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Review article

Human gene therapy approaches for the treatment of Parkinson's disease: An overview of current and completed clinical trials

Frederick L. Hitti[a,*], Andrew I. Yang[a], Pedro Gonzalez-Alegre[b], Gordon H. Baltuch[a]

[a] Department of Neurosurgery, Pennsylvania Hospital, University of Pennsylvania, 800 Spruce St, Philadelphia, PA 19107, USA
[b] Department of Neurology, Pennsylvania Hospital, University of Pennsylvania, 800 Spruce St, Philadelphia, PA 19107, USA

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ABSTRACT

Gene therapy has been employed in the human brain for a number of disorders in clinical trials and may serve as an avenue for the treatment of Parkinson's disease (PD). Several gene therapy treatment strategies have been developed and evaluated in patients with PD. Three main strategies have been used-enhancement of dopamine synthesis, expression of trophic factors, and neuromodulation. Typically, genes are delivered via viral vectors and expressed within neurons in PD-relevant areas of the brain such as the striatum. These methods of gene delivery have the potential for long-term expression and may only need to be delivered once.

Notably, current gene therapy strategies do not address the non-motor symptoms of PD and do not curtail α-synuclein aggregation/spread. Furthermore, many of the completed trials were open-label trials and are subject to placebo effects and bias. Clinical trials have, however, demonstrated safety and studies are ongoing. Here, we review the current landscape of the development of gene therapy for PD and discuss the future of this novel treatment strategy.

1. Introduction

PD is a neurodegenerative disorder characterized by aggregation of α-synuclein, and the hallmark of the disease is loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) [1]. These pathological changes result in the characteristic motor symptoms (resting tremor, rigidity, and bradykinesia) and non-motor symptoms (sleep-wake dysregulation, cognitive decline, mood disorders, and autonomic failure) of PD [2].

L-3,4-dihydroxyphenylalanine (L-DOPA) is the mainstay of PD treatment and ameliorates the majority of the motor manifestations of PD [3,4]. However as the disease progresses, its efficacy wanes and debilitating side-effects may develop. Newly developed delivery methods such as continuous infusion of levodopa-carbidopa intestinal gel [5] have improved efficacy, however these and other pharmacological agents do not slow the progression of the disease [3].

Deep brain stimulation (DBS) is a non-pharmacological neuromodulatory therapy for PD that improves motor manifestations of PD, improves quality of life, and often enables reduction of medication doses [6–10]. Some symptoms of PD, such as tremor, respond very well to DBS even at long-term follow-up of five [11] or even greater than ten years [12]. However, other motor symptoms, such as freezing and dysarthria, respond incompletely [11,13].

The aforementioned limitations of currently available treatments have prompted the development of novel therapies. Gene transfer is a relatively novel modality for the treatment of PD. This approach is very versatile and a number of different avenues have been explored to treat this disease. Several clinical trials have been completed or are currently underway. In this review, we cover the emerging gene therapy approaches for the treatment of PD.

2. Human gene therapy

Gene therapy entails the delivery of genetic material to alter the expression of endogenous genes or to introduce exogenous genes [14]. To augment cellular function, gene therapy approaches may be used to provide a transgene. Commonly, a sequence encoding a wildtype human isoform of an enzyme is delivered. This therapeutic strategy may be used to provide a functional gene in patients with a mutated non-functional gene or an under-expressed gene. This technique can also be used to express proteins such as growth factors to enhance cell survival.

Gene therapy may also be used to knockdown pathological genes. Several strategies exist for gene knockdown. One strategy entails the use of antisense oligonucleotides (AON). In this technique, a short sequence (~20 nucleotides long) complementary to the mRNA of interest

* Corresponding author. University of Pennsylvania, Department of Neurosurgery, 3rd floor Silverstein, 3400 Spruce Street, Philadelphia, PA 19104, USA.
E-mail address: Frederick.Hitti@uphs.upenn.edu (F.L. Hitti).

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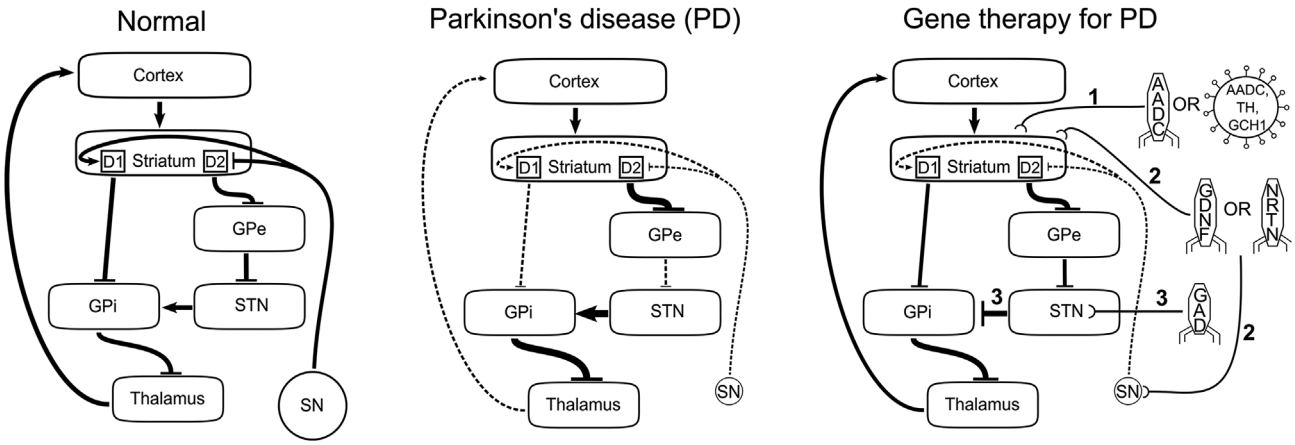

Fig. 1. Basal ganglia circuity and gene therapy approaches to treat PD. (Left panel) The normal basal ganglia circuit is depicted. (Middle panel) In PD, loss of SN dopaminergic neurons results in decreased dopaminergic tone. This results in decreased activity of the direct pathway (striatum to GPi to thalamus) and increased activity of the indirect pathway (striatum to GPe to STN to GPi to thalamus). (Right panel) Gene therapy approaches to treat PD and their effects on the basal ganglia circuit. Approach #1 entails delivery of the AADC gene alone (AAV vector) or the AADC, TH, and GCH1 genes together (lentivirus) to the striatum. Approach #2 involves delivery of growth factors (GDNF or NRTN) to the striatum or striatum/SN via an AAV vector. The goal of this approach is enhanced neuronal survival. Approach #3 is neuromodulatory in nature and involves AAV-mediated delivery of GAD to the STN. This converts STN output from excitatory to inhibitory, thereby decreasing activity of GPi. Legend: Pointed arrowheads represent excitatory connections and flat arrowheads represent inhibitory connections. Dashed lines indicate diminished activity of a pathway. Relatively thicker lines indicate increased activity of a pathway.

is generated. Hybridization of the AON with the transcript of interest results in gene knockdown [15]. Alternatively, viruses may be used to deliver small interfering RNA (siRNA) or microRNA (miRNA) for gene knockdown [16].

This versatile treatment modality has been employed for a number of diseases including neurological disorders such as PD. Notably, several gene therapy treatments have recently been approved by the Food and Drug Administration (FDA). These include tisagenlecleucel, voretigene neparvovec, and nusinersen [17–20]. Tisagenlecleucel is a CAR T-cell treatment for leukemia that is generated using gene therapy [19]. Voretigene neparvovec is a treatment for RPE65-mediated Leber congenital amaurosis that entails delivery of the RPE65 gene via subretinal injections of a viral vector carrying RPE65 [20]. Finally, nusinersen is a treatment for spinal muscular atrophy that can improve motor function and survival via delivery of AON [17,18].

The most common gene therapy approach employs engineered viruses as delivery vectors. Viruses can now be engineered to carry a gene or genes of interest with extremely little viral DNA [14,21]. The small amount of viral DNA contained in most engineered viruses is solely used for viral packaging (generation of the engineered viral particles) and does not result in the expression of any viral proteins [21].

A number of different viruses have been used as vectors for gene therapy in humans. These vectors include adenovirus, adeno-associated virus (AAV), lentivirus, and herpesvirus [14,21–24]. Each of these viruses has advantages and disadvantages. Adenovirus and herpesvirus are pathogenic in humans and can instigate an immune response after delivery. These viruses are most often used for oncologic applications, because an immune anti-tumor response may actually increase therapeutic efficacy [25]. Lentivirus and AAV, on the other hand, are not immunogenic. Furthermore, these viruses have demonstrated long-term expression, on the order of years [26,27]. These properties make these vectors ideal for non-oncologic applications.

Both lentivirus and AAV have been used in the treatment of PD. Lentivirus is a single-stranded RNA retrovirus that integrates its genetic payload into the host genome. Advantageously, this enables long-term expression of the transgene (exogenous gene) introduced by the lentivirus. Integration, however, carries the risk of insertional mutagenesis (disruption of an endogenous gene after integration). Conversely, AAV is a single-stranded DNA virus that does not integrate into the genome. The DNA introduced by the virus remains largely extra-chromosomal, so endogenous genes are not disrupted. Despite the lack of integration, AAV transduction still results in long-term gene expression due to the stability of the extra-chromosomal construct [21]. Another difference between these two vectors is the carrying capacity. Lentiviruses can carry 8–9 kB of genetic material while AAV can only carry < 5 kB. Hence, size limitations may preclude the use of AAV in some applications.

In addition to the use of engineered viruses, another method of gene transfer is plasmid transfection. Plasmid transfection techniques often involve the delivery of circular DNA constructs (plasmids) carried by liposomes, nanoparticles, electroporation, or other physical means [28]. This form of gene therapy has been applied for the treatment of amyotrophic lateral sclerosis (ALS) using a plasmid encoding a zinc-finger protein targeted at increasing vascular endothelial growth factor levels (NCT00748501). Another group delivered a plasmid carrying the hepatocyte growth factor gene as an investigational treatment for ALS (NCT02039401) [29]. It has also been used in the treatment of Canavan Disease [30]. Plasmid transfection has not been applied to PD treatment, however, due to the much more robust and sustained expression that can be achieved with viral transduction. In the remainder of this review, we will focus on viral-based gene therapies that have been employed in PD gene therapy clinical trials.

3. Gene therapy for PD

3.1. Approach #1 – enhancement of dopamine synthesis

As detailed in the introduction, the mainstay of pharmacological treatment for PD is L-DOPA. Due to the loss of dopaminergic neurons in the SNc, dopamine levels are decreased in patients with PD. L-DOPA is a precursor to dopamine in the dopamine synthesis pathway [31]. By increasing levels of L-DOPA, dopamine levels may be augmented. With this in mind, several groups have pursued using gene therapy to augment dopamine levels [32–35]. This strategy entails delivering genes that code for enzymes in the biosynthesis pathway of dopamine (Fig. 1, Table 1). The most common approach is delivery of the aromatic L-amino acid decarboxylase (AADC) gene. This enzyme converts L-DOPA to dopamine in the final step of the dopamine synthesis pathway. All of the groups using this strategy are targeting the striatum/putamen for gene delivery. By expressing AADC in the striatum/putamen, L-DOPA may be converted to dopamine at the site where it is normally released.

Table 1
Gene therapy for Parkinson's disease – completed trials.

Vector	Injection site	Mechanism	Institution/Company	Phase	Outcome	Trial #	Year	Ref
AAV2-CMV-hAADC-2	Striatum	Enhance DA synthesis	Genzyme	I	Safe	NCT00229736	2008, 2009	[32, 33]
AAV2-CMV-hAADC-2	Putamen	Enhance DA synthesis	Jichi Medical University	I	Safe	n/a	2010	[34]
LV-CMV-TH-AADC-GCH1	Putamen	Enhance DA synthesis	Oxford BioMedica	I/II	Safe	NCT00627588	2014	[35]
AAV2-CMV-hAADC	Putamen	Enhance DA synthesis	Voyager Therapeutics, multicenter	I	Safe	NCT01973543	2019	[39]
AAV2-CAG-NTN	Putamen	Growth factor	Sangamo/UCSD, Rush	I	Safe	NCT00252850	2008	[53]
AAV2-CAG-NTN	Putamen	Growth factor	Sangamo, multicenter	II	Safe, not effective	NCT00400634	2010	[54]
AAV2-CAG-NTN	SN and putamen	Growth factor	Sangamo, multicenter	I/II	Safe, not effective	NCT00985517	2013, 2015	[56, 57]
AAV2-CAG-GAD	STN	Neuromodulation	Neurologix	I	Safe	NCT00195143	2007	[64]
AAV2-CAG-GAD	STN	Neuromodulation	Neurologix	II	Safe, effective	NCT00643890	2011	[65]

Abbreviations: AADC (Aromatic L-amino acid decarboxylase), AAV (Adeno-associated virus), GCH1 (GTP cyclohydrolase 1), DA (dopamine), LV (Lentivirus), Neurturin (NTN), SN (substantia nigra), SNc (substantia nigra pars compacta), STN (subthalamic nucleus), TH (tyrosine hydroxylase), UCSD (University of California San Diego).
Vectors are listed as virus-promoter (if information was available)-gene.

Table 2
Gene therapy for Parkinson's disease – ongoing trials.

Vector	Injection site	Mechanism	Institution/Company	Phase	Status	Trial #
AAV2-hAADC	Putamen	Enhance DA synthesis	Voyager Therapeutics, multicenter	II	Active, recruiting	NCT03562494
AAV-hAADC-2	Putamen	Enhance DA synthesis	Jichi Medical University	I/II	Active, recruiting	NCT02418598
LV-CMV-TH-AADC-GCH1	Putamen	Enhance DA synthesis	Oxford BioMedica	I/II	Active, not recruiting	NCT01856439
AAV2-CMV-GDNF	Putamen	Growth factor	NINDS	I	Active, not recruiting	NCT01621581

Abbreviations: AADC (Aromatic L-amino acid decarboxylase), AAV (Adeno-associated virus), GCH1 (GTP cyclohydrolase 1), GDNF (glial cell line-derived neurotrophic factor), DA (dopamine), LV (Lentivirus), NINDS (National Institute of Neurological Disorders and Stroke), TH (tyrosine hydroxylase).
Vectors are listed as virus-promoter (if information was available)-gene.

This therapeutic strategy may enhance the efficacy of L-DOPA and may allow medication doses to be lowered.

Several groups have pursued the dopamine augmentation approach (Fig. 1, approach #1). These groups include Voyager Therapeutics, Genzyme, Jichi Medical University, and Oxford BioMedica (Table 1, Table 2) [32–35]. Other than Oxford BioMedica, these investigators have used AAV vectors to deliver the AADC gene to the striatum/putamen. These groups have completed phase I trials that have demonstrated safety of the therapy.

The Genzyme group has published the results of their phase I trial. They injected AAV-AADC into the putamen of 10 patients using a Leksell frame for stereotactic targeting [32,33]. Unified Parkinson's Disease Rating Scale (UPDRS) scores were obtained at baseline and at 6 month follow-up (Table 3). There was improvement in mean UPDRS scores and “on” time (Table 3). Furthermore, there was increased uptake of the AADC tracer on positron emission tomography (PET) at follow-up. One symptomatic and two asymptomatic intracranial hemorrhages were observed in this cohort. Fortunately, the authors reported that the symptomatic patient has recovered almost entirely. They noted that the hemorrhages occurred along the catheter tract and not at the injection site. The hemorrhages, therefore, likely occurred upon catheter placement and were not due to the injection itself. This hemorrhage rate of 30% is quite high compared to rates reported for DBS lead implantation (< 4%) [36–38]. In the study conducted by Christine et al. (2009), all patients underwent post-operative brain MRI. Many centers do not routinely get post-operative imaging after DBS implantation. Therefore, the asymptomatic hemorrhages seen in the Christine et al. (2009) study are possibly a result of increased detection/diagnosis of hemorrhage and not increased incidence. These studies were phase I studies, so the UPDRS and PET data must be interpreted with caution.

Muramatsu et al. from Jichi Medical University also published the results of their phase I study of AAV-AADC gene therapy as a treatment for PD [34]. These authors used the same vector used in the Christine et al. (2009) study. They performed stereotactic putaminal injections of AAV-AADC in 6 patients and found improvements in total and motor UPDRS scores (Table 3). PET imaging using an AADC tracer revealed a 56% increase in uptake at 6 month follow-up. Muramatsu et al. reported one transiently symptomatic hemorrhage in the subcortical white matter. This event was attributed to the surgery itself and not the injection/delivery of vector, because it occurred along the catheter tract and not at the injection site. As with the above studies, no control group was included in this trial so the efficacy of this approach remains unknown.

In a recent study, Christine et al. (2019) used MRI-compatible skull aiming devices to inject AAV-AADC into the putamen bilaterally [39]. Unlike the studies discussed above that used traditional stereotactic techniques, the technique used by the Voyager Therapeutics group allows for direct visualization of vector infusion since the injections are done in an MRI scanner and the vector is mixed with the contrast agent gadolinium [39,40]. They injected 15 patients at three different dose levels. They increased the volume of vector delivered based on interim results that showed incomplete coverage of the putamen. The initial low-dose cohort had 21% mean coverage of the putamen while the highest-dose cohort had a mean coverage of 42%. The procedure was safe and well tolerated. There was only one serious adverse event - deep venous thrombosis (DVT) and pulmonary embolus in one patient. They are now using MRI-compatible sequential compression devices for DVT prophylaxis. The highest-dose group (cohort 3) exhibited a 79% mean increase in ^{18}F-Dopa uptake 6 months after injection. Medication reduction and increase in “on” time were observed (Table 3) [39]. These results have prompted initiation of a phase II trial (NCT03562494) using this delivery technique and AADC vector. While this approach is safe, efficacy remains unknown pending the results of future studies.

The Oxford BioMedica group has developed a lentiviral vector that carries several of the genes involved in the dopamine biosynthesis pathway [35]. This single vector carries the genes for AADC in addition to the genes for tyrosine hydroxylase (TH) and GTP cyclohydrolase 1 (GCH1). TH is the rate-limiting enzyme in the biosynthesis pathway of dopamine, and GCH1 is the rate-limiting enzyme in the production of tetrahydrobiopterin (THB), a necessary co-factor for TH and phenylalanine hydroxylase (PAH). This strategy enables endogenous production of dopamine without providing exogenous L-DOPA, because delivery of the TH gene should result in endogenous production of L-

Table 3
Gene therapy for Parkinson's disease – results of completed trials.

Vector	Ref	Phase	Trial design	Study size	Outcome (mean)	Follow-up (months)	Complications
AAV2-CMV-hAADC-2	[32]	I	Safety, no control	10	'off' total UPDRS: 31% ↓ 'off' UPDRS III: 36% ↓ 'on' time: 3.3h ↑ 'off' time: 3.1h ↓	6	3 intracranial hemorrhages
AAV2-CMV-hAADC-2	[34]	I	Safety, no control	6	'off' total UPDRS: 28% ↓ 'off' UPDRS III: 46% ↓	6	1 intracranial hemorrhage
AAV2-CMV-hAADC	[39]	I	Safety, no control	15	'off' UPDRS III: 41% ↓ 'on' time: 1.5 h ↑ L-DOPA equivalent dose: 42% ↓	12	1 DVT/PE
LV-CMV-TH-AADC-GCH1	[35]	I/II	Safety/dose escalation, no control	15	'off' total UPDRS: 76% ↓ 'off' UPDRS III: 71% ↓	12	1 subdural hematoma
AAV2-CAG-NTN	[53]	I	Safety, no control	12	'off' total UPDRS: 29% ↓ 'off' UPDRS III: 36% ↓	12	no permanent adverse events
AAV2-CAG-NTN	[54]	II	double-blind randomized control	58 (20 control)	'off' UPDRS III (treatment): 18% ↓ 'off' UPDRS III (control): 18% ↓ PDQ-39 (treatment): 3.83 ↓ PDQ-39 (control): 3.06 ↑	12	1 MI, 1 PE, 1 GBM, 1 hemorrhage
AAV2-CAG-NTN	[57]	I/II	double-blind randomized control	51 (27 control)	'off' UPDRS III (treatment): 20% ↓ 'off' UPDRS III (control): 14% ↓	15	2 intracranial hemorrhages
AAV2-CAG-GAD	[64]	I	Safety, no control	12	'off' UPDRS III: 24% ↓	12	None
AAV2-CAG-GAD	[65]	II	double-blind randomized control	45 (23 control)	'off' UPDRS III (treatment): 23% ↓ 'off' UPDRS III (control): 13% ↓	6	Catheter misplacement

DOPA. This approach may therefore prove more effective than providing AADC alone. A unique feature of this group's vector design is the use of two internal ribosome entry site (IRES) sequences to allow for the expression of multiple proteins (polycistronic gene expression) [41]. This group has published the results of their phase I/II trial [35]. They injected their vector bilaterally into the putamen of 3 cohorts of patients (5 PD patients per cohort) in a dose-escalation study. Patients demonstrated improvement in total and motor UPDRS scores (Table 3). The majority of adverse events in the study were increased medication-induced dyskinesias. These events were successfully treated with medication reduction and were possibly indicative of treatment effect. Only one intracranial hemorrhage was noted (subdural hematoma) that was not clinically significant. Other adverse events included right inguinal hernia, large vessel vasculitis, fall due to dyskinesia, aspiration pneumonia, and uncontrolled diabetes. These events were deemed unrelated to the study drug or procedure.

Given that there was no control group in this study, determination of efficacy is pending completion of future trials. Furthermore, the aforementioned dopamine augmentation strategy (approach 1) does not address non-motor symptoms of PD nor does it directly address α-synuclein pathology.

3.2. Approach #2 – delivery of trophic factors

Given that the hallmark of PD is the loss of dopaminergic neurons, several groups have pursued delivery of growth factors for the treatment of PD (Fig. 1, approach #2). The expression of growth factor genes has been demonstrated to enhance neuronal survival. Glial cell line-derived neurotrophic factor (GDNF) is one growth factor that has been extensively explored for the treatment of PD. Studies were initially conducted using direct intracerebral infusion of the GDNF protein. Preclinical experiments in rodent and primate models of PD showed excellent efficacy of direct infusion of GDNF protein [42,43]. Human phase I studies showed safety of this therapy, and this prompted further investigation [44]. Randomized-controlled trials of GDNF protein infusion in the ventricular system or in the putamen directly, however, were not clinically effective [45,46]. Further studies suggested that the trial failures were possibly due to very limited tissue spread of the protein [47]. A recent randomized clinical trial of intraputaminal delivery of GDNF aimed to mitigate the problem of limited tissue spread via intermittent convection-enhanced delivery through a novel implanted device [48]. The study investigators injected gadolinium into the device to determine tissue spread. Only patients that had ≥40% coverage of the motor putamen on post-injection MRI were included for randomization. Improvement in UPDRS motor scores was seen in both treatment and control groups at 40 weeks. While there was a trend towards greater improvement in the treatment group, the difference was not statistically significant. The authors postulate that this may be due to the low dose of GDNF administered in the study due to safety concerns, insufficient treatment/follow-up time of 40 weeks, a greater than expected improvement in the control group due to a possible lesion effect of sham treatment, and small sample size. Interestingly, there was a subgroup of patients that demonstrated an improvement of greater than 10 points in UPDRS "off" motor scores in the treatment group but not in the control group. While subgroup analysis was unrevealing, these findings lend some support for further investigation. Furthermore, an open-label extension study (while not placebo-controlled) did demonstrate continued clinical improvement with 40 weeks of additional treatment, thus supporting a clinical trial with an 80 week rather than 40 week endpoint [49].

Given the limitations of direct GDNF protein infusions, delivery of GDNF via gene therapy is currently being investigated. Compared to direct protein infusion, a gene therapy approach offers the advantage of continuous protein production. GDNF gene therapy, therefore, offers a means of providing a persistent trophic signal after a single infusion. The National Institute of Neurological Disorders and Stroke (NINDS) is currently conducting a phase I trial to assess the safety and potential efficacy of putaminal injections of an AAV vector carrying the GDNF gene (Table 2).

Another group is exploring the use of the Neurturin (NRTN) gene for

the treatment of PD (Table 1). This gene is a homolog of GDNF and is expressed in the SN and striatum [50]. Furthermore, rodent and primate studies have shown that NRTN gene therapy protects dopamine neurons from cell death and improves motor function in animal models of PD [51,52]. Based on this pre-clinical data, Marks et al. performed bilateral putaminal injections of AAV-NRTN in 12 PD patients in a phase I study [53]. The injections were done with traditional frame-based stereotactic techniques. Marks et al. did not observe any intracranial hemorrhages. They did have one case of suspected procedural air embolus that was treated with irrigation and re-positioning. This patient stabilized and received the treatment vector. At 12 month follow-up, no permanent adverse events were noted in the patient cohort. Furthermore, Marks et al. found improvements in UPDRS scores (Table 3), however these results must be interpreted cautiously since this trial did not include a control group.

These phase I studies prompted a double-blind randomized trial [54]. Marks et al. randomized 38 PD patients to receive AAV-NRTN and 20 PD patients to undergo sham surgery. The treatment group received bilateral putaminal injections of AAV-NRTN using traditional stereotactic techniques while the sham group underwent bilateral partial-thickness burr hole placement. The primary endpoint of the study was off-medication UPDRS part III scores at 12 month follow-up (Table 3). No difference was seen in the primary endpoint, however PDQ-39, a secondary outcome favored AAV2-NRTN treatment (Table 3). One patient in the treatment group died from a myocardial infarction 47 days post-operatively and one patient died of a pulmonary embolus on post-operative day 91. These deaths were not attributed to the treatment. One glioblastoma multiforme (GBM) was diagnosed in the treatment group, however it occurred at a location distinct from the injection site and was present on pre-operative imaging upon more careful review. Additionally, tissue biopsy did not show evidence of AAV-NRTN. Hence, the GBM was not attributed to the treatment. One intracranial hemorrhage was observed in the treatment group, however it did not result in permanent neurological deficit. These data supported the safety of the treatment, and suggested that longer follow-up times may be needed to see the full effects of treatment. Additionally, this study demonstrated the importance of surgical sham groups in future trials. These results caution against overly optimistic interpretation of phase I trial data, because placebo effects may be quite robust.

In both of the aforementioned trials, the putamen was targeted. While pre-clinical studies demonstrated efficacy of this approach, the predominant area of cell loss is the SNc. Moreover, pre-clinical studies in an animal model of PD undergoing active neurodegeneration have demonstrated increased efficacy of combined striatum/SN injections of AAV-NRTN [55]. Therefore, the Sangamo group conducted a phase I/II trial in which the AAV-NRTN vector was injected into both the putamen and SN (Table 1) [56]. Bartus et al. demonstrated the safety of this approach in 6 PD patients. They did not observe any clinically significant adverse events related to treatment. A double-blind randomized controlled trial was then conducted to fully assess the efficacy of AAV-NRTN delivery to the putamen and SN [57]. Olanow et al. randomized 24 patients to the treatment group and 27 patients to the sham surgery group. No difference was seen between the two groups in the primary endpoint of the study, UPDRS part III scores off-medication (Table 3). Secondary outcomes were also not significantly different between the treatment and sham groups. With regards to adverse events, there were two intracranial hemorrhages in the treatment group that resolved without permanent neurological deficit. The remainder of the adverse events were unrelated to the procedure or vector delivery. These results once again demonstrated the relative safety of this approach but failed to demonstrate efficacy. It is possible that the failure of this trial was due to incomplete targeting of the putamen and/or SN. Higher doses of vector are likely required to completely target these structures. This could be addressed using techniques that allow for real-time imaging of vector infusion with MRI. The lack of clinical efficacy may also be due to the advanced disease of the patient cohort in the Olanow et al. study. The majority of the patients in the study had PD for more than 5 years, and a substantial number of patients (48%) had PD for over 10 years [57]. Indeed, subgroup analysis presented by the authors suggested that patients who were treated within 5 years of diagnosis exhibited greater improvement in UPDRS scores compared to the sham subgroup. The findings from this study are informative and will aid in the design of future trials. While the above growth factor therapies aim to enhance cell survival, they do not address α-synuclein pathology directly.

3.3. Approach #3 – neuromodulation via gene therapy

Dopamine is produced in the SNc and released in the striatum (Fig. 1) [58]. In a simplified view of basal ganglia circuity, dopamine excites D1 neurons in the striatum in the “direct pathway”. These neurons then inhibit the GPi, and thus the thalamus becomes disinhibited and provides excitation to the cortex. This provides a “go signal” for movement. Dopamine inhibits the D2 neurons of the “indirect pathway” and thus disinhibits the globus pallidus externa (GPe). Disinhibition of the GPe thereby results in inhibition of the subthalamic nucleus (STN). Decreased activity of the STN results in decreased GPi activity, which facilities movement via disinhibition of the thalamus (Fig. 1). In the PD state, there is degeneration of dopaminergic neurons in the SN and dopamine levels decrease. This results in decreased excitation of the direct pathway and decreased inhibition of the indirect pathway. The net result is diminished thalamic excitation of the cortex. This circuit model predicts STN hyperactivity in the PD state, and STN hyperactivity is the putative target of ablative techniques and DBS [59].

Gene transfer is a versatile technique that allows for the delivery of nearly any gene. One group has devised a clever gene therapy approach to modulate activity of the STN (Fig. 1, approach #3). Normally, the STN contains predominately glutamatergic neurons. Glutamate decarboxylase (GAD) converts glutamate to gamma-Aminobutyric acid (GABA) [60]. Hence, expression of GAD in the STN was proposed to transform the glutamatergic neurons to GABAergic neurons and convert STN output from excitatory to inhibitory. To achieve this, an AAV vector carrying the GAD gene was engineered.

Pre-clinical work demonstrated that administration of AAV-GAD to the STN results in increased levels of GABA in SN output regions as assayed by microdialysis [61]. Inhibition of STN target structures was also observed electrophysiologically. This approach was successful despite the fact that the vesicular GABA-transporter (VGaT) gene was not administered with GAD. While not tested directly in the pre-clinical studies, this may be due to endogenous low levels of vGaT expression or by induction of vGaT expression by GAD production. Indeed, recent work has demonstrated co-release of disparate neurotransmitters from single neurons [62]. Furthermore, there is evidence that neurons in the STN can express both vesicular glutamate transporters and vGaT [63]. Regardless of the precise mechanism, the pre-clinical data supported increased inhibition to targets of the STN.

Kaplitt et al. conducted a phase I trial in which 12 PD patients received AAV-GAD in the STN unilaterally at three dose levels [64]. The viral vector was injected using frame-based stereotaxy. No adverse events related to the procedure or virus were observed. In addition, post-operative imaging did not demonstrate any intracranial hemorrhage. Improvement was seen in UPDRS part III scores at 12 month follow-up (Table 3).

The results detailed above spurred a phase II double-blind randomized controlled trial [65]. LeWitt et al. randomized 22 patients to the treatment group and 23 patients to the control group. Of these patients, 16 in the treatment group and 21 in the control group were included in the final analysis. Patients were excluded from the final analysis due to catheter misplacement, catheter malfunction, or missed surgical target. The intended treatment was bilateral infusion of AAV-GAD into the STN, and the control group underwent sham surgery with partial-thickness burr hole placement. At 6 month follow-up, a statistically

significant greater improvement in UPDRS part III scores was observed in the treatment group (Table 3). Regarding adverse events, only minor events such as nausea and headache were attributed to the procedure and were self-limited. Bowel obstruction was seen in one patient in the treatment group, but this event was not attributed to the treatment. Finally, one patient was excluded from the final analysis due to inappropriate placement of two stereotactic catheters in the same STN. This resulted in a double dose of AAV-GAD to the same hemisphere. In response to this event, the surgical procedure was revised to include an intra-procedure time out to verify the stereotactic coordinates when switching to the second hemisphere. Despite these adverse events, this trial demonstrated both the efficacy and safety of this treatment. Furthermore, long-term outcomes of the trial have recently been reported [66]. The therapeutic efficacy seen at 6 months post-treatment was also seen at 12 month follow-up. There was a significantly greater percentage of responders in the treatment group (62.5%) compared to the sham group (23.8%) at the 12 month time point [66]. The authors defined "responders" as patients who had an improvement of at least 9 points in motor UPDRS scores. Additionally, functional network connectivity as assayed by FDG PET revealed network changes 12 months after treatment relative to pre-treatment baseline [67]. Namely, increased metabolic activity was observed in the premotor cortex, motor cortex, and supramarginal gyrus. Reduced metabolism was seen in the caudate, anterior putamen, globus pallidus, ventral anterior thalamus, medial dorsal thalamus, and inferior frontal gyrus. These network changes were associated with treatment effect. These results demonstrate that neuromodulation via a gene therapy approach may be a durable and effective form of treatment for PD. Regrettably, the further clinical development of the AAV-GAD treatment paradigm has been halted due to a lack of funding.

3.4. Limitations

While gene therapy provides a novel approach to treat PD, we must review some limitations of this approach and the current data. The majority of the trials detailed above were open-label trials. Therefore, the results of these trials are subject to placebo effects and bias from the clinicians scoring symptoms and findings. These factors and others may produce type I error, so the results must interpreted with caution.

The gene therapy strategies outlined in this review are mainly targeted at augmenting the dopaminergic circuit implicated in the motor manifestations of PD. The non-motor symptoms of late PD such as cognitive decline, however must be considered because they are a significant cause of morbidity that results in decreased quality of life. The therapeutic strategies described here would not be expected to mitigate cognitive decline. Further studies regarding the pathogenesis of the non-motor symptoms of PD must be undertaken to reveal possible therapeutic strategies to curtail symptoms such as cognitive decline.

Another limitation of the approaches detailed above is that they mainly target the symptoms of PD and not cell survival. Additionally, although the growth factor strategies (approach #2) aim to promote cell survival, ongoing accumulation and spread of α-synuclein may make this approach ineffective in the long-term. Furthermore, these gene transfer approaches must be compared to the currently available symptomatic therapies in the domains of efficacy and also cost-effectiveness.

Many of the clinical trials described above used a sham surgery group as the control group. This group continued medical management of their PD. It will be important to compare these novel therapies to the currently accepted standard of care in surgical treatment of PD, DBS. Furthermore, if the patient does not demonstrate improvement with the novel treatment should they be excluded from future DBS implantation since the risks of repeat intervention in the same brain region are not entirely known? Mittermeyer et al. reported that four patients who received AADC gene therapy were implanted with DBS after their gene therapy treatment [68]. This argues against excluding gene therapy patients from future treatment with DBS. Finally, the efficacy of these genetic therapies must always be compared to the evolving landscape of novel PD therapies. For example, novel DBS stimulation paradigms and closed-loop systems may enhance DBS efficacy [69,70]. Furthermore, novel drugs and drug delivery routes such as inhaled levodopa are becoming available for clinical use [71]. The parallel development of multiple treatment approaches necessitates consideration of all treatment avenues when determining the clinical utility of an emerging therapy.

3.5. Future directions

Gene therapy is a burgeoning field with diverse applications. We have reviewed the gene therapy approaches currently in development for the treatment of patients with PD. The dopamine augmentation strategy is safe and preliminary studies have suggested clinical efficacy. Final determination of the clinical utility of this approach is pending larger randomized-controlled trials.

While the delivery of growth factor proteins has not been effective in randomized clinical trials, delivery of growth factor genes may overcome the limitations inherent with the delivery of recombinant proteins, such as the necessity of multiple injections. A major advantage of this approach is the continual production of the growth factor. This may curtail further cell loss and clinical deterioration. If successful, this treatment would be paradigm shifting, because no currently available treatments halt progression of dopaminergic cell loss. While randomized trials of growth factor gene therapy have not yet demonstrated efficacy versus sham, an important consideration for future study enrollment is the length of time since disease diagnosis. The advanced nature of the neurodegeneration in patients included in prior studies may have precluded a biological response to the growth factors. Furthermore, some pre-clinical work has demonstrated lack of efficacy of GDNF in α-synuclein overexpression animal models of PD [72]. Hence, a disease state with high α-synuclein burden may not respond to GDNF/NRTN treatment. While Decressac and colleagues demonstrated lack of efficacy of GDNF therapy in an α-synuclein overexpression model, there is evidence that argues against the validity of this model [73]. Su et al. found that α-synuclein expression is not elevated in the SN of early PD patients compared to controls. Furthermore, other growth factors, such as cerebral dopamine neurotrophic factor (CDNF), are being investigated that may prove effective despite the high α-synuclein burden present in advanced PD [74]. A phase I/II clinical trial is currently underway to investigate the safety of intraputamenal administration of CDNF in patients with PD (NCT03295786).

Since α-synuclein aggregation has been heavily implicated in the neurodegeneration seen in PD, several groups have developed therapeutic strategies to target α-synuclein. Antibodies directed against α-synuclein are currently under investigation in clinical trials [75]. The Protena group demonstrated safety, a reduction in serum free α-synuclein levels, and dose-dependent increases in CSF antibody levels [75]. Efficacy will be investigated in future studies. α-synuclein knockdown is an emerging gene therapy approach for treating the α-synuclein aggregation seen in PD. Several groups have developed strategies to knockdown α-synuclein in animal models using RNAi [76–78]. These groups have demonstrated a reduction in dopaminergic neuron degeneration and a decrement in PD-like motor deficits. While some have cautioned that excessive knockdown of α-synuclein may be detrimental [79], other groups have not observed untoward effects of robust knockdown [80]. Further development of these therapies is ongoing.

Clinical trials have demonstrated the safety and potential efficacy of neuromodulatory gene therapy for the treatment of PD. This neuromodulatory approach could be explored for the treatment of other disease processes as well.

The gene therapy strategies discussed here are not mutually exclusive. Future studies could investigate the combination of these therapies for the treatment of PD. For example, one could inject a vector

carrying the AADC gene into the putamen, a growth factor vector into the SN, and a neuromodulatory vector into the STN. By working via different mechanisms, treatment with a combination of approaches may demonstrate non-linear synergy.

The studies described above utilized viral vectors for gene delivery. With direct injections into the immunoprivileged brain, robust immune responses are generally not observed. The clinical trials reviewed here did not use immunosuppression to enable gene delivery. Neutralizing antibodies to the vectors, however may be produced after administration and may affect future treatments if multiple injections become necessary. Furthermore, some patients may already have antibodies to the vectors before administration. Careful study of the immune response before and after treatment will be essential.

In addition to vector design, optimizing the delivery of gene therapy vectors is an important area for further development. New injection catheter designs could limit reflux and enhance target tissue spread [81]. All of the early gene therapy trials used standard stereotactic techniques for target localization and vector administration. Enhanced tissue spread has been achieved by convection-enhanced delivery (the application of positive pressure to deliver the therapeutic agent) [81]. While convection-enhanced delivery enables greater tissue distribution, traditional stereotactic techniques are limited in their ability to monitor spread of the therapeutic vector. Novel approaches allow for real-time targeting and injection in a magnetic resonance imaging (MRI) machine. The ClearPoint platform (MRI Interventions, Inc.) is an FDA approved device that allows for stereotactic targeting in an MRI machine [82]. This device has been used for accurate drug infusion in patients with diffuse intrinsic pontine glioma (DIPG). Furthermore, it has also been used for gene therapy applications such as the infusion of AAV-GDNF into the putamen for the treatment of PD [82]. Notably, if the vector is mixed with contrast agent, vector spread may be imaged during delivery. Appropriate delivery of the vector can be ensured with this technique. Furthermore, studies could be designed in which the amount of vector that is injected is dictated by the dose needed for complete coverage of the intended target. As described above, current studies are underway using this technique for the delivery of AAV-AADC. These strategies have the potential to eliminate trial failures due to improper administration or incomplete spread of the therapeutic vector.

The methods for delivery of gene therapy vectors detailed above entail placement of intracranial drug infusion catheters. Focused ultrasound (FUS) is an emerging treatment for essential tremor and other movement disorders [83,84]. FUS at high frequencies is used to produce thermal lesions of the thalamus to treat tremor. At lower frequencies, FUS may be used to open the blood-brain barrier. Pre-clinical work has demonstrated effective delivery of GDNF expression plasmids using this technique in a rodent model of PD [85]. This approach may provide a non-invasive means of targeted gene therapy. One limitation of this method is the decreased longevity of plasmid-based gene therapy relative to viral-based gene therapy. This limitation may necessitate repeated treatments but warrants further study.

4. Conclusions

Gene therapy has been demonstrated to be relatively safe in PD clinical trials and merits continued investigation for the treatment of PD. Multiple treatment approaches are currently under investigation including enhancement of dopamine synthesis, growth factor administration to curb neurodegeneration, and neuromodulation via enzyme expression. These treatments have the potential to change standard of care for the management of PD and render it a less debilitating disease. Additional strategies may be necessary to address non-motor symptoms of PD and α-synuclein pathology directly.

5. Contributors

FLH conducted the literature search, wrote the manuscript, and made the figures. AIY and PG critically revised the manuscript. GHB critically revised the manuscript and supervised the project. All authors have seen and approved the final version of the manuscript.

Declaration of interests

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