Journal of Parkinson's Disease 11 (2021) S173–S182
DOI 10.3233/JPD-212724
IOS Press

Review

An Update on Gene Therapy Approaches for Parkinson's Disease: Restoration of Dopaminergic Function

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Accepted 14 July 2021
Pre-press 4 August 2021

Abstract. At present there is a significant unmet need for clinically available treatments for Parkinson's disease (PD) patients to stably restore balance to dopamine network function, leaving patients with inadequate management of symptoms as the disease progresses. Gene therapy is an attractive approach to impart a durable effect on neuronal function through introduction of genetic material to reestablish dopamine levels and/or functionally recover dopaminergic signaling by improving neuronal health. Ongoing clinical gene therapy trials in PD are focused on enzymatic enhancement of dopamine production and/or the restoration of the nigrostriatal pathway to improve dopaminergic network function. In this review, we discuss data from current gene therapy trials for PD and recent advances in study design and surgical approaches.

Keywords: Gene therapy, Parkinson's disease, aromatic-L-amino-acid decarboxylase, glial cell line-derived neurotrophic factor, image-guided convection-enhanced delivery, clinical trial design

INTRODUCTION

For over 6 decades, the mainstay treatment for the motor symptoms associated with Parkinson's disease (PD) has remained dopamine (DA) replacement via administration of the precursor levodopa (L-DOPA). While efficacious, this treatment can cause several side effects, which are only moderately managed by reformulations of the drug or co-administration of drugs that prolong L-DOPA efficacy and mitigate side effects. While multiple other strategies have been developed for the symptomatic management of PD, including deep brain stimulation and continuous enteral or subcutaneous dopaminergic infusions, these are all limited by the employment of non-physiological mechanisms of action. Further, these treatments do not address the underlying neurodegeneration, and their efficacy wanes as neuron loss progresses. Thus, there remains a need for a therapy that slows disease progression and/or provides a functional benefit that is both robust and long-lasting to meaningfully improve patients' quality of life. A gene therapy approach with direct brain delivery offers the potential for a durable effect to not

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Table 1
Take Home Messages

Gene Supplementation Therapy for Parkinson's Disease
– Gene supplementation therapy consists of using a vector, usually an adeno-associated virus (AAV) or a lentivirus (LV), to deliver complementary DNA (cDNA) sequences coding for one or more genes involved in disease-specific pathogenic mechanisms.
Clinical Applications in Parkinson's Disease
– Gene therapies are primarily focused on two paradigms:
A. Direct enhancement of key enzymes in dopamine production (i.e., TH, AADC, GCH1) to increase the bioavailability of dopamine in the nigrostriatal synapsis
B. Restoration of neurotrophic support essential for dopaminergic pathway (i.e., NRTN, GDNF) to promote the survival and functionality of dopaminergic neurons and the sprouting of remaining axonal projections.
Initial Results
– Both gene therapy strategies demonstrated a robust safety profile and evidence of dopamine restoration. However, *post-hoc* volumetric analyses and post-mortem autopsy data highlighted the need for higher infusion volumes and vector titers to achieve optimal putaminal coverage.
Open Challenges and Future Developments
– Upcoming technical improvements will need to optimize the reproducibility of intracranial infusions and reduce the overall procedure time to standardize outcomes across centers.
– Optimization of targeted gene therapy delivery to the CNS via systemic administration would be aided by developing novel capsids capable of evading the blood-brain barrier and enhancers and cell-specific promoters that increase brain region specificity.

only improve upon current dopaminergic therapy, but also alter the course of the disease. Gene therapy aims to accomplish this by restoring the physiological functionality of the nigrostriatal dopaminergic synapsis with potential benefits on long-term complications due to maladaptive brain plasticity and medication- or stimulation-induced side effects. In this brief update, we summarize the (1) ongoing gene therapy trials focused on dopaminergic network function and evidence for their efficacy, (2) advances in vector delivery and PD gene therapy study design, and (3) future perspectives on gene therapy for PD and other neurological diseases (Table 1).

SUMMARY OF ONGOING GENE THERAPY TRIALS FOR RESTORATION OF DA NETWORK FUNCTION

Direct enzymatic enhancement of dopamine production

The most direct way of addressing DA restoration is to increase DA production at the site of greatest deficiency—the putamen. To this end, several gene therapy studies have focused on increasing expression of enzymes in the DA synthesis pathway, namely tyrosine hydroxylase (TH; the rate limiting step in DA production), aromatic L-amino acid decarboxylase (AADC; conversion of L-DOPA to DA), and GTP cyclohydroxylase 1 (GCH1; the rate limiting step in the production of the TH enzyme cofactor tetrahydrobiopterin) (Fig. 1A).

Preclinical studies in non-human primate (NHP) parkinsonian models explored striatal viral transduction and expression of AADC alone, AADC with TH, or the combination of AADC, TH, and GCH. Those studies demonstrated safe gene transfer and robust expression, restoration of DA signaling, and improvements in motor behavior, and have been thoroughly reviewed elsewhere [1, 2]. Key clinical trials assessing dose escalation, safety, and efficacy in PD patients that have moved forward and are currently active include AAV2-hAADC and lentiviral-GCH1-TH-AADC.

AAV2-hAADC

Clinical phase. To date, there have been a total of six Phase 1 open-label clinical studies and one Phase 2 placebo-controlled study utilizing adeno-associated serotype 2 viral vectors for expressing human AADC (AAV2-hAADC) administered via bilateral intraputaminal infusions [3–8], with four of the studies reporting long-term data from the same cohort of patients [3–6]. At present two studies are active, but not recruiting, VY-AADC01 (Phase 1; NCT03065192) and VY-AADC02 (Phase 2; NCT03562494). A long-term observational extension study for the participants who previously received AAV2-hAADC therapy is enrolling by invitation only (NCT03733496).

Efficacy. Key findings from the Phase 1 studies thus far show evidence of DA signaling restoration based on imaging and clinical evidence. Positron emission tomography (PET) imaging analysis using the radiotracer [18F]fluoro-l-m-tyrosine (^{18}FMT; a substrate

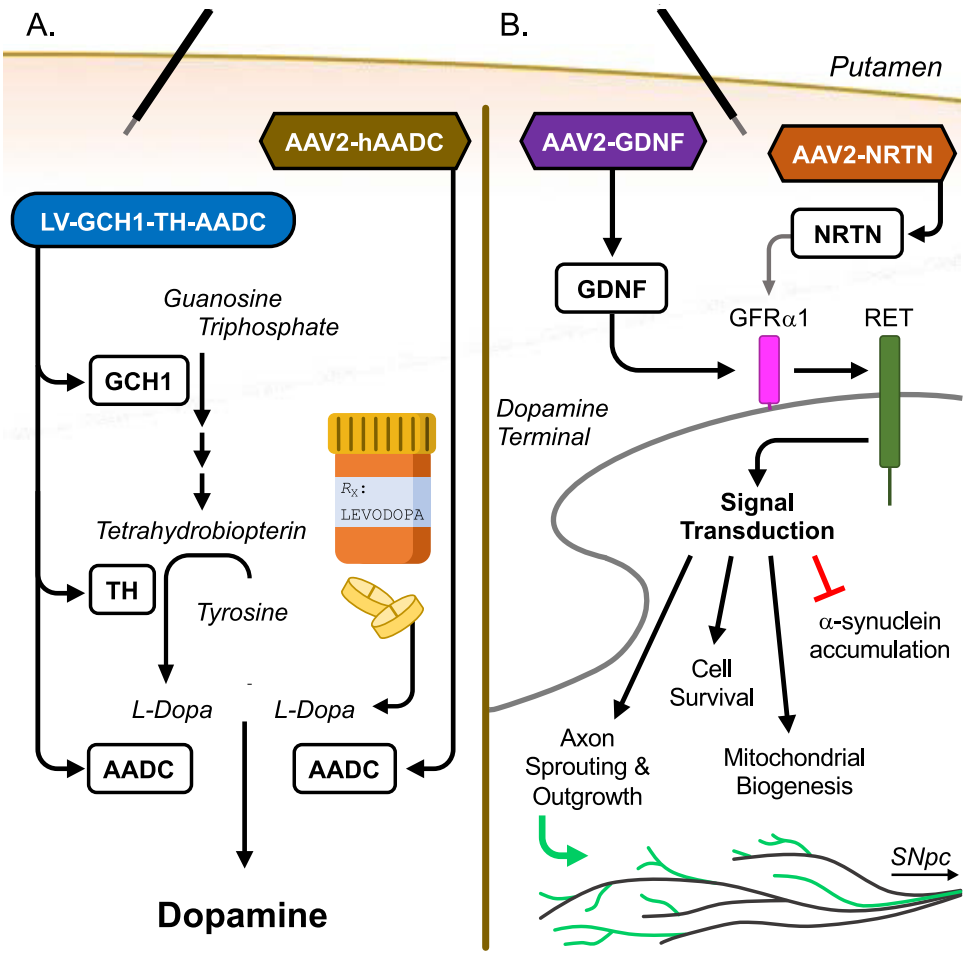

Fig. 1. Methods of Action for Current Gene Therapies. A) Enhancement of dopamine production. LV-GCH1-TH-AADC transduction of putaminal neurons restores key enzymes of the DA production pathway, leading to increased production of the TH co-factor tetrahydrobiopterin (via GCH1), increased production of levodopa from tyrosine (via TH), and enhanced conversion of levodopa (L-DOPA) to readily available DA (via AADC). AAV2-hAADC transduction of putaminal neurons leads to the increased local production of AADC to enhance the conversion of L-DOPA to readily available DA. Both therapies durably enhance the amount and consistent production of DA, from both endogenously produced and medication derived L-DOPA, within the putamen with the goal of reducing "Off" time symptoms [1, 2]. B) Restoration of neurotrophic signaling. Transduction of putaminal neurons by AAV2-GDNF or AAV2-NRTN leads to increased expression of glial cell line-derived neurotrophic factor (GDNF) and neurturin (NRTN), respectively, both of which are decreased in PD brain. These neurotrophic factors exert their effects by binding to GDNF family receptor α (GFRα) members on the surface of the DA neuron terminals. GDNF has a high affinity for GFRα1, which is highly expressed on DA neurons. NRTN can also bind to GFRα1, though with a lower affinity. The receptor/ligand complex attracts and activates the transmembrane receptor RET, a receptor tyrosine kinase, triggering a cell survival signaling cascade within the DA neurons. Evidence from animal models of PD have shown that enhanced neurotrophic factor expression in the striatum can protect against nigrostriatal DA neuron loss, reduce α-synuclein accumulation in DA neurons, improve mitochondrial biogenesis and function, and encourage sprouting and growth of DA axons [14, 15, 58].

of AADC decarboxylase activity), was reported in initial studies, and demonstrated increased putaminal uptake from baseline (25–75%) [4–7]. [18F] Fluorodopa (^{18}F-DOPA) radiotracer PET imaging in subjects at 6 months was reported in the VY-AADC01 study and showed a significant increase in putaminal uptake and AADC activity (conversion of ^{18}F-DOPA to ^{18}F-DA and its subsequent storage) as compared to baseline [8]. Clinically, patients demonstrated a reduced total L-DOPA equivalent daily dose (LEDD) and improvement in UPDRS-III Off-state scores at 6 months (21–36%) [4, 7]. One study found this improvement was durable out to 3 years in follow-up [8, 9]. No changes were observed for UPDRS-III On-state scores. Patients also demonstrated an improvement in PDQ-39 scores in one study [8]. Evidence from PET imaging studies also suggested that greater putaminal coverage by the

AAV2-hAADC drug infusion correlated with better clinical outcomes [8, 9].

Safety. AAV2-hAADC has shown to be safe and tolerable, with 1–4 years of follow-up [5, 7, 8]. With 31 participants administered the drug across the six open-label studies, there were five reported serious adverse events (SAEs) related to the surgical procedure (four intracranial hemorrhages, two of which were asymptomatic, and one deep vein thrombosis) [3–5, 7, 8]. Eight participants reported transient dyskinesia increases [5, 8]. Of note, the Phase 2 VY-AADC02 study (NCT03562494) is currently on clinical hold pending evaluation of undisclosed MRI abnormalities in test subjects [10].

LV-GCH1-TH-AADC

Clinical phase. There is one completed and one active clinical trial utilizing a lentiviral vector expressing GCH1, TH, and AADC (LV-GCH1-TH-AADC), referred to as ProSavin [11, 12]. The completed Phase 1/2 study was an open-label, dose-escalation study (NCT00627588). The same cohort of patients administered ProSavin are being evaluated in an ongoing long-term safety and efficacy study (NCT01856439).

OXB-102 (AXO-Lenti-PD), is a modified lentiviral vector designed to transduce and express GCH1-TH-AADC, but with the addition of an optimized gene expression cassette to further enhance protein expression, and thereby increase DA production. This optimized vector is currently in an active Phase 1/2 safety and dose escalation study (AXO-Lenti-PD study, NCT03720418) [13].

Efficacy. Imaging of study participants treated with ProSavin showed no significant difference in ^{18}F-DOPA PET putaminal signal at 6-month follow-up [12]. Clinically, participants showed evidence of improved DA function with significant improvement in Off-state UPDRS-III at 6–12-month follow-up (11.8-point improvement), and a majority of patients that could be followed past 2 years continued to show significantly improved scores in long-term follow-up [11, 12]. A majority of patients also demonstrated a lower LEDD in long-term follow-up [11]. Improvements in both UPDRS-II (2 points in On-state and 4 points in Off-state scores) and PDQ-39 (5.7 points) were observed at 6 months, but these improvements were not confirmed in the long-term follow-up [11, 12]. Similarly, no significant differences were observed in UPDRS-I or -IV in long-term follow-up [11]. While the therapy is promising given the UPDRS-III Off-state scores, concerns with long-term durability and efficacy in other clinical outcomes following ProSavin administration were partially attributed to issues with delivery and vector design, prompting the OXB-102 vector development and subsequent AXO-Lenti-PD study [11].

A press release of 6-month data from the first 4 treated participants in the high dose cohort 2 of the AXO-Lenti-PD study (NCT03720418) demonstrated significant improvements in UPDRS-III Off scores (-21 point improvement) and 2.2-hour improvement of On time as compared to baseline from participant reported diaries [13]. The study is planned to continue enrolling for cohort 3, utilizing a higher dose and also incorporating procedural changes including higher delivery volume and increased flow rates to improve coverage and reduce procedural time [13].

Safety. ProSavin was found to be safe and well tolerated, with long-term observation reported out to 8 years in some participants. Three SAEs associated with the therapy were reported, including cases of dyskinesia, acute psychosis, and a nervous system disorder (etiology unspecified) [12]. Eight patients required deep-brain stimulation (DBS) surgical intervention after 2 years due to continued disease progression [11]. Two deaths were reported, one after 4-year post-infusion (attributed to cardio-respiratory arrest) and one after 6 years (attributed to peritonitis), but were considered unrelated to the therapy [11]. OXB-102 has also shown a favorable safety and tolerability profile at 6 month follow-up with no therapy-related SAEs [13].

Restoring neurotrophic signaling to the dopaminergic network

Though evidence suggests vector-mediated enhancement of DA production is an improvement over current pharmacotherapy, this approach does not address disease progression. Preservation and regeneration of the nigrostriatal pathway is crucial to extending PD patient quality of life and even reversing symptoms of the disease. In addition to DAergic neuron loss, PD patient brains also exhibit significant loss of endogenous neuronal growth factors in affected brain regions [14–19]. Thus, extensive research on restoring growth factors to encourage sustained DAergic health has been carried out. Preclinical work in NHP models of PD has shown significant promise from intraparenchymal infusions of growth factor-based protein or gene therapy to stabilize and even restore DAergic signaling via

sprouting of remaining DA projections, thereby ultimately improving motor symptoms [1, 20, 21].

Two growth factors, glial cell line-derived neurotrophic factor (GDNF) and neurturin (NRTN), have garnered significant attention. GDNF and family member NRTN both work in pathways that activate the REarranged during Transfection (RET) receptor tyrosine kinase triggering a cascade of intracellular signaling, including activation of Nurr1, an intranuclear receptor that regulates the development of DAergic neurons and expression of AADC, TH, dopamine transporter (DAT) and vesicular monoamine transporter 2 (VMAT2) [22] (Fig. 1B). AAV2-NRTN (CERE-120) has been evaluated in multiple Phase 1 and Phase 2 studies examining both bilateral putaminal infusions, and bilateral putaminal-plus-nigral infusions [21, 23–29], however there are no trials currently active for NRTN gene therapy. Despite successful Phase 1 trials, patients in the follow-up Phase 2 trials presented with mixed results and failed to meet the studies respective primary endpoints. Postmortem studies demonstrated increased density of TH fibers; however, this was restricted to the areas of NRTN expression, which were limited by the low putaminal coverage and modest nigral coverage of the vector [25, 30]. This suggests that issues with coverage and delivery of the drug contributed to the lack of efficacy. Only AAV2-GDNF is currently active in two clinical gene therapy trials for PD.

AAV2-GDNF

Clinical phase. AAV2-GDNF has been used in one Phase 1 dose-escalation study that is nearing completion (NCT01621581), and an actively recruiting Phase 1b study utilizing MRI-guided bilateral putaminal delivery of the study drug (NCT04167540).

Efficacy. PET imaging data from the Phase 1 study has demonstrated a radiographic improvement with significant increase in ^{18}F-DOPA putaminal signal at 6 months (+36%) and 18 months (+54%) following treatment, suggesting restoration of DA function via an increase of AADC activity. Scores for UPDRS-I, -II On or Off, -III On or Off, or -IV remained stable with no significant changes reported following 18 months of follow-up [31]. However, it is notable that patient motor symptoms did not significantly deteriorate either, as would be expected for idiopathic PD [31].

These results encouraged the currently ongoing Phase 1b trial, with modifications in the study design to improve the vector coverage via increased delivery volume and a posterior trajectory to infuse along the long axis of the putamen. Another key change is the inclusion of early-stage PD participants to explore the regenerative capacity of GDNF when more surviving neurons are available to respond to neurotrophic factor-based therapy. These findings also prompted the initiation of a similar gene therapy approach for Multiple System Atrophy, a related parkinsonian disorder associated with loss of putaminal neurotrophic support (NCT04680065).

Safety. AAV2-GDNF administration has so far demonstrated a robust safety profile. Of the 13 participants in the Phase 1 study, only one SAE was reported related to the surgical procedure, which was a scalp wound dehiscence requiring debridement. No other SAEs were attributed to either the surgical procedure or the AAV2-GDNF drug.

INTRAPARENCHYMAL DELIVERY

Targeted CNS delivery of gene therapies aims to restore DA in the putamen as it is the most affected by the progressive DAergic denervation in PD. Accurate and reproducible delivery is a critical aspect to gene therapy, particularly for neurologic disorders, as has been evidenced by prior clinical experience with intraparenchymal delivery. Suboptimal coverage of the putamen by delivering conservative volumes has been identified as one of the most common reasons underlying the lack of success in the earliest gene therapy clinical trials [3, 11, 13, 25, 26, 31]. The lessons learned from the earlier and first-in-human clinical trials, which relied primarily on the passive diffusion of the therapeutic, triggered extensive preclinical research in animal models to optimize multiple aspects of intraparenchymal delivery such as delivery devices and infusion monitoring [32, 33]. Thus, with the emergence of intraoperative MR imaging (MRI) and the design of new devices, the latest clinical trials utilize a real-time, image-guided convection-enhanced delivery (CED), which uses a pressurized infusion method to improve infusate distribution within the target brain structure. This delivery platform includes use of reflux-resistant cannulae to contain the infusate within the putamen, and co-infusion of the therapeutic with an MR tracer (gadolinium) for continuous visualization of the infusion in near real-time [34]. Both preclinical and clinical studies identified perivascular spread of the infusate as one of the most critical challenges in targeted, intraparenchymal delivery to the

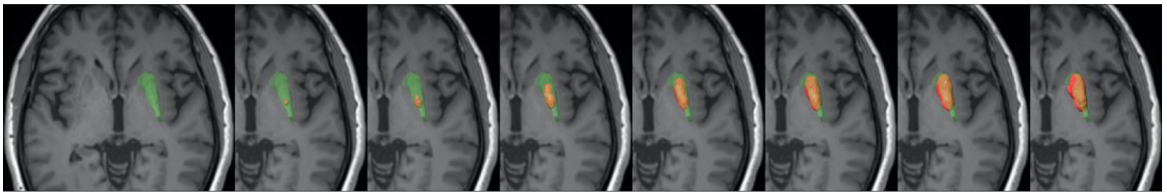

Fig. 2. Representative distribution of gene delivery (AAV2 vector) into the putamen. Expression of transgene after CED of AAV2-GDNF and AAV2-AADC vectors has been shown to co-distribute with MR tracer gadolinium [34, 59]. Near real-time monitoring by MR imaging of AAV2/gadolinium infusions into the putamen of PD and MSA patients allows us to calculate volume of distribution of the tracer as a function of volume that has been infused. This figure depicts the relationship between volume and distribution of the gene therapy infusate (red) needed to achieve meaningful coverage of the putamen (green) in a PD participant that received AAV2-GDNF. The area in dark orange indicates the area of putamen covered by the AAV2-GDNF infusion. Minimal infusate leakage (medial red-only area) occurred beyond the putamen as there are no physical boundaries (i.e., membranes) surrounding brain structures like the putamen that can contain drug delivered into the brain. Most of the previous clinical studies in PD that have employed direct delivery of the therapeutics in the putamen (e.g., GDNF protein, gene therapy) used suboptimal volumes that could have resulted in insufficient therapeutic drug delivery.

highly vascularized putamen [34–36]. When the tip of cannula is placed in the vicinity of a blood vessel, the infusate has been visualized to follow the course of least resistance through perivascular potential spaces, inevitably leaving the target structure [36], which can be mitigated with adjustments to the surgical delivery. Based on real-time imaging observations, these studies demonstrated the need for higher volumes of infusion to achieve greater putaminal coverage and to account for perivascular spread of the infusate. As modeled in the NHP brain [37], using an occipital approach to deliver AAV2-hAADC along the antero-posterior axis of the putamen resulted in greater coverage of the putamen and yielded greater clinical benefit as compared to increasing the vector dose [8] (Fig. 2). This CED platform has now proven safe in intracranial drug delivery in multiple clinical trials with follow-up, including gene therapy trials of AAV2-hAADC for PD, AAV2-GDNF for PD, and AAV2-hAADC for AADC deficiency [8, 9, 31, 38], and brain tumor drug delivery trials [39–43].

REFINEMENT OF TRIAL DESIGN ELEMENTS OF PD GENE THERAPY CLINICAL STUDIES

Genetic status in selection of parkinsonian patients for gene therapy studies

The genetics of PD and other neurodegenerative disorders is a rapidly developing field that has the potential to guide our understanding of the pathogenesis, and potentially a personalized approach to the treatment of these complex diseases. Due to the advancements in technology, genetic testing has become more affordable and widely available [44]. Many patients may also be aware of their genetic status prior to enrollment in studies as the lower cost and use of send-away personal DNA kits has made gene testing more attainable. Other PD-focused groups and collaborations with commercially available genetic testing companies (PD GENEration [45] and Fox Insight [46]) have accelerated gene testing of patients with PD in an effort to improve disease management and future treatments.

Ethical concerns in mind, this new genetic landscape should be considered in the development of PD gene therapy trials. It is now feasible to consider selected enrollment for a particular gene mutation or group of mutations with similar pathogenic mechanism, depending on the proposed mechanism of action of a chosen transgene. Such criteria have been included in a few PD clinical trials (GBA mutations with AAV9-GBA (NCT04127578), LRRK2 mutations for LRRK2 targeted therapies (NCT03976349, NCT03710707), and exclusion of known PD-related mutations (NCT04167540). This selectivity is anticipated to become more widespread [47] as selection based on genetics allows determination of a drug effect in a more homogenous population, which may reveal which genetic mutations are more or less likely to respond to a particular treatment. A precision medicine approach based on genetic status has the potential to improve detection of a clinically mean-

ingful treatment effect and speed development of gene therapies for patients with PD.

Differences in population focus for enzymatic enhancement of DA production versus neurotrophic restoration of DA system

Selection for enzymatic enhancement

Moderate to severe PD is frequently accompanied by motor fluctuations and unpredictability of response to medications. These symptoms are in part related to complications of long-term use of DAergic medications, but also ongoing neurodegeneration and subsequent loss of key enzymes for DA production [48, 49]. This advanced parkinsonian phenotype is expected to have the greatest benefit with a gene therapy approach to enzymatically reconstitute DA production, as opposed to a *de novo* or earlier stage PD patient when the remaining endogenous DA machinery is still able to compensate. However, significantly advanced PD is more likely to experience other complications of therapy such as impulse control disorders and DA dysregulation syndrome, which have the potential to worsen with supratherapeutic levels of DA. Enrollment of these individuals should be considered carefully for an unmodifiable gene therapy approach.

Selection for neurotrophic restoration

Decades of exploration of neurotrophic factors to modify the disease trajectory for PD patients has strongly suggested that the neurotrophic restoration approach has greatest chance for success in early disease stages. Evaluation of postmortem PD tissue has demonstrated a near total loss of DAergic terminal markers in putamen as early as 4 years from diagnosis [48], and preclinical evidence indicates that the degree of functional restoration elicited by increasing neurotrophic factors is dependent on the degree of nigrostriatal degeneration at the time of treatment [20, 50]. Lessons from these preclinical parkinsonian models and other clinical studies investigating neurotrophic factors (AAV2-NRTN, recombinant GDNF protein infusions, AAV2-GDNF) have highlighted the importance of targeting an earlier stage of PD [29, 31, 51] with a neurotrophic intervention when there is a sufficient number of neurons remaining to respond to therapy. In addition, these studies will necessitate a longer duration of monitoring as the restorative effects of neurotrophic factors are expected to take minimum 12 months for optimal benefit.

With the inclusion of early PD patients (e.g., < 5 years from diagnosis), diagnostic confidence is critical to ensure enrollment of patients with PD and not an atypical parkinsonism. Criteria have been established to define "clinically probable" PD as a means to guide clinical trials involving early disease [52], but diagnostic confidence is improved with time and objective measures. Integration of technology, multimodal imaging, and other ancillary studies to distinguish PD from other parkinsonisms are important design elements to consider in PD gene therapy studies, particularly for studies enrolling participants with early or *de novo* PD. The use of FDG PET with MIBG cardiac scintigraphy, other imaging of the DAergic system, or MRI findings can support clinical impressions to bolster diagnostic certainty and to differentiate PD from atypical parkinsonisms [53, 54]. Other "low-tech" assessments like olfactory testing can also easily be included to distinguish PD from other atypical forms [55]. The ability to accurately distinguish PD from other parkinsonisms is expected to be increasingly important as earlier stages of PD and even prodromal disease are considered in the development of novel disease modifying and neuroprotective therapies for PD.

FUTURE DIRECTIONS: IMPROVED DELIVERY METHODS, ENHANCED TARGETING, AND NOVEL VECTOR DESIGN

Remarkable progress has been made over the past decade with regards to improved delivery and percent of vector coverage of targeted brain regions. In spite of these advancements, further exploration of methods to improve delivery, regulate distribution of transgene expression, and novel vector designs are being pursued in order to optimize clinical impact for CNS disorders.

Surgical delivery of gene therapy has rapidly advanced and is expected to rapidly continue in its evolution to streamline direct brain delivery into an outpatient or overnight procedure. Improvements to targeting devices and catheter design have the potential to improve the reproducibility of infusions, simplify neurosurgical training and device adoption, and reduce the overall procedure time. Taking advantage of advancements in imaging technology, the use of bedside MR scanning is expected to revolutionize the availability and feasibility of intraparenchymal delivery on a wider scale [56].

Another key innovation for addressing neurologic disease is the development of novel capsids that are able to evade the blood-brain barrier, a technological leap forward that would provide targeted delivery to the CNS via systemic administration. The addition of enhancers and other cell-specific promoters are expected to further improve cell and brain region specificity with the potential to regulate expression and limit off-target effects [57]. For PD and other parkinsonian disorders specifically, it is anticipated that novel genetic targets and brain regions will be explored to address motor complications of long-standing disease, and possibly also targeting features of non-motor symptoms.

CONCLUSIONS

Gene therapy remains a promising therapeutic approach to significantly improve PD patient quality of life. While no gene therapy has yet been approved for PD treatment, promising results from concluded and ongoing clinical trials help direct refinement of the approach. Broad coverage of the targeted brain region and patient selection based on mechanism of action of a particular vector design are keys to successful outcomes in PD gene therapy studies. With regard to growth factor-based gene therapy, earlier stage participants and longer duration of observation are expected to be required to determine ability to modify disease progression. Innovations in surgical approach and vector design continue to be explored to optimize gene therapy approaches and better treat PD.

CONFLICT OF INTEREST

Krystof Bankiewicz is a founder and consultant to Brain Neurotherapy Bio, Ask Bio subsidiary. The other authors have no conflict of interest to report.

REFERENCES

[1] Axelsen TM, Woldbye DPD (2018) Gene therapy for Parkinson's disease, an update. *J Parkinsons Dis* **8**, 195-215.

[2] Merola A, Van Laar A, Lonser R, Bankiewicz K (2020) Gene therapy for Parkinson's disease: Contemporary practice and emerging concepts. *Expert Rev Neurother* **20**, 577-590.

[3] Eberling JL, Jagust WJ, Christine CW, Starr P, Larson P, Bankiewicz KS, Aminoff MJ (2008) Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology* **70**, 1980-1983.

[4] Christine CW, Starr PA, Larson PS, Eberling JL, Jagust WJ, Hawkins RA, VanBrocklin HF, Wright JF, Bankiewicz KS, Aminoff MJ (2009) Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology* **73**, 1662-1669.

[5] Mittermeyer G, Christine CW, Rosenbluth KH, Baker SL, Starr P, Larson P, Kaplan PL, Forsayeth J, Aminoff MJ, Bankiewicz KS (2012) Long-term evaluation of a phase 1 study of AADC gene therapy for Parkinson's disease. *Hum Gene Ther* **23**, 377-381.

[6] Valles F, Fiandaca MS, Eberling JL, Starr PA, Larson PS, Christine CW, Forsayeth J, Richardson RM, Su X, Aminoff MJ, Bankiewicz KS (2010) Qualitative imaging of adeno-associated virus serotype 2-human aromatic L-amino acid decarboxylase gene therapy in a phase I study for the treatment of Parkinson disease. *Neurosurgery* **67**, 1377-1385.

[7] Muramatsu S, Fujimoto K, Kato S, Mizukami H, Asari S, Ikeguchi K, Kawakami T, Urabe M, Kume A, Sato T, Watanabe E, Ozawa K, Nakano I (2010) A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. *Mol Ther* **18**, 1731-1735.

[8] Christine CW, Bankiewicz KS, Van Laar AD, Richardson RM, Ravina B, Kells AP, Boot B, Martin AJ, Nutt J, Thompson ME, Larson PS (2019) Magnetic resonance imaging-guided phase 1 trial of putaminal AADC gene therapy for Parkinson's disease. *Ann Neurol* **85**, 704-714.

[9] Christine C, Richardson R, Van Laar A, Thompson M, Herbert K, Li C, Liang G, Fine E, Larson P (2020) Three-year safety and clinical outcomes from the PD-1101 trial of AADC gene therapy for advanced Parkinson's disease. *Mov Disord* **35**(suppl 1), https://www.mdsabstracts.org/abstract/three-year-safety-and-clinical-outcomes-from-the-pd-1101-trial-of-aadc-gene-therapy-for-advanced-parkinsons-disease/.

[10] Voyager Therapeutics I, Voyager Therapeutics Provides Update On NBIb-1817 (VY-AADC) Gene Therapy Program, https://ir.voyagertherapeutics.com/news-releases/news-release-details/voyager-therapeutics-provides-update-nbib-1817-vy-aadc-gene

[11] Palfi S, Gurruchaga JM, Lepetit H, Howard K, Ralph GS, Mason S, Gouello G, Domenech P, Buttery PC, Hantraye P, Tuckwell NJ, Barker RA, Mitrophanous KA (2018) Long-term follow-up of a Phase I/II study of ProSavin, a lentiviral vector gene therapy for Parkinson's disease. *Hum Gene Ther Clin Dev* **29**, 148-155.

[12] Palfi S, Gurruchaga JM, Ralph GS, Lepetit H, Lavisse S, Buttery PC, Watts C, Miskin J, Kelleher M, Deeley S, Iwamuro H, Lefaucheur JP, Thiriez C, Fenelon G, Lucas C, Brugieres P, Gabriel I, Abhay K, Drouot X, Tani N, Kas A, Ghaleb B, Le Corvoisier P, Dolphin P, Breen DP, Mason S, Guzman NV, Mazarakis ND, Radcliffe PA, Harrop R, Kingsman SM, Rascol O, Naylor S, Barker RA, Hantraye P, Remy P, Cesaro P, Mitrophanous KA (2014) Long-term safety and tolerability of ProSavin, a lentiviral vector-based gene therapy for Parkinson's disease: A dose escalation, open-label, phase 1/2 trial. *Lancet* **383**, 1138-1146.

[13] Sio Gene Therapies I, Axovant Announces Positive Six-Month Follow-Up Data From Second Cohort of SUNRISE-PD Phase 2 Trial of AXO-Lenti-PD Gene Therapy, http://investors.axovant.com/news-releases/news-release-details/axovant-announces-positive-six-month-follow-data-second-cohort

[14] Kramer ER, Liss B (2015) GDNF-Ret signaling in midbrain dopaminergic neurons and its implication for Parkinson disease. *FEBS Lett* **589**, 3760-3772.

[15] Hadaczek P, Wu G, Sharma N, Ciesielska A, Bankiewicz K, Davidow AL, Lu ZH, Forsayeth J, Ledeen RW (2015)

GDNF signaling implemented by GM1 ganglioside; failure in Parkinson's disease and GM1-deficient murine model. *Exp Neurol* **263**, 177-189.

[16] Sampaio TB, Savall AS, Gutierrez MEZ, Pinton S (2017) Neurotrophic factors in Alzheimer's and Parkinson's diseases: Implications for pathogenesis and therapy. *Neural Regen Res* **12**, 549-557.

[17] Nagatsu T, Sawada M (2007) Biochemistry of postmortem brains in Parkinson's disease: Historical overview and future prospects. *J Neural Transm Suppl*, pp. 113-120.

[18] Chauhan NB, Siegel GJ, Lee JM (2001) Depletion of glial cell line-derived neurotrophic factor in substantia nigra neurons of Parkinson's disease brain. *J Chem Neuroanat* **21**, 277-288.

[19] Howells DW, Porritt MJ, Wong JY, Batchelor PE, Kalnins R, Hughes AJ, Donnan GA (2000) Reduced BDNF mRNA expression in the Parkinson's disease substantia nigra. *Exp Neurol* **166**, 127-135.

[20] Kells AP, Eberling J, Su X, Pivirotto P, Bringas J, Hadaczek P, Narrow WC, Bowers WJ, Federoff HJ, Forsayeth J, Bankiewicz KS (2010) Regeneration of the MPTP-lesioned dopaminergic system after convection-enhanced delivery of AAV2-GDNF. *J Neurosci* **30**, 9567-9577.

[21] Bartus RT, Herzog CD, Chu Y, Wilson A, Brown L, Siffert J, Johnson EM, Jr., Olanow CW, Mufson EJ, Kordower JH (2011) Bioactivity of AAV2-neurturin gene therapy (CERE-120): Differences between Parkinson's disease and nonhuman primate brains. *Mov Disord* **26**, 27-36.

[22] Jankovic J, Chen S, Le WD (2005) The role of Nurr1 in the development of dopaminergic neurons and Parkinson's disease. *Prog Neurobiol* **77**, 128-138.

[23] Bartus RT, Baumann TL, Siffert J, Herzog CD, Alterman R, Boulis N, Turner DA, Stacy M, Lang AE, Lozano AM, Olanow CW (2013) Safety/feasibility of targeting the substantia nigra with AAV2-neurturin in Parkinson patients. *Neurology* **80**, 1698-1701.

[24] Bartus RT, Kordower JH, Johnson EM, Jr., Brown L, Kruegel BR, Chu Y, Baumann TL, Lang AE, Olanow CW, Herzog CD (2015) Post-mortem assessment of the short and long-term effects of the trophic factor neurturin in patients with α-synucleinopathies. *Neurobiol Dis* **78**, 162-171.

[25] Chu Y, Bartus RT, Manfredsson FP, Olanow CW, Kordower JH (2020) Long-term post-mortem studies following neurturin gene therapy in patients with advanced Parkinson's disease. *Brain* **143**, 960-975.

[26] Marks WJ Jr, Bartus RT, Siffert J, Davis CS, Lozano A, Boulis N, Vitek J, Stacy M, Turner D, Verhagen L, Bakay R, Watts R, Guthrie B, Jankovic J, Simpson R, Tagliati M, Alterman R, Stern M, Baltuch G, Starr PA, Larson PS, Ostrem JL, Nutt J, Kieburtz K, Kordower JH, Olanow CW (2010) Gene delivery of AAV2-neurturin for Parkinson's disease: A double-blind, randomised, controlled trial. *Lancet Neurol* **9**, 1164-1172.

[27] Marks WJ Jr., Baumann TL, Bartus RT (2016) Long-term safety of patients with Parkinson's disease receiving rAAV2-neurturin (CERE-120) gene transfer. *Hum Gene Ther* **27**, 522-527.

[28] Marks WJ Jr., Ostrem JL, Verhagen L, Starr PA, Larson PS, Bakay RA, Taylor R, Cahn-Weiner DA, Stoessl AJ, Olanow CW, Bartus RT (2008) Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: An open-label, phase I trial. *Lancet Neurol* **7**, 400-408.

[29] Warren Olanow C, Bartus RT, Baumann TL, Factor S, Boulis N, Stacy M, Turner DA, Marks W, Larson P, Starr PA, Jankovic J, Simpson R, Watts R, Guthrie B, Poston K, Henderson JM, Stern M, Baltuch G, Goetz CG, Herzog C, Kordower JH, Alterman R, Lozano AM, Lang AE (2015) Gene delivery of neurturin to putamen and substantia nigra in Parkinson disease: A double-blind, randomized, controlled trial. *Ann Neurol* **78**, 248-257.

[30] Bartus RT, Kordower JH, Johnson EM, Jr., Brown L, Kruegel BR, Chu Y, Baumann TL, Lang AE, Olanow CW, Herzog CD (2015) Post-mortem assessment of the short and long-term effects of the trophic factor neurturin in patients with alpha-synucleinopathies. *Neurobiol Dis* **78**, 162-171.

[31] Heiss JD, Lungu C, Hammoud DA, Herscovitch P, Ehrlich DJ, Argersinger DP, Sinharay S, Scott G, Wu T, Federoff HJ, Zaghloul KA, Hallett M, Lonser RR, Bankiewicz KS (2019) Trial of magnetic resonance-guided putaminal gene therapy for advanced Parkinson's disease. *Mov Disord* **34**, 1073-1078.

[32] Fiandaca MS, Forsayeth JR, Dickinson PJ, Bankiewicz KS (2008) Image-guided convection-enhanced delivery platform in the treatment of neurological diseases. *Neurotherapeutics* **5**, 123-127.

[33] Richardson RM, Bankiewicz KS, Christine CW, Van Laar AD, Gross RE, Lonser R, Factor SA, Kostyk SK, Kells AP, Ravina B, Larson PS (2020) Data-driven evolution of neurosurgical gene therapy delivery in Parkinson's disease. *J Neurol Neurosurg Psychiatry* **91**, 1210-1218.

[34] Richardson RM, Kells AP, Rosenbluth KH, Salegio EA, Fiandaca MS, Larson PS, Starr PA, Martin AJ, Lonser RR, Federoff HJ, Forsayeth JR, Bankiewicz KS (2011) Interventional MRI-guided putaminal delivery of AAV2-GDNF for a planned clinical trial in Parkinson's disease. *Mol Ther* **19**, 1048-1057.

[35] Brady ML, Raghavan R, Alexander A, Kubota K, Sillay K, Emborg ME (2013) Pathways of infusate loss during convection-enhanced delivery into the putamen nucleus. *Stereotact Funct Neurosurg* **91**, 69-78.

[36] Varenika V, Dickinson P, Bringas J, LeCouteur R, Higgins R, Park J, Fiandaca M, Berger M, Sampson J, Bankiewicz K (2008) Detection of infusate leakage in the brain using real-time imaging of convection-enhanced delivery. *J Neurosurg* **109**, 874-880.

[37] Sudhakar V, Naidoo J, Samaranch L, Bringas JR, Lonser RR, Fiandaca MS, Bankiewicz KS (2019) Infuse-as-you-go convective delivery to enhance coverage of elongated brain targets: Technical note. *J Neurosurg*, doi: 10.3171/2019.4.JNS19826

[38] Pearson TS, Gupta N, San Sebastian W, Imamura-Ching J, Viehoever A, Grijalvo-Perez A, Fay AJ, Seth N, Lundy SM, Seo Y, Pampaloni M, Hyland K, Smith E, de Oliveira Barbosa G, Heathcock JC, Minnema A, Lonser R, Elder JB, Leonard J, Larson P, Bankiewicz KS (2021) Gene therapy for aromatic L-amino acid decarboxylase deficiency by MR-guided direct delivery of AAV2-AADC to midbrain dopaminergic neurons. *Nat Commun* **12**, 4251.

[39] Bander ED, Ramos AD, Wembacher-Schroeder E, Ivasyk I, Thomson R, Morgenstern PF, Souweidane MM (2020) Repeat convection-enhanced delivery for diffuse intrinsic pontine glioma. *J Neurosurg Pediatr*, doi: 10.3171/2020.6.PEDS20280

[40] Debinski W, Tatter SB (2009) Convection-enhanced delivery for the treatment of brain tumors. *Expert Rev Neurother* **9**, 1519-1527.

[41] Ellingson BM, Sampson J, Achrol AS, Aghi MK, Bankiewicz K, Wang C, Bexon M, Brem S, Brenner A, Chowdhary S, Floyd JR, Han S, Kesari S, Randazzo D, Vogelbaum MA, Vrionis F, Zabek M, Butowski N, Coello M, Merchant N, Merchant F (2021) Modified RANO, immunotherapy RANO, and standard RANO response to convection-enhanced delivery of IL4R-targeted immunotoxin MDNA55 in recurrent glioblastoma. *Clin Cancer Res* **27**, 3916-3925.

[42] Jahangiri A, Chin AT, Flanigan PM, Chen R, Bankiewicz K, Aghi MK (2017) Convection-enhanced delivery in glioblastoma: A review of preclinical and clinical studies. *J Neurosurg* **126**, 191-200.

[43] Singleton WGB, Bienemann AS, Woolley M, Johnson D, Lewis O, Wyatt MJ, Damment SJP, Boulter LJ, Killick-Cole CL, Asby DJ, Gill SS (2018) The distribution, clearance, and brainstem toxicity of panobinostat administered by convection-enhanced delivery. *J Neurosurg Pediatr* **22**, 288-296.

[44] Horton RH, Lucassen AM (2019) Recent developments in genetic/genomic medicine. *Clin Sci (Lond)* **133**, 697-708.

[45] Fox Insight, https://foxinsight.michaeljfox.org,

[46] Parkinson's Foundation, https://www.parkinson.org/PDGENEration,

[47] Payne K, Walls B, Wojcieszek J (2019) Approach to assessment of Parkinson disease with emphasis on genetic testing. *Med Clin North Am* **103**, 1055-1075.

[48] Kordower JH, Olanow CW, Dodiya HB, Chu Y, Beach TG, Adler CH, Halliday GM, Bartus RT (2013) Disease duration and the integrity of the nigrostriatal system in Parkinson's disease. *Brain* **136**, 2419-2431.

[49] Ciesielska A, Samaranch L, San Sebastian W, Dickson DW, Goldman S, Forsayeth J, Bankiewicz KS (2017) Depletion of AADC activity in caudate nucleus and putamen of Parkinson's disease patients; implications for ongoing AAV2-AADC gene therapy trial. *PLoS One* **12**, e0169965.

[50] Quintino L, Avallone M, Brannstrom E, Kavanagh P, Lockowandt M, Garcia Jareno P, Breger LS, Lundberg C (2019) GDNF-mediated rescue of the nigrostriatal system depends on the degree of degeneration. *Gene Ther* **26**, 57-64.

[51] Whone A, Luz M, Boca M, Woolley M, Mooney L, Dharia S, Broadfoot J, Cronin D, Schroers C, Barua NU, Longpre L, Barclay CL, Boiko C, Johnson GA, Fibiger HC, Harrison R, Lewis O, Pritchard G, Howell M, Irving C, Johnson D, Kinch S, Marshall C, Lawrence AD, Blinder S, Sossi V, Stoessl AJ, Skinner P, Mohr E, Gill SS (2019) Randomized trial of intermittent intraputamenal glial cell line-derived neurotrophic factor in Parkinson's disease. *Brain* **142**, 512-525.

[52] Berg D, Adler CH, Bloem BR, Chan P, Gasser T, Goetz CG, Halliday G, Lang AE, Lewis S, Li Y, Liepelt-Scarfone I, Litvan I, Marek K, Maetzler C, Mi T, Obeso J, Oertel W, Olanow CW, Poewe W, Rios-Romenets S, Schaffer E, Seppi K, Heim B, Slow E, Stern M, Bledsoe IO, Deuschl G, Postuma RB (2018) Movement disorder society criteria for clinically established early Parkinson's disease. *Mov Disord* **33**, 1643-1646.

[53] Brumberg J, Schroter N, Blazhenets G, Frings L, Volkmann J, Lapa C, Jost WH, Isaias IU, Meyer PT (2020) Differential diagnosis of parkinsonism: A head-to-head comparison of FDG PET and MIBG scintigraphy. *NPJ Parkinsons Dis* **6**, 39.

[54] Langston JW, Wiley JC, Tagliati M (2018) Optimizing Parkinson's disease diagnosis: The role of a dual nuclear imaging algorithm. *NPJ Parkinsons Dis* **4**, 5.

[55] Fujita H, Suzuki K, Numao A, Watanabe Y, Uchiyama T, Miyamoto T, Miyamoto M, Hirata K (2016) Usefulness of cardiac MIBG scintigraphy, olfactory testing and substantia nigra hyperechogenicity as additional diagnostic markers for distinguishing between Parkinson's disease and atypical parkinsonian syndromes. *PLoS One* **11**, e0165869.

[56] Sheth KN, Mazurek MH, Yuen MM, Cahn BA, Shah JT, Ward A, Kim JA, Gilmore EJ, Falcone GJ, Petersen N, Gobeske KT, Kaddouh F, Hwang DY, Schindler J, Sansing L, Matouk C, Rothberg J, Sze G, Siner J, Rosen MS, Spudich S, Kimberly WT (2020) Assessment of brain injury using portable, low-field magnetic resonance imaging at the bedside of critically ill patients. *JAMA Neurol*, doi: 10.1001/jamaneurol.2020.3263

[57] Domenger C, Grimm D (2019) Next-generation AAV vectors-do not judge a virus (only) by its cover. *Hum Mol Genet* **28**, R3-R14.

[58] Chmielarz P, Er S, Konovalova J, Bandres L, Hlushchuk I, Albert K, Panhelainen A, Luk K, Airavaara M, Domanskyi A (2020) GDNF/RET signaling pathway activation eliminates Lewy body pathology in midbrain dopamine neurons. *Mov Disord* **35**, 2279-2289.

[59] Su X, Kells AP, Salegio EA, Salegio EA, Richardson RM, Hadaczek P, Beyer J, Bringas J, Pivirotto P, Forsayeth J, Bankiewicz KS (2010) Real-time MR imaging with Gadoteridol predicts distribution of transgenes after convection-enhanced delivery of AAV2 vectors. *Mol Ther* **18**, 1490-1495.