Gene Therapy for Parkinson’s Disease

Subjects: Medicine, Research & Experimental

Contributor: Karim E. Shalaby, Omar M. A. El-Agnaf

Parkinson’s disease (PD) is the second most common neurodegenerative disorder with a prevalence that increases steadily with age. Gene therapy is a modern medical practice that theoretically, and practically, has demonstrated its capability in joining the battle against PD and other complex disorders on most if not all fronts.

Keywords: neurodegeneration ; Parkinson’s disease ; gene therapy

1. Introduction

The high potential of gene therapy gives immense hope for curing thousands of diseases with limited treatment options, such as cancer and monogenic diseases [1][2]. Gene therapy is either carried out ex vivo, involving the genetic modification of cultured cells and transplanting them into the patient, or in vivo, involving the use of vectors to deliver nucleic acids (DNA, RNA, etc.) or genome-modifying components such as CRISPR-Cas to cells to correct a mutation or to regulate a gene’s expression. Gene delivery vectors can either be viral or non-viral. The former harnesses viruses’ natural ability to infect host cells with their genome and are genetically modified to remove pathogenic genes and replace them with the sequence of interest. The most commonly used viral vectors are lentiviruses and adeno-associated viruses (AAVs). Lentiviruses, a subspecies of retroviruses, integrate their cargo into the host’s genome, assuring long-term expression of the delivered gene, but carrying the risk of random insertional mutagenesis. While AAVs offer a rather limited carriage capacity (~4.5 kb), they are deemed large enough for most genes used in therapy. They are also considered a much safer alternative because their genomes exist as an independent episome in the transfected cell, and they are also capable of conferring long-term expression in non-dividing neurons [3][4]. Furthermore, the use of certain serotypes (e.g., AAV9) carries certain advantages, such the ability to cross the BBB [5], a major obstacle in the way of vectors to the brain.

Conventional gene therapy utilizes viral vectors for the delivery of therapeutic transgenes. The first viral-mediated gene therapy was approved for clinical use in 1990 for adenosine deaminase-severe combined immunodeficiency (ADA-SCID). In it, two young girls were treated with autologous T-lymphocytes genetically modified with retroviruses carrying a wild-type ADA gene ex vivo [6][7]. Evidence of modest feasibility encouraged subsequent gene therapy trials to take place, to which major limitations ensued. For example, gene therapy trials for SCID resulted in the development of therapy-related leukemia in several young children, leading to the death of one patient [8][9]. Investigations of the possible cause of leukemia revealed insertional mutagenesis of the therapeutic gene into a proto-oncogene locus [10]. Consequently, various bodies modeled by the US Recombinant DNA Advisory Committee and the Food and Drug Administration have been established to acknowledge this risk and to standardize protocols for gene therapy in humans globally [11].

Non-viral vectors are synthetic, which gives them a unique flexibility to be customized for use from a range of different compounds, such as lipids and proteins. The components of synthetic vectors are inspired from the composition of viral envelopes that enable viruses to specifically bind to host cells. However, they offer a safer alternative to viral vectors with possibly lesser mutagenic and immunogenic responses in the host [12]. It has been shown that by integrating a fragment derived from the rabies virus glycoprotein (RVG), these vectors can be modified to cross the BBB and deliver their cargo to specific neuronal cells for the treatment of Parkinson’s disease (PD) [13][14]. Thus far, all gene therapy clinical trials for PD have been carried out using viral vectors, since non-viral vectors need to be optimized to provide equal delivery efficiency to viral vectors.

Gene editing approaches, such as using CRISPR, are capable of inducing genetic insertions and corrections at a specific locus and offer an alternative to introducing a therapeutic gene into a random genomic locus. The emergence of such approaches further helps to overcome the critical limitation of insertional mutagenesis.

2. Gene Therapy Trials for PD

Most gene therapy clinical trials have fulfilled Phase I, safety and efficacy profiles, but the majority failed when advanced to controlled, blinded Phase II trials to achieve results beyond placebo effect or better than those seen with current treatments (**Table 1**). Importantly, these studies represent a proof of concept of how PD can be tackled at the genetic level. Ongoing trials focus either on symptomatic benefit through balancing physiological basal ganglia circuitry or enhancing the dopamine biogenesis pathway, or on disease modification through providing neuronal protection or preventing α-synuclein aggregation or accumulation. The principles, progress and results of these trials are discussed below to highlight how gene therapy could be used to tackle PD on these different levels, emphasizing its broad scope of intervention.

Table 1. Current status of gene therapies for Parkinson's disease.

	Mode of Delivery	Phase	Duration	Primary Endpoint	Outcome(s)	Reference(s)
Restoring the physiological balance of the basal ganglia						
GABA						
AAV2-*GAD*	**IP (subthalamic nucleus)**	**I**	**2003–2005**	**Safety**	**UPDRS improvement persisted for 12 months, and reduced thalamic activity as assessed by ^{18}F-FDG PET in all patients.**	**NCT00195143** [15]
AAV2-*GAD*	**IP (subthalamic nucleus)**	**II**	**2008–2010**	**Disease severity and progression**	**UPDRS improvement over sham control group, diminished PD symptoms with no adverse events for 12 months in all patients. No improvement over conventional PD treatment.**	**NCT00643890** [16][17]
Enhancing dopamine synthesis						
AADC						
AAV2-*AADC*	**IP (striatum)**	**I**	**2004–2013**	**Safety and tolerability**	**Clinical improvements in first 12 months in all patients followed by slow deterioration. One symptomatic and two asymptomatic intracranial hemorrhages followed. Increased ON time and reduced OFF time. Increased AADC activity as measured by ^{18}FMT PET.**	**NCT00229736** [18]
AAV2-*AADC*	**IP (striatum)**	**I**	**2013–2020**	**Safety and tolerability**	**Stable increase in AADC activity as measured by ^{18}F-Levodopa PET at 6 months, and clinical improvements at 12 months with no serious adverse events in all patients. Improvements were stable or improved at 12, 24 and 36 months.**	**NCT01973543** [19][20]
AAV2-*AADC*	**IP (putamen)**	**I/II**	**2015–2018**	**Safety**	**N/A**	**NCT02418598**
AAV2-*AADC*	**IP (striatum)**	**I**	**2017–2021**	**Safety and suicide risk**	**Increase in AADC activity as measured by PET and increase in ON time without troublesome dyskinesia.**	**NCT03065192**

	Mode of Delivery	Phase	Duration	Primary Endpoint	Outcome(s)	Reference(s)
AAV2-*AADC*	IP (striatum)	II	2018–2022	Change in ON time without troublesome dyskinesia	N/A	NCT03562494 [21]
AADC, TH, CGH1						
Lentivirus-*TH/AADC/CGH1*	IP (striatum)	I/II	2008–2012	Safety	Significant UPDRS improvement at 6 months. Mild to moderate but no serious drug-related adverse events were reported during the first 12 months.	NCT00627588 [22]
Lentivirus-TH/AADC/CGH1	IP (striatum)	I/II	2011–2022	Long-term safety and tolerability	N/A	NCT01856439 [22]
Neuroprotection						
GDNF						
AAV2-*GDNF*	IP (putamen)	I	2013–2022	Safety and tolerability	Stable motor scores throughout study period. Increase in ^{18}F-DOPA uptake as assessed by PET at 6 and 18 months in 10/13 and 12/13 patients, respectively.	NCT01621581 [21]
AAV2-*GDNF*	IP (putamen)	I	2020–2026	Safety and tolerability	N/A	NCT04167540
NRTN						
AAV2-*NTN*	IP (putamen)	I	2005–2007	Safety and tolerability	Improvement of 14 points in off-medication motor score of UPDRS and increase of 2.3 h in ON time without troublesome dyskinesia at 12 months. Non-significant improvements in several secondary measures. No change in ^{18}F-levodopa uptake as assessed by PET	NCT00252850 [23]
AAV2-*NTN*	IP (putamen)	II	2006–2008	Disease severity and progression	No significant improvement in UPDRS over sham surgery control group at 12 months. Serious adverse events in 13/38 treated and in 4/20 control individuals. Three patients in the treated group and two in the sham surgery group developed tumors.	NCT00400634 [24]
AAV2-*NTN*	IP (substantia nigra + putamen)	I/II	2009–2017	Disease severity and progression	No serious adverse events in all patients.	NCT00985517 [25]
Targeting disease genes						
AAV9-*Gcase*	IC	I/II	2020–2027	Safety and immunogenicity	N/A	NCT04127578

GABA: gamma-aminobutyric acid; GAD: glutamic acid decarboxylase; IP: intraparenchymal; UPDRS: Unified Parkinson's Disease Rating Scale; FDG: fluorodeoxyglucose; PET: positron emission tomography; PD: Parkinson's disease; AADC: aromatic l-amino acid decarboxylase; TH: tyrosine hydroxylase; CGH1: cyclohydroxylase; GDNF: glial-derived neurotrophic factor; DOPA: dihydroxyphenylalnine; NRTN/NTN: neurturin; Gcase: glucocerebrocidase; IC: intracranial.

2.1. Restoring the Physiological Balance of the Basal Ganglia

The loss of dopaminergic neurons in PD leads to a hyperactive STN, which can be reversed by injections of an agonist of γ-aminobutyric acid (GABA), the inhibitory neurotransmitter of the STN, as shown previously in non-human primate (NHP)

PD models [26].

GABA

PD-like symptoms were alleviated in rat and NHP models via overexpression of glutamic acid decarboxylase (GAD), the enzyme involved in the synthesis of GABA, in the STN [27][28]. Two genetically distinct GAD isoforms, GAD65 and GAD67, were both used in a trial to increase GAD expression in the STN [15]. The trial's aim was to increase GABA production and the inhibition of the STN, thus attaining the same effect of DBS and improving motor deficits. In Phase I, all patients who received a unilateral AAV2-GAD injection to the STN showed improvements on their unified Parkinson's disease rating scale (UPDRS) scores which persisted throughout the 12-month duration of the study [15]. Subsequent positron emission tomography (PET) scans using [^{18}F] fluoro-deoxyglucose as a tracer showed a significant reduction in glucose uptake in the thalamus of the treated side, indicating a reduction in thalamic metabolic activity, in line with the improved motor functions [15]. In a double-blinded Phase II trial, patients who received AAV2-GAD injection had improvements in UPDRS scores over the sham control group [16]. Although gene therapy ameliorated PD symptoms in these trials with no adverse events reported in any of the patients and effects persisting for a year [17], they showed no greater improvement over current standards of care. It is important to note that these studies serve as a proof-of-principle approach for a generally safe and efficacious operation for gene therapy which is valuable for optimizing the design of larger clinical trials in the future.

2.2. Enhancing Dopamine Synthesis

Dopamine is synthesized in dopaminergic neurons from the amino acid tyrosine derived through diet. Tyrosine is first converted to L-DOPA by tyrosine hydroxylase (TH), and aromatic amino acid decarboxylase (AADC) converts L-DOPA to dopamine. Guanosine triphosphate cyclohydroxylase I (GCH1) is the rate-limiting enzyme in synthesizing the TH co-factor tetrahydrobiopterine (BH4). These enzymes are transported from the SNc to the striatum through the nigrostriatal pathway in an anterograde manner. In advanced PD, severe dopaminergic nerve loss is associated with a significant reduction in the activity of these enzymes in the striatum. In addition, as the disease progresses, L-DOPA dosage requirements for patients increase and the resulting elevated levels of dopamine outside the basal ganglia lead to dyskinesia.

AADC

Gene transfer of AADC has been used to enhance the pathway of dopamine biogenesis, by rescuing AADC levels dropping with the degeneration of nigral dopaminergic neurons [18][29][30][31][32][33][34]. After assessment in animal models [29][30][31], Phase I clinical trials using AAV2 [18][32][33] concluded that AAV-AADC gene transfer is safe and stable and patients showed clinical improvements in the first 12 months; however, this improvement slowly deteriorated. This was attributed to the restricted distribution of AADC expression, and the relatively small final volume of vector infused. Consequently, researchers have developed a novel mechanism of delivery, with the aim of increasing the coverage of the striatum, using an MRI-guided convection enhanced delivery (CED) to monitor the delivery of the transgene in non-human primates in real time [34]. Accurate positioning of the cannula was confirmed by MRI images indicating increased coverage of the targeted affected mid-brain neurons in all animals. Further assessment by immunohistochemical staining confirmed the increased expression of AADC in the SNc compared to control animals, which correlated with an increase in AADC concentration in the striatum, indicating successful axonal transport throughout the nigrostriatal pathway. Indeed, as this trial has achieved safety and efficacy and an MRI-CED delivery of AAV2-AADC, it should be assessed for efficiency in a human clinical trial.

TH*, *AADC* and *GCH1

A lentiviral-vector-based gene therapy aimed at increasing the efficiency of patients' metabolism of L-DOPA, and thus decreasing the dose of L-DOPA required and avoiding treatment-related side effects [22][35]. Initially, triple gene transfer of CGH1, TH and AADC in a single lentiviral transcriptional unit was first demonstrated to successfully increase dopamine production and reduce motor asymmetry in rat models of PD [35]. Recently, in a dose-escalation clinical trial, patients who received the Lenti-TH-AADC-GCH treatment demonstrated dose-dependent improvements in their UPDRS scores after 12 months, with the group receiving the highest dosage displaying the most improvement [22]. The study was extended, and patients who were still part of the study showed further improvements after 24 and 36 months [22]. Overall, the trial achieved safety and efficacy, but no control group was included, and the improvements were within the placebo effect seen in other trials [22]. This trial, however, represents the first where lentiviral vectors have been successfully applied to the treatment of a neurological disorder. Moreover, this approach does not depend on dopaminergic neurons, but rather assumes that the transfected striatal neurons will develop the ability to synthesize dopamine. However, striatal neurons lack other mechanisms intrinsic to dopaminergic neurons, such as the capacity to store synthesized dopamine in synaptic

vesicles and to take it back up after release through dopamine transporters, which is perhaps why there was no significant improvement.

2.3. Neuroprotection and Regeneration

The loss of SNc dopaminergic neurons caused by PD pathology results in the reduction in dopamine levels in the striatum. Thus, providing support and protection to dopaminergic neurons from degeneration is an attractive strategy to prevent the manifestation of downstream complications such as physiological imbalances, and symptoms such as rigidity and bradykinesia associated with the disease.

GDNF, *NRTN*

Gene therapy trials offering neuroprotection have focused primarily on the delivery of members of the glial cell family of ligands (GFLs), which are known to play a role in cell protection and survival [36]. For example, the delivery of neurturin (NRTN) to the putamen using AAV2 was well tolerated in a Phase I clinical trial [23], but no improvements in motor function were observed [24]. Analyses of postmortem tissue showed an increase in NRTN expression in the putamen; however, due to a failure of retrograde transport, it was not upregulated in the SN [37]. Moreover, no improvements in motor function were seen in a clinical trial using higher doses of AAV-NRTN to address this issue [25]. On another hand, in animal models, glial-derived neurotrophic factor (GDNF) expression protected nigrostriatal neurons and improved motor function. However, it was found that its long-term expression causes aberrant axonal sprouting and downregulation of TH [38][39]. GDNF is a growth factor that was the first GFL to be discovered, and it functions in promoting the survival of dopaminergic neurons [40]. Further studies demonstrated that injections of AAV-GDNF were well tolerated [41][42]; however, bilateral SN injections resulted in weight loss in aged monkeys [42]. Two ongoing Phase I clinical trials are currently testing the safety of bilateral injections of AAV2-GDNF into the putamen (NCT01621581 and NCT04167540). Importantly, a system whereby the expression of GDNF can be controlled after delivery was developed and showed neuroprotection and improvement in motor function in rodent models [43][44]. In this method, the destabilizing domain of *E. coli* dihydrofolate reductase was fused to GDNF and delivered to neurons using a lentiviral vector. The expression was controlled by the temporal administration of Trimethopram, a drug that crosses the BBB to stabilize the destabilizing domain and activate GDNF expression [43]. The treated group showed improved motor function and a higher level of TH expression than rodents receiving a normal GDNF transfusion. This chemical method sets an adaptable protocol using destabilizing domains to control the expression of other genes of interest. Some PD patients exhibit downregulation of Ret, the receptor for GDNF and NRTN, which could explain the lack of success seen in the above neurotrophic growth factor gene therapies [45]. Additionally, the degenerative state of the PD brain may affect the transport of growth factors, suggesting that such gene therapies may improve patients receiving therapy earlier in the disease course.

Neural Regeneration

An exciting gene therapy approach tackling PD is generating new neurons through converting astrocytes, abundant in the brain, into induced dopamine-releasing neurons [46][47]. Two teams showed that depletion of PTB, an RNA-binding protein which suppresses neuronal differentiation, using short hairpin RNA (shRNA) [47] or through viral delivery of CasRx, an RNA-targeting CRISPR-Cas, to the brain [46], converted resident astrocytes into neurons and rescued neurochemical and motor deficits in mice [46][47].

2.4. Targeting Disease Genes

SNCA, *LRRK2* and *GBA*

As discussed in previous sections, evidence links mutations increasing the expression of α-synuclein to PD and to the formation of Lewy bodies associated with neurodegeneration [48][49][50][51]. It is thus thought that providing neuronal protection could be achieved through downregulating *SNCA*. Knocking down α-synuclein by shRNA or anti-sense oligonucleotides (ASOs) was reported to prevent neurodegeneration in PD models [52][53][54]. AAV-mediated delivery of shRNA-targeting endogenous *SNCA* in rats attenuated rotenone-induced progressive motor deficits and neurodegeneration [52]. Furthermore, *SNCA* downregulation ameliorated neurological deficits in mice models expressing human α-synuclein [53]. Cole et al. (2021) recently showed that ASO-mediated reduction of α-synuclein reversed PD pathology and rescued dopaminergic neuronal function in rodent models of PD [54]. Furthermore, the study reported decreased human α-synuclein levels in the cerebrospinal fluid of non-human primates as a demonstration of the translational potential of the approach [54]. Downregulation of SNCA using CRISPR was also shown to improve cell viability in a PD patient's iPSC-derived dopaminergic neurons [55]. However, other studies found lowering SNCA levels to be associated with further pathologies in vivo, an issue which is yet to be solved [56][57]. Short interfering RNA (siRNA) delivered into rats' brains using AAV2/5 vectors to block the translation of α-synuclein in the SNc resulted in even worse

motor and behavioral deficits accompanied by reduced TH levels and nigral dopaminergic neurodegeneration [56]. This indicates that both the overexpression and downregulation of SNCA may have a negative effect on neuronal survival, and hints at essential functions of the α-synuclein protein in a healthy brain. It is necessary that alternative strategies tackling α-synuclein are explored. For example, expressing six mutant versions of α-synuclein that block the aggregation of wild-type α-synuclein has shown promising results, but has yet to be validated in vivo [58]. Using CRISPR-interference (dCas9-KRAB), Heman-Ackah et al. (2016) knocked down α-synuclein in iPSCs carrying the *SNCA* triplication mutation [59]. Another group used an epigenetic approach (dCas9-DNMT3A) by hyper-methylating *SNCA*'s intron 1 to restore normal mRNA levels in iPSC-derived dopaminergic neurons [55].

LRRK2 mutations cause familial PD or may increase the risk of developing sporadic PD [60]. G2019S and other LRRK2 mutations associated with PD lead to an increased kinase activity, and thus, knocking it out is a sound therapeutic strategy. However, studies have shown that LRRK2 depletion may lead to pathological consequences in other tissues where LRRK2 is expressed, such as the lungs, kidneys and spleen [61][62]. Alternatively, direct intracerebral injections of ASOs depleted LRRK2 protein levels, reduced fibril-induced α-synuclein inclusions and protected TH^+ neurons in the brain of mice, with no detected pathological phenotype in other tissues [63]. A Phase I clinical trial using LRRK2 ASO intrathecal injections is currently ongoing in patients with PD (NCT03976349). However, it is essential to employ routes of delivery capable of brain-specific targeting to apply the LRRK2 gene therapy in a less invasive setting.

A potential gene therapy approach for tackling loss-of-function forms of PD is to overexpress the functional protein. A significant proportion of recessive PD is associated with loss-of-function mutations in PRKN and PINK1 genes involved in mitochondrial function and mitophagy [48][64][65][66]. However, since motor symptoms in such forms of PD are efficiently treated by levodopa, it may be unnecessary to develop a gene therapy to restore Parkin function as an alternative treatment.

Mutations in GBA cause Gaucher's disease, a lysosomal storage disorder, and also represent the most common risk factor for developing PD [67]. A study reported that direct AAV-GBA1 injections in the brains of rodent models of PD reduced α-synuclein levels and pathology [68]. Moreover, intravenous injections of AAV-PHP.B-GBA1 alleviated α-synuclein pathology and produced significant behavioral recovery in A53T mouse models of PD [69]. Intracisternal injection of AAV9-GBA1 to treat PD patients is currently undergoing a Phase I/II clinical trial (NCT04127578).

References

1. Amer, M.H. Gene therapy for cancer: Present status and future perspective. Mol. Cell. Ther. 2014, 2, 27.
2. Prakash, V.; Moore, M.; Yáñez-Muñoz, R.J. Current Progress in Therapeutic Gene Editing for Monogenic Diseases. Mol. Ther. 2016, 24, 465–474.
3. Weinberg, M.S.; Samulski, R.J.; McCown, T.J. Adeno-associated virus (AAV) gene therapy for neurological disease. Neuropharmacology 2013, 69, 82–88.
4. Nanou, A.; Azzouz, M. Gene therapy for neurodegenerative diseases based on lentiviral vectors. Prog. Brain Res. 2009, 175, 187–200.
5. Duque, S.; Joussemet, B.; Riviere, C.; Marais, T.; Dubreil, L.; Douar, A.-M.; Fyfe, J.; Moullier, P.; Colle, M.-A.; Barkats, M. Intravenous Administration of Self-complementary AAV9 Enables Transgene Delivery to Adult Motor Neurons. Mol. Ther. 2009, 17, 1187–1196.
6. Blaese, R.M.; Culver, K.W.; Miller, A.D.; Carter, C.S.; Fleisher, T.; Clerici, M.; Shearer, G.; Chang, L.; Chiang, Y.; Tolstoshev, P.; et al. T Lymphocyte-Directed Gene Therapy for ADA− SCID: Initial Trial Results After 4 Years. Science 1995, 270, 475–480.
7. Bordignon, C.; Notarangelo, L.D.; Nobili, N.; Ferrari, G.; Casorati, G.; Panina, P.; Mazzolari, E.; Maggioni, D.; Rossi, C.; Servida, P.; et al. Gene Therapy in Peripheral Blood Lymphocytes and Bone Marrow for ADA− Immunodeficient Patients. Science 1995, 270, 470–475.
8. Cavazzana-Calvo, M.; Hacein-Bey, S.; Basile, G.D.S.; Gross, F.; Yvon, E.; Nusbaum, P.; Selz, F.; Hue, C.; Certain, S.; Casanova, J.-L.; et al. Gene Therapy of Human Severe Combined Immunodeficiency (SCID)-X1 Disease. Science 2000, 288, 669–672.
9. Herzog, R.W. Gene Therapy for SCID-X1: Round 2. Mol. Ther. 2010, 18, 1891.
10. Hacein-Bey-Abina, S.; Von Kalle, C.; Schmidt, M.; McCormack, M.P.; Wulffraat, N.; Leboulch, P.; Lim, A.; Osborne, C.S.; Pawliuk, R.; Morillon, E.; et al. LMO2-Associated Clonal T Cell Proliferation in Two Patients after Gene Therapy

for SCID-X1. Science 2003, 302, 415–419.

11. Hammer, M.J.; Eckardt, P.; Barton-Burke, M. Informed Consent: A Clinical Trials Perspective. Oncol. Nurs. Forum 2016, 43, 694–696.
12. Shalaby, K.; Aouida, M.; El-Agnaf, O. Tissue-Specific Delivery of CRISPR Therapeutics: Strategies and Mechanisms of Non-Viral Vectors. Int. J. Mol. Sci. 2020, 21, 7353.
13. Javed, H.; Menon, S.A.; Al-Mansoori, K.M.; Al-Wandi, A.; Majbour, N.K.; Ardah, M.T.; Varghese, S.; Vaikath, N.N.; Haque, M.E.; Azzouz, M.; et al. Development of Nonviral Vectors Targeting the Brain as a Therapeutic Approach For Parkinson's Disease and Other Brain Disorders. Mol. Ther. 2016, 24, 746–758.
14. Hwang, D.W.; Son, S.; Jang, J.; Youn, H.; Lee, S.; Lee, D.; Lee, Y.-S.; Jeong, J.M.; Kim, W.J.; Lee, D.S. A brain-targeted rabies virus glycoprotein-disulfide linked PEI nanocarrier for delivery of neurogenic microRNA. Biomaterials 2011, 32, 4968–4975.
15. Kaplitt, M.G.; Feigin, A.; Tang, C.; Fitzsimons, H.L.; Mattis, P.; A Lawlor, P.; Bland, R.J.; Young, D.; Strybing, K.; Eidelberg, D.; et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: An open label, phase I trial. Lancet 2007, 369, 2097–2105.
16. LeWitt, P.A.; Rezai, A.R.; Leehey, M.A.; Ojemann, S.G.; Flaherty, A.W.; Eskandar, E.N.; Kostyk, S.; Thomas, K.; Sarkar, A.; Siddiqui, M.S.; et al. AAV2-GAD gene therapy for advanced Parkinson's disease: A double-blind, sham-surgery controlled, randomised trial. Lancet Neurol. 2011, 10, 309–319.
17. Niethammer, M.; Tang, C.C.; LeWitt, P.A.; Rezai, A.R.; Leehey, M.A.; Ojemann, S.G.; Flaherty, A.W.; Eskandar, E.N.; Kostyk, S.K.; Sarkar, A.; et al. Long-term follow-up of a randomized AAV2-GAD gene therapy trial for Parkinson's disease. JCI Insight 2017, 2, e90133.
18. Christine, C.W.; Starr, P.A.; Larson, P.S.; Eberling, J.L.; Jagust, W.J.; Hawkins, R.A.; VanBrocklin, H.F.; Wright, J.F.; Bankiewicz, K.S.; Aminoff, M.J. Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. Neurology 2009, 73, 1662–1669.
19. Christine, C.W.; Bankiewicz, K.S.; Van Laar, A.D.; Richardson, R.M.; Ravina, B.; Kells, A.P.; Boot, B.; Martin, A.J.; Nutt, J.; Ms, M.E.T.; et al. Magnetic resonance imaging–guided phase 1 trial of putaminal AADC gene therapy for Parkinson's disease. Ann. Neurol. 2019, 85, 704–714.
20. Christine, C.W.; Richardson, R.M.; Van Laar, A.D.; Thompson, M.E.; Fine, E.M.; Khwaja, O.S.; Li, C.; Liang, G.S.; Meier, A.; Roberts, E.W.; et al. Safety of AADC Gene Therapy for Moderately Advanced Parkinson Disease. Neurology 2022, 98, e40–e50.
21. McFarthing, K.; Prakash, N.; Simuni, T. Clinical trial highlights: 1. gene therapy for parkinson's, 2. phase 3 study in focus—intec pharma's accordion pill, 3. clinical trials resources. J. Park. Dis. 2019, 9, 251–264.
22. Mittermeyer, G.; Christine, C.W.; Rosenbluth, K.H.; Baker, S.L.; Starr, P.; Larson, P.; Kaplan, P.L.; Forsayeth, J.; Aminoff, M.J.; Bankiewicz, K.S. Long-Term Evaluation of a Phase 1 Study of AADC Gene Therapy for Parkinson's Disease. Hum. Gene Ther. 2012, 23, 377–381.
23. Azzouz, M.; Martin-Rendon, E.; Barber, R.D.; Mitrophanous, K.A.; Carter, E.E.; Rohll, J.B.; Kingsman, S.M.; Kingsman, A.J.; Mazarakis, N.D. Multicistronic Lentiviral Vector-Mediated Striatal Gene Transfer of Aromatic l-Amino Acid Decarboxylase, Tyrosine Hydroxylase, and GTP Cyclohydrolase I Induces Sustained Transgene Expression, Dopamine Production, and Functional Improvement in a Rat Model of Parkinson's Disease. J. Neurosci. 2002, 22, 10302–10312.
24. Palfi, S.; Gurruchaga, J.M.; Ralph, G.S.; Lepetit, H.; Lavisse, S.; Buttery, P.C.; Watts, C.; Miskin, J.; Kelleher, M.; Deeley, S.; et al. 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 2014, 383, 1138–1146.
25. Marks, W.J.; Ostrem, J.L.; Verhagen, L.; A Starr, P.; Larson, P.S.; Bakay, R.A.; Taylor, R.; Cahn-Weiner, D.A.; Stoessl, A.J.; Olanow, C.W.; et al. 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. 2008, 7, 400–408.
26. Baron, M.S.; Wichmann, T.; Ma, D.; Delong, M.R. Effects of Transient Focal Inactivation of the Basal Ganglia in Parkinsonian Primates. J. Neurosci. 2002, 22, 592–599.
27. Emborg, M.E.; Carbon, M.; Holden, J.E.; During, M.J.; Ma, Y.; Tang, C.; Moirano, J.M.; Fitzsimons, H.; Roitberg, B.Z.; Tüccar, E.; et al. Subthalamic Glutamic Acid Decarboxylase Gene Therapy: Changes in Motor Function and Cortical Metabolism. J. Cereb. Blood Flow Metab. 2007, 27, 501–509.
28. Luo, J.; Kaplitt, M.G.; Fitzsimons, H.L.; Zuzga, D.S.; Liu, Y.; Oshinsky, M.L.; During, M.J. Subthalamic GAD Gene Therapy in a Parkinson's Disease Rat Model. Science 2002, 298, 425–429.

29. Leff, S.; Spratt, S.; Snyder, R.; Mandel, R. Long-term restoration of striatal l-aromatic amino acid decarboxylase activity using recombinant adeno-associated viral vector gene transfer in a rodent model of Parkinson's disease. Neuroscience 1999, 92, 185–196.

30. Bankiewicz, K.S.; Eberling, J.L.; Kohutnicka, M.; Jagust, W.; Pivirotto, P.; Bringas, J.; Cunningham, J.; Budinger, T.F.; Harvey-White, J. Convection-Enhanced Delivery of AAV Vector in Parkinsonian Monkeys; In Vivo Detection of Gene Expression and Restoration of Dopaminergic Function Using Pro-drug Approach. Exp. Neurol. 2000, 164, 2–14.

31. Bankiewicz, K.S.; Forsayeth, J.; Eberling, J.L.; Sanchez-Pernaute, R.; Pivirotto, P.; Bringas, J.; Herscovitch, P.; Carson, R.E.; Eckelman, W.; Reutter, B.; et al. Long-Term Clinical Improvement in MPTP-Lesioned Primates after Gene Therapy with AAV-hAADC. Mol. Ther. 2006, 14, 564–570.

32. Muramatsu, S.-I.; Fujimoto, K.-I.; Kato, S.; Mizukami, H.; Asari, S.; Ikeguchi, K.; Kawakami, T.; Urabe, M.; Kume, A.; Sato, T.; et al. A Phase I Study of Aromatic L-Amino Acid Decarboxylase Gene Therapy for Parkinson's Disease. Mol. Ther. 2010, 18, 1731–1735.

33. Sebastian, W.S.; Richardson, R.M.; Kells, A.P.; Lamarre, C.; Bringas, J.; Pivirotto, P.; Salegio, E.A.; DeArmond, S.J.; Forsayeth, J.; Bankiewicz, K.S. Safety and Tolerability of Magnetic Resonance Imaging-Guided Convection-Enhanced Delivery of AAV2-hAADC with a Novel Delivery Platform in Nonhuman Primate Striatum. Hum. Gene Ther. 2012, 23, 210–217.

34. Collier, T.J.; Sortwell, C.E. Therapeutic potential of nerve growth factors in Parkinson's disease. Drugs Aging 1999, 14, 261–287.

35. Marks, W.J.; Bartus, R.T.; Siffert, J.; Davis, C.S.; Lozano, A.; Boulis, N.; Vitek, J.; Stacy, M.; Turner, D.; Verhagen, L.; et al. Gene delivery of AAV2-neurturin for Parkinson's disease: A double-blind, randomised, controlled trial. Lancet Neurol. 2010, 9, 1164–1172.

36. Bartus, R.T.; Herzog, C.D.; Chu, Y.; Wilson, A.; Brown, L.; Siffert, J.; Johnson, E.M., Jr.; Olanow, C.W.; Mufson, E.J.; Kordower, J.H. Bioactivity of AAV2-neurturin gene therapy (CERE-120): Differences between Parkinson's disease and nonhuman primate brains. Mov. Disord. 2010, 26, 27–36.

37. Bartus, R.T.; Baumann, T.L.; Siffert, J.; Herzog, C.D.; Alterman, R.; Boulis, N.; Turner, D.A.; Stacy, M.; Lang, A.E.; Lozano, A.M.; et al. Safety/feasibility of targeting the substantia nigra with AAV2-neurturin in Parkinson patients. Neurology 2013, 80, 1698–1701.

38. Georgievska, B. Aberrant Sprouting and Downregulation of Tyrosine Hydroxylase in Lesioned Nigrostriatal Dopamine Neurons Induced by Long-Lasting Overexpression of Glial Cell Line Derived Neurotrophic Factor in the Striatum by Lentiviral Gene Transfer. Exp. Neurol. 2002, 177, 461–474.

39. Rosenblad, C.; Georgievska, B.; Kirik, D. Long-term striatal overexpression of GDNF selectively downregulates tyrosine hydroxylase in the intact nigrostriatal dopamine system. Eur. J. Neurosci. 2003, 17, 260–270.

40. Airaksinen, M.S.; Saarma, M. The GDNF family: Signalling, biological functions and therapeutic value. Nat. Rev. Neurosci. 2002, 3, 383–394.

41. Chen, Y.; Harvey, B.K.; Hoffman, A.; Wang, Y.; Chiang, Y.; Lupica, C. MPTP-induced deficits in striatal synaptic plasticity are prevented by glial cell line-derived neurotrophic factor expressed via an adeno-associated viral vector. FASEB J. 2007, 22, 261–275.

42. Su, X.; Kells, A.P.; Huang, E.; Lee, H.S.; Hadaczek, P.; Beyer, J.; Bringas, J.; Pivirotto, P.; Penticuff, J.; Eberling, J.; et al. Safety Evaluation of AAV2-GDNF Gene Transfer into the Dopaminergic Nigrostriatal Pathway in Aged and Parkinsonian Rhesus Monkeys. Hum. Gene Ther. 2009, 20, 1627–1640.

43. Quintino, L.; Manfré, G.; Wettergren, E.E.; Namislo, A.; Isaksson, C.; Lundberg, C. Functional Neuroprotection and Efficient Regulation of GDNF Using Destabilizing Domains in a Rodent Model of Parkinson's Disease. Mol. Ther. 2013, 21, 2169–2180.

44. Iwamoto, M.; Björklund, T.; Lundberg, C.; Kirik, D.; Wandless, T.J. A General Chemical Method to Regulate Protein Stability in the Mammalian Central Nervous System. Chem. Biol. 2010, 17, 981–988.

45. Decressac, M.; Kadkhodaei, B.; Mattsson, B.; Laguna, A.; Perlmann, T.; Björklund, A. α-Synuclein–Induced Down-Regulation of Nurr1 Disrupts GDNF Signaling in Nigral Dopamine Neurons. Sci. Transl. Med. 2012, 4, 163ra156.

46. Zhou, H.; Su, J.; Hu, X.; Zhou, C.; Li, H.; Chen, Z.; Xiao, Q.; Wang, B.; Wu, W.; Sun, Y.; et al. Glia-to-Neuron Conversion by CRISPR-CasRx Alleviates Symptoms of Neurological Disease in Mice. Cell 2020, 181, 590–603.e16.

47. Qian, H.; Kang, X.; Hu, J.; Zhang, D.; Liang, Z.; Meng, F.; Zhang, X.; Xue, Y.; Maimon, R.; Dowdy, S.F.; et al. Reversing a model of Parkinson's disease with in situ converted nigral neurons. Nature 2020, 582, 550–556.

48. Blauwendraat, C.; Nalls, M.A.; Singleton, A.B. The genetic architecture of Parkinson's disease. Lancet Neurol. 2020, 19, 170–178.

49. Maraganore, D.M.; De Andrade, M.; Elbaz, A.; Farrer, M.J.; Ioannidis, J.P.; Krüger, R.; Rocca, W.A.; Schneider, N.K.; Lesnick, T.G.; Lincoln, S.J.; et al. Collaborative Analysis of α-Synuclein Gene Promoter Variability and Parkinson Disease. JAMA 2006, 296, 661–670.

50. Lee, V.M.-Y.; Trojanowski, J.Q. Mechanisms of Parkinson's Disease Linked to Pathological α-Synuclein: New Targets for Drug Discovery. Neuron 2006, 52, 33–38.

51. Tofaris, G.; Spillantini, M.G. Physiological and pathological properties of α-synuclein. Experientia 2007, 64, 2194–2201.

52. Zharikov, A.D.; Cannon, J.R.; Tapias, V.; Bai, Q.; Horowitz, M.P.; Shah, V.; El Ayadi, A.; Hastings, T.G.; Greenamyre, J.T.; Burton, E. shRNA targeting alpha-synuclein prevents neurodegeneration in a Parkinson's disease model. J. Clin. Investig. 2015, 125, 2721–2735.

53. Uehara, T.; Choong, C.-J.; Nakamori, M.; Hayakawa, H.; Nishiyama, K.; Kasahara, Y.; Baba, K.; Nagata, T.; Yokota, T.; Tsuda, H.; et al. Amido-bridged nucleic acid (AmNA)-modified antisense oligonucleotides targeting α-synuclein as a novel therapy for Parkinson's disease. Sci. Rep. 2019, 9, 7567.

54. Cole, T.A.; Zhao, H.; Collier, T.J.; Sandoval, I.; Sortwell, C.E.; Steece-Collier, K.; Daley, B.F.; Booms, A.; Lipton, J.; Welch, M.; et al. α-Synuclein antisense oligonucleotides as a disease-modifying therapy for Parkinson's disease. JCI Insight 2021, 6, e135633.

55. Kantor, B.; Tagliafierro, L.; Gu, J.; Zamora, M.E.; Ilich, E.; Grenier, C.; Huang, Z.Y.; Murphy, S.; Chiba-Falek, O. Downregulation of SNCA Expression by Targeted Editing of DNA Methylation: A Potential Strategy for Precision Therapy in PD. Mol. Ther. 2018, 26, 2638–2649.

56. Gorbatyuk, O.S.; Li, S.; Nash, K.; Gorbatyuk, M.; Lewin, A.S.; Sullivan, L.F.; Mandel, R.J.; Chen, W.; Meyers, C.; Manfredsson, F.P.; et al. In Vivo RNAi-Mediated alpha-Synuclein Silencing Induces Nigrostriatal Degeneration. Mol. Ther. 2010, 18, 1450–1457.

57. Benskey, M.J.; Sellnow, R.C.; Sandoval, I.M.; Sortwell, C.E.; Lipton, J.W.; Manfredsson, F.P. Silencing Alpha Synuclein in Mature Nigral Neurons Results in Rapid Neuroinflammation and Subsequent Toxicity. Front. Mol. Neurosci. 2018, 11, 36.

58. Koo, H.-J.; Choi, M.Y.; Im, H. Aggregation-defective α-synuclein mutants inhibit the fibrillation of Parkinson's disease-linked α-synuclein variants. Biochem. Biophys. Res. Commun. 2009, 386, 165–169.

59. Heman-Ackah, S.M.; Bassett, A.R.; Wood, M.J.A. Precision Modulation of Neurodegenerative Disease-Related Gene Expression in Human iPSC-Derived Neurons. Sci. Rep. 2016, 6, 28420.

60. Gandhi, P.N.; Chen, S.G.; Wilson-Delfosse, A.L. Leucine-rich repeat kinase 2 (LRRK2): A key player in the pathogenesis of Parkinson's disease. J. Neurosci. Res. 2009, 87, 1283–1295.

61. Ness, D.; Ren, Z.; Gardai, S.; Sharpnack, D.; Johnson, V.J.; Brennan, R.J.; Brigham, E.F.; Olaharski, A.J. Leucine-Rich Repeat Kinase 2 (LRRK2)-Deficient Rats Exhibit Renal Tubule Injury and Perturbations in Metabolic and Immunological Homeostasis. PLoS ONE 2013, 8, e66164.

62. Fuji, R.N.; Flagella, M.; Baca, M.; Baptista, M.A.S.; Brodbeck, J.; Chan, B.K.; Fiske, B.K.; Honigberg, L.; Jubb, A.M.; Katavolos, P.; et al. Effect of selective LRRK2 kinase inhibition on nonhuman primate lung. Sci. Transl. Med. 2015, 7, 273ra15.

63. Zhao, H.T.; John, N.; Delic, V.; Ikeda-Lee, K.; Kim, A.; Weihofen, A.; Swayze, E.; Kordasiewicz, H.B.; West, A.; Volpicelli-Daley, L.A. LRRK2 Antisense Oligonucleotides Ameliorate α-Synuclein Inclusion Formation in a Parkinson's Disease Mouse Model. Mol. Ther.-Nucleic Acids 2017, 8, 508–519.

64. Clark, I.E.; Dodson, M.W.; Jiang, C.; Cao, J.H.; Huh, J.R.; Seol, J.H.; Yoo, S.J.; Hay, B.A.; Guo, M. Drosophila pink1 is required for mitochondrial function and interacts genetically with parkin. Nature 2006, 441, 1162–1166.

65. Park, J.; Lee, S.B.; Lee, S.; Kim, Y.; Song, S.; Kim, S.; Bae, E.; Kim, J.; Shong, M.; Kim, J.-M.; et al. Mitochondrial dysfunction in Drosophila PINK1 mutants is complemented by parkin. Nature 2006, 441, 1157–1161.

66. Narendra, D.; Tanaka, A.; Suen, D.-F.; Youle, R.J. Parkin is recruited selectively to impaired mitochondria and promotes their autophagy. J. Cell Biol. 2008, 183, 795–803.

67. Sidransky, E.; Nalls, M.A.; Aasly, J.O.; Aharon-Peretz, J.; Annesi, G.; Barbosa, E.R.; Bar-Shira, A.; Berg, D.; Bras, J.; Brice, A.; et al. Multicenter Analysis of Glucocerebrosidase Mutations in Parkinson's Disease. N. Engl. J. Med. 2009, 361, 1651–1661.

68. Sardi, S.P.; Clarke, J.; Viel, C.; Chan, M.; Tamsett, T.J.; Treleaven, C.M.; Bu, J.; Sweet, L.; Passini, M.A.; Dodge, J.C.; et al. Augmenting CNS glucocerebrosidase activity as a therapeutic strategy for parkinsonism and other Gaucher-related synucleinopathies. Proc. Natl. Acad. Sci. USA 2013, 110, 3537–3542.

69. Morabito, G.; Giannelli, S.G.; Ordazzo, G.; Bido, S.; Castoldi, V.; Indrigo, M.T.; Cabassi, T.; Cattaneo, S.; Luoni, M.; Cancellieri, C.; et al. AAV-PHP.B-Mediated Global-Scale Expression in the Mouse Nervous System Enables GBA1 Gene Therapy for Wide Protection from Synucleinopathy. Mol. Ther. 2017, 25, 2727–2742.