brain, T2 imaging in monitoring of intraparenchymal real-time CED, interventional MRI-guided putaminal delivery of AAV2-GDNF for clinical trial in PD, gene and cell delivery to the degenerated striatum in preclinical efforts in primate models, future application of gene-based therapy are noteworthy. A number of groups have contributed to this and are tabulated (Table 1).

Table 1: Key milestones including i) theory of intervention, ii) target validation, iii) gene expression, iv) efficacy and v) complications

Key milestone	Inquiry	Findings	Reference
1. Theory of intervention (Palliative/Neuroprotective/Neurorestorative)			
[AAV]	Clinical trials in neurological disorders: Promises and challenges	All trials depend on the <i>de novo</i> expression of an enzyme or a trophic factor to view on neuropathology. Strategies used for the PD, Canavan's and lateral sclerosis, are discussed	86
NHP AAV2	Evaluate infusion device & delivery parameters for intraputamenal gene therapy	AADC distribution throughout the putamen without significant difference. Clinical practicality of novel device employing non-ramped protocol and its adaptability for human diseases	33
NHP Liposomes	Effects of the perivascular space and CED	CED in putamen linked with perivascular transport of liposomes, all through CNS arteries. Perivascular space serves as a channel for distribution of endogenous molecules including interstitially infused agents.	87
[rAAV]	Therapeutic agents to treat neurological disorders	Use of rAAV in early stage clinical trials for neurodegenerative disorders, preclinical data, and possible limitations for rAAV-based gene therapy are discussed.	88
A review	Real-time imaging of liposome delivery quantification	Practical aspects, liposomes as colloidal systems, real-time distribution and its quantification and further improvements are highlighted	89
Rat [AAV]	Role of overexpressed E3 ligase, parkin in PD	Enhanced levels of striatal tyrosine hydroxylase (TH) and dopamine (DA) following nigral parkin overexpression; no protective magnitude on the NA DA system, yet increase in striatal TH parkin extended competence of NS DA transmission in intact nigral DA neurons.	90
NHP [Liposomes]	Safety of rCED	High predictability, procedural repeatability with identical results and no long-term brain pathologies.	91
A review [primate models]	Status of preclinical efforts	Advancement in new preclinical models while addressing the questions rising from clinical data.	92
Rat [Human interleukin-10 gene]	Human IL-10 gene transfer is protective	Transcriptional analyses revealed alteration of a few genes by AAV2-hIL-10 may contribute to neuroprotection.	93
A review	Combating the inflexible PD progression	Exclusive features with outstanding look for developing PD therapy; failure of conventional pharmacotherapies and surgical interventions; development of the treatment providing not only palliative relief but lifelong cure also.	94
[AAV/HSV/LV]	Properties and clinical grade production	Characteristics of rAV, rAAV, HSV, and LV are defined and compared.	95

Key milestone	Inquiry	Findings	Reference
A review	Choice of viral vector for future applications	Use the viral vector that predicts enzymatic or neurotrophic activity in the recipient tissue.	96
NHP [AAV] A review	Specific dissemination of viral vectors from the parenchymal infusion site.	Vector distribution is mediated by CSF within perivascular space, vector transferring along axonal projections, vector receptor followed by cell transduction in distant areas.	97
NHP [Liposomes]	CED of liposomes	Improved cannula design that reduced significantly infusate reflux; introduced MRI contrast agent Gd facilitates tracking of liposomes infusions into brain parenchyma.	98
A review	Gene therapy for PD: NHP to humans.	Achievements and challenges during 2008-10 are discussed.	99
A review	Animal to human trials	Translation of gene therapy from animal model to human revealing "where we are now and where are we going".	100
A review	Animal to human trials	Use of AAV9 is "over the fence and into the woods" is commendable.	101
A review	Gene therapy strategies: Phase I or Phase II clinical trials	i) Enhancing endogenous DA levels or intensifying the function of levodopa; ii) normalizing basal ganglia circuitry by reducing the PD-related over-activity of specific brain structures; iii) leading to symptomatic benefit; iv) potential of gene delivery of trophic factors.	102
NHP [AAV2-GDNF]	Anterograde distribution of AAV2 vectors in the brain via CED	Widespread distribution of vector-GDNF within the putamen and transport to the severely lesioned SN specifies anterograde transport by SN connections validates non-clinical neurorestoration.	103
2. Target validation			
NHP Liposomes	Gadolinium-loaded liposomes allow for real-time MRI of CED	An integrated strategy combining liposome, nanoparticle technology, CED, and MRI for the treatment of brain tumors.	104
Human and non-human primates	Striatal volume differences	The volumetric ratio of size and species of the monkeys used are liable for variances in ratios for each structure between monkeys and humans should be considered for clinical therapies	105
NHP [AAV1/AAV2]	Real-time MRI of AAVV delivery in brain	AAV1 correlates better than AAV2 with MRI delivery monitoring and this may be due to tissue specificity of serotypes	106
Rat	Cannula design optimization and CED placement	Stepped cannula with a 1-mm tip assures reliable distribution of gene transfer, local protein delivery or cellular replacement.	107
NHP	Implications for clinical delivery of therapeutics	Cannula placement recommendations from translational NHP studies and use in clinical trials.	108
[AAV2-GDNF]	Use of Gd and MRI	To monitor AAV2 infusion and envisage the distribution of GDNF protein.	109
Human and non-human primates	Putamen optimal region for image-guided CED	Cannula placement and optimal stereotactic coordinates incriminated in justifying effective delivery.	110
NHP [AAVV]	T2 imaging in monitoring intra-parenchymal rCED	For detection of intra-parenchymal delivery and distribution of a transgene	111
NHP	Preclinical validation in nonhuman primate brain	Developed a unified delivery platform with no infusions shaped occlusion, cannula reflux, leakage, or signs of unpredicted age.	112
AAV ₂ -GDNF	Interventional MRI-guided putamenal delivery AAV2-GDNF for clinical trial.	Factors essential for vigorous expression of vector-GDNF in the putamenal motor area and afferent SN of PD patients are discussed.	54
NHP [AAV-GDNF] {putamen}	Image guided CED of GDNF protein	Reflux-resilient cannula may permit reconsideration of direct GDNF infusion into parenchyma.	113

Key milestone	Inquiry	Findings	Reference
NHP AAV	Guided delivery of vector	MRI and new stereotactic aiming devices as convincing tools for gene-based therapy	114
NHP AAV2-hAADC	Safety and tolerability of MRI-guided CED	The approach comprising of directed accurate cannula placement, desired vector distribution and with no hostile effects of high dose.	115
3. Gene Expression/Location/Durability			
NHP [TH enriched <i>ex vivo</i> engineered autologous monkey fibroblasts]	Location of gene expression	MPTP-treated monkeys resulted in expression up to four months; gene expression at three weeks and three months limited to the striatum neurons.	116
NHP [AAV ₂ -AADC]	Gene expression and restoration of dopaminergic function using pro-drug	Positron emission tomography and AADC tracer, 6-[(18)F] fluoro-1-m-tyrosine can be used for monitoring gene therapy	117
Rat [AAV-TK]	Vector distribution following intracranial CED	Tissues receiving high doses of AAV-TK exhibited the vector in brain hemispheres, spinal cord, spleen, kidney following three weeks of infusion.	118
Rat [rAAV]	Sustained long-term expression of transgenes	Characterized new rAAV serotypes with improved transduction efficiencies in various regions of the brain and spinal cord.	119
Rodent [AAV _{1,2,5}]	Cell tropism, transgene expression duration, distribution of viral transduction and immunity	Following delivery to different regions of the CNS, rAAV2/1 and rAAV2/5 revealed significant transduction frequencies than rAAV2/2.	120
NHP [L-dopa]	Origin of Dyskinesias	Increased levels of focal DA in response to L-dopa administration can result in dyskinesias in patients that developed off-drug	121
NHP (PD/MPTP) [AAV ₂ -hAADC]	Durability of AAV transgene expression	Infusion of AAV vector into brain results in at least six years of transgene expression	122
NHP [AAV ₂] {striatum}	CED into striatum and transport in brain	CED is efficient method for delivery of the AAV2 vector, detection of the transgenes.	123
NHP (PD/Fabry) [AAV ₂ -hAADC]	Bio-distribution of vector by CED in brain	A dose-dependent increase in vector DNA; following six months of infusion 99 per cent in the target site; high dose AAV2-hAADC recipients or control AAV2-GFP control groups exhibited no significant increase in neutralizing antibody titers.	124
4. Efficacy			
<i>In vitro</i> [HIV-2 derived LV vectors]	Gene transfer of aromatic acid decarboxylase (AADC)	i) Enhanced efficiency of transduced cells to convert L-dopa into dopamine; both monocistronic and bicistronic vectors effective; self-packaged vectors and the cross-packaged hybrid vectors effective in gene transfer.	125
NHP _m (6-OHDA) [AAV ₂ -GDNF]	Protection against 6-OHDA lesion	Exhibits behavioral and anatomical efficacy of GDNF delivered via rAAV vector, a possible scenario for PD treatment.	126
NHP	PET imaging of gene expression	Several aspects of molecular therapy are discussed	127
Human	STNDBS and dopaminergic therapy share similar functional mechanisms	Both therapies showed significant metabolic decrease in the putamen, globus pallidus, sensorimotor cortex and cerebellar vermis.	128
[AAV ₂ -hAADC]	Phase I safety trial of gene therapy	Increase of 30 per cent FMT uptake (K _i (c)) in the putamen following gene transfer demonstrates therapy safety.	129

Key milestone	Inquiry	Findings	Reference
NHP [AAV-hAADC]	Dose ranging of viral vector	Implicated for the design and interpretation of clinical studies of AAV-hAADC gene therapy	130
NHP [AAV ₂ -NTN] {striatum}	Use of CERE-120	Provided a substantial evidence for a novel treatment for PD	131
NHP, rhesus [AAV ₂ -GDNF]	Effects of vector-GDNF on dopaminergic nigrostriatal pathway	Functional recovery in the nigrostriatal pathway, no adverse effects and safefor delivery of therapeutic agent over a six-month time.	132
NHP [AAV ₁]	Transduction of brain with vector: antigen-presenting cells within the CNS	Carefulwhile studying transgene therapy with vectors possess-ing broader tropism than AAV2	133
NHP (PD/MPTP) [AAV ₂ -hAADC]	Clinical improvement in MPTP-lesioned animals	L-dopa therapeutic window resulting from AADC gene therapy is evident and persistent for many years	134
Rat [GDNF / NTN]	Pharmacokinetics and bioactivity of GDNF and NTN	Daily or continuous dosing not necessary for delivery of growth factors into the CNS.	135
Human [AAV ₂ -NTN]	A double blind, randomized, controlled trial	Intrapataminal AAV2-NTN not superior to sham surgery when assessed at 12 months using the UPDRS motor score	136
NHP [AAV ₂ -NTN]	NTN-expression linked with sporadic TH-induction in the striatum: a comparative study	i) Infrequent evidence NTN in SNc cell bodies; ii) strong signal of nigral-NTN in all monkeys; iii) on the other hand NTN exhibited strong TH-induction all over the nigrostriatal neurons in primates.	75
NHP [AAV9]	Systemic gene delivery: AAV9deliv-ery via CSF	The results support the use of AAV9 for gene transfer to the CNS for disorders in pediatric populations	137
NHP [AAV/LV]	Role of difference in measurements of brains of NHPs and humans	Obtaining improved translation of stereotactic targeting coordinates with promise to enhance efficacy in human clinical trials.	138

5. Complications

NHP & Canines	Infusate leakage via real-time imaging of CED	Real-time MRI during CED can lead to accurate andvigorous dis-tribution of delivered therapeutic agents.	139
NHP _R [AAV2-GDNF]	CED method and volume distribu-tion within putamen	Increased FMT-PET uptake in the ipsilateral putamen and lo-comotor activity; high dose gene transfer caused GDNF fiber and extracellular immunoreactivity; retrograde and anterograde transport of GDNF to other regions; non-significant effect on DA in the ipsilateral putamen.	140
NHP [AAV2-GDNF]	AAV2-GDNF by CED in putamen: Safety evaluation of gene transfer	Nonclinical neurorestoration after putaminal infusion. However, its administration in nigra resulted in a significant weight loss raised question.	141
Rat [AAV ₂ -GDNF] {basal ganglia}	Axonal transport of AAV2-GDNF in basal ganglia	Anterograde carriage of AAV2 leads GDNF expression in basal ganglia the area affected besides SNc in PD. Vector delivery to SN does not straight GDNF expression in ST.	142
Rat [α-synuclein AAV gene]	Toxicity of human shRNA to dop-amine neurons.	i) High levels of SNCA gene human shRNA were toxic to DA neu-rons; ii) surrounding neurons exposed to lower levels protected by hSNCA gene silencing, a promise for novel PD therapy.	143
Rat, mice, dog, sheep, rabbits, porcine [AAV1, AAV2, AAV6, AAV9]	Neutralizing antibodies (NA) against AAV serotypes inmodels of large animal species.	i) High occurrence of NA in humans; ii) lowest levels in rats; iii) naive mice held NA; iv) distinct AAV6 transduction in dog; v) modest neutralization of AAV transduction in sheep and rabbit; vi) strongly inhibited transduction in porcine by all se-rotypes. Highincidence of NA in humans is obstacle for gene-based therapy.	144

Considering transgene studies in the host, the pioneering work of Bankiewicz and Oldfield group gets academic popularity not only in gene-based but cell-based therapies as well. Several aspects studied by this group have a special relevance such as transient behavioral recovery in hemiparkinsonian primates, trophism, transplantation, and animal models; application of gene-based therapy using NHP model, CED of AAV vector in parkinsonian monkeys, *in vivo* detection of gene expression, long term clinical improvement in MPTP-lesioned primates after gene-based therapy with AAV-hAADC, *ex vivo* and *in vivo* gene-based therapy for PD, and several others comprising of grafting genetically engineered cells into the striatum of NHP yielded a qualitative and quantitative data in field of preclinical models of PD (Table 1).

The lead in research on GDNF- and NTN-viral vector delivery by CED gave a credit to Kordower group. Hadaczek, Bringas, and Bankiewicz work on AAV2-GDNF vector expression, eight years of clinical improvement in MPTP-lesioned primates after AAV-hAADC gene-based therapy, transduction of NHP brain with AAV-1, vector trafficking and immune response, pharmacokinetics of GDNF and NTN in rat brain, CED of AAV2 in monkey striatum, limited efficacy of gene transfer in HSV-gene-based therapy filled the gap in the research on PD. Investigation of CED method in depth explored several avenues to enrich the literature with physics of infusion, modifications for improvement of protocols, and highlights of MRI intertherapeutic interventions was breakthrough from Sampson, Raghavan, Brady and their colleagues. An attempt to study efficacy of various therapeutic agents for control or reversal of PD in addition to control in early stage was considered by Hadaczek, Oldfield, Eberling, Asanuma, Kaplitt and their teams. They have shown improvement in the treatment modalities, however, complete success is still awaited (Table 1).

Conclusions

In the last two decades researchers have undertaken challenging studies to offer neuroprotective therapies in PD. Since systemic delivered therapy has fallen short of hopes for neuroprotection and neuroregeneration employing gene-based therapies such as GDNF- and NTN-viral vector system via CED have emerged as alternative strategies. Using this route,

several studies have shown that trophic factors can be locally produced at the target site after delivery over the long term, thereby providing the opportunity for prolonged neuroprotection and the possibility of providing an environment conducive of regeneration.

This review highlights auspicious interventional therapeutic agents and biological targets presently under evaluation for transitory to mainstream PD treatment. Although preclinical results have been encouraging, significant progress to the clinic will depend on several factors such as understanding genetic mutations or susceptibility factors that lead to PD, effective translation between preclinical animal models and clinical research, and optimum design of future clinical trials. Exploring protocols, appropriate viral vectors delivered to suitable targets under MRI rCED may allow for optimization of transgene delivery, maximum safety, improved efficacy for future clinical trials.

Independent verification of rCED trial infusion protocols is warranted with benchmark testing of published parameters in applicable models such as gel phantoms, *ex vivo* tissue and *in vivo* preclinical animal models to effectively inform planned and future clinical therapies. Further understanding of the multi factorial nature of viral vector infusions suggests the need for real-time assessment of vector distribution for optimizing clinical efficacy and safety. In addition to longitudinal observation of improvements in clinically relevant patient outcomes rating scales, post-mortem autopsy and histological analysis if co-registered with imaging based verification of extent and location of delivery will help confirm efficacy and evaluate any unforeseen limitations to future trials and adoption of these therapies in the clinic. Careful follow-up of the observations from ongoing research on gene-based therapy over the next years will enhance our outlook and provide a foundation to translate these findings in the clinic.

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