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Cellular and Molecular Aspects of Parkinson Treatment: Future Therapeutic Perspectives

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Abstract

Parkinson's disease is a neurodegenerative disorder accompanied by depletion of dopamine and loss of dopaminergic neurons in the brain that is believed to be responsible for the motor and non-motor symptoms in this disease. The main drug prescribed for Parkinsonian patients is L-dopa, which can be converted to dopamine by passing through the blood-brain barrier. Although L-dopa is able to improve motor function and improve the quality of life in the patients, there is inter-individual variability and some patients do not achieve the therapeutic effect. Variations in treatment response and side effects of current drugs have convinced scientists to think of treating Parkinson's disease at the cellular and molecular level. Molecular and cellular therapy for Parkinson's disease include (i) cell transplantation therapy with human embryonic stem (ES) cells, human induced pluripotent stem (iPS) cells and human fetal mesencephalic tissue, (ii) immunological and inflammatory therapy which is done using antibodies, and (iii) gene therapy with AADC-TH-GCH gene therapy, viral vector-mediated gene delivery, RNA interference-based therapy, CRISPR-Cas9 gene editing system, and alternative methods such as optogenetics and chemogenetics. Although these methods currently have a series of challenges, they seem to be promising techniques for Parkinson's treatment in future. In this study, these prospective therapeutic approaches are reviewed.

Keywords Parkinson's disease · Transplantation therapy · Molecular mechanisms · L-dopa · Gene therapy

Key Message The new therapeutic approaches described in this study include cell transplantation, gene therapy and immunotherapy, and are promising strategies for effective PD therapy in the near horizon.

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Introduction

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder that occurs in both genders and in all ethnic groups throughout the world [1, 2]. PD is the second most common neurodegenerative disease after Alzheimer's disease [3–5]. The incidence of PD has been reported to be around 2% among people over 50 years of age and 4% among the population older than age 85. The onset of PD can be family or sporadic, early or delayed, with or without symptoms [1, 6–8]. The symptoms of PD usually occur in five steps including early, primary motor, secondary motor, and primary and secondary non-motor symptoms [9]. These symptoms are accompanied by motor disorders such as muscle stiffness, tremor, bradykinesia, postural instability, soft speech, slow handwriting, lack of limb movement, sleep problems, and decreased associated movements such as body abnormal movements, and changes in facial expressions when talking (Table 1) [10–12].

In PD patients, 50–70% of dopaminergic neurons in the substantia nigra are destroyed and there is an aggregation of alpha-synuclein in Lewy bodies (LBs), which are abnormal protein-rich aggregates in the remaining neurons [13, 14]. Also, there are cytoplasmic inclusions in neurons of specific brain regions including the cortical and brainstem [15, 16]. Hence, the main features of PD are depletion of dopamine and loss of dopaminergic neurons that are believed to be responsible for the motor and non-motor symptoms in PD [17, 18]. Factors or mechanisms involved in PD are associated with mitochondrial dysfunction, oxidative stress, and activation of apoptotic pathways, which ultimately lead to degeneration of dopaminergic neuromuscular diseases [19–22]. These factors include increased age, environmental factors, and genetic factors (Fig. 1) [3, 23, 24].

In total, 15% of PD patients have family history but only 5–10% of cases have a monogenic form of PD with Mendelian pattern [25, 26]. Hence, the majority of PD patients are sporadic and the etiology of the disease is unknown and these forms are usually created by a combination of genetic and environmental factors. So far, 23 gene loci and 19 genes have been identified for PD. Of these, 10 genes are responsible for PD with dominant autosomal forms and 9 genes are responsible for autosomal recessive forms [27].

A successful PD therapy should target motor function, autonomic function, cognitive and communicative skills, and psychiatric symptoms, and support self-sustainment in the activities of daily living and disability and the need of support. Also, PD therapy should be focused to preserve independence and social competence in patients and they should earn the capability to work and or regain health-related quality of life [18].

Currently, drug treatment is the main strategy used to control the symptoms of PD to enhance the level of dopamine in the brain but this approach in all patients has not had the same response and also some patients may not be treated effectively. Furthermore, it has been demonstrated that this drug therapy may have many side effects in patients [28, 29]. Therefore, the diversity of treatment response and drug side effects have led scientists to treat PD at the molecular and cellular levels. In this review, we aimed to discuss therapeutic aspects of PD treatment but our main focus is on new promising aspects and prospective therapeutic approaches of Parkinson treatment at the molecular and cellular levels.

Current Therapies for Parkinson's Disease

Unfortunately, there are currently no effective therapies for PD and available treatments to slow down the progression of the disease include drug treatment and surgery [30–32]. Deep brain stimulation (DBS), a surgical treatment for PD accomplished by implanting an electrode into the targeted brain area, can mimic the effect of a lesion without the need for destroying brain tissue [33]. Dopaminergic drugs such as L-DOPA is the most commonly used drug and it is very effective in reducing the resting-tremors and other primary symptoms, but it is unable to stop further progression of PD [34]. L-dopa can be converted to dopamine by passing through the blood-brain barrier and compensate the shortage of dopamine [35–37]. This drug is able to improve motor function and improve the quality of life in these patients, but in all patients the same response is not received and some patients do not achieve the therapeutic effect [28, 29]. Additionally, it has been shown that administration of L-

Table 1 Symptoms of Parkinson's disease

Early symptoms	Primary motor	Secondary motor	Primary non-motor	Secondary non-motor
Mild tremors, soft speech, slow handwriting, movement problem, unusual face, loss of focus in thought and speed, fatigue, irritability, depression, and posture difficulty	Resting Tremor, Rigidity, slow movement, bradykinesia, and postural instability	Difficulties in swallowing and chewing, sexual dysfunction, dystonia, and muscle cramps	Depression, cognitive dysfunction, dementia, insomnia, pain, and fatigue	Sweating, urinary problem, hypotension, and emotional changes

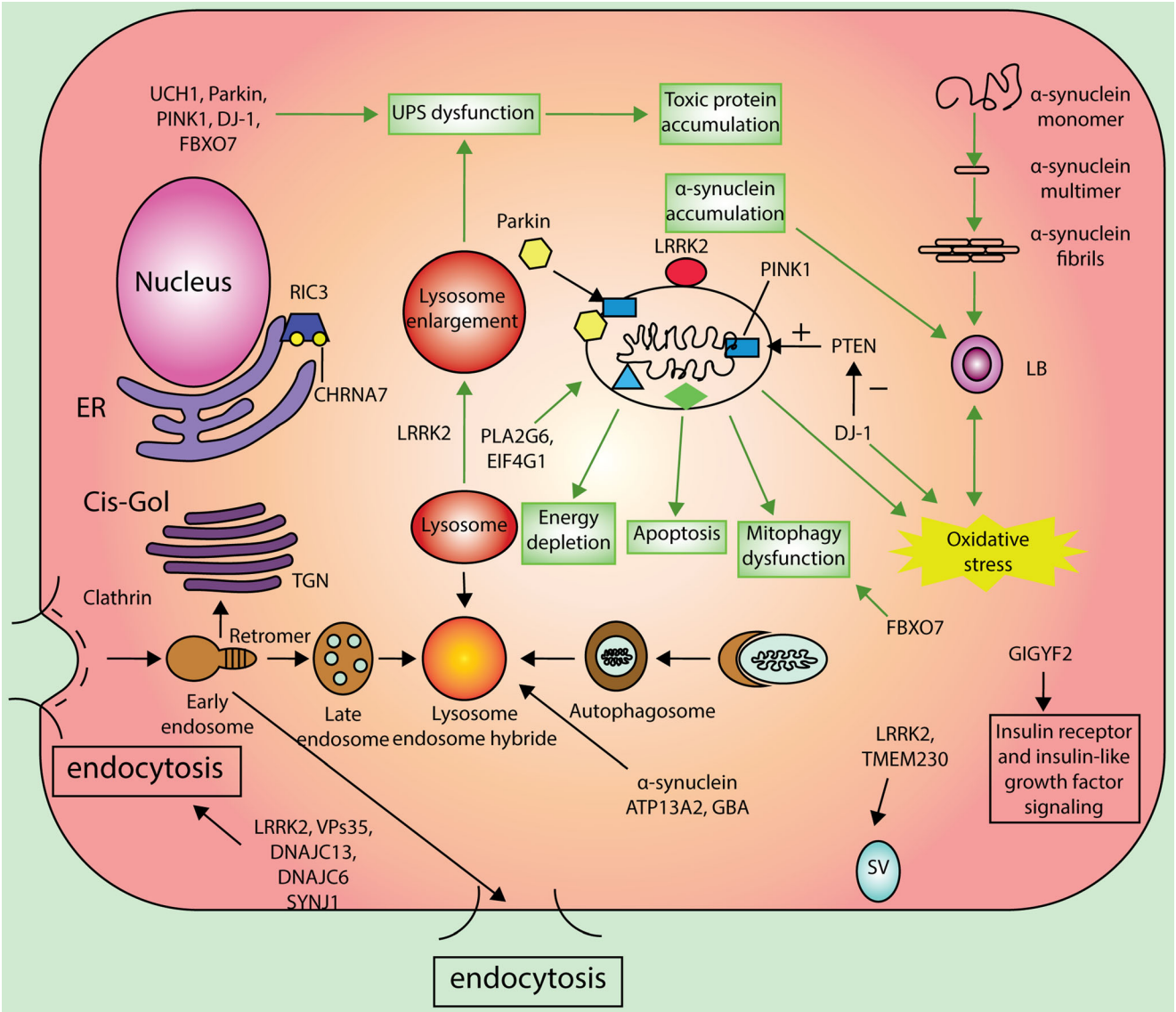

Fig. 1 Main molecular pathways related to PD pathogenesis. Abbreviations: UPS, ubiquitin-proteasome system; LB, Lewy body; TGN, trans-Golgi network; SV, synaptic vesicle; ER, endoplasmic reticulum; UPSCHRNA7, neuronal nicotinic acetylcholine receptor subunit α-7

dopa with other auxiliary drugs or combination therapy has some side effects on patients [28, 38]. These some side effects are including low blood pressure, nausea, restlessness, dyskinesia, drowsiness, and vomiting and when it reaches to the targeted area, its power and efficiency is reduced, although it can be used with other medications to prevent this defect. COMT inhibitors, MAO-B inhibitors, dopamine agonists, and anticholinergic drugs are other dopaminergic drug used for PD [9, 39]. Drug treatments available for PD until now are of symptomatic nature and currently, there is no therapy available that reduces the progression of Parkinson's disease or even to prevent its manifestation [18].Considering the incomplete efficacy of these drugs and their side effects, we will continue to discuss non-drug therapeutic options for PD.

Natural Products

Some natural products and herbs have proved to be effective and more reliable than the usual synthetic drugs for PD treatment [40–46]. These natural products have some anti-PD properties such as anti-inflammatory, anti-oxidative, and protective effects on protein misfolding, iron accumulation, maintenance of proteasome degradation, and mitochondrial homeostasis [47, 48].

Until now, approximately 37 natural products have been discovered to have significant anti-PD effects including flavonoid and polyphenol compounds, phenylpropanoid compounds, quinone compounds, saponin compounds, alkaloid compounds, and terpenoid compounds that are summarized in Table 2 [49, 50]. However, many of these natural products

outlined in Table 2 could not be directly used as drug for treatment of PD until compelling evidence from randomized controlled trials are available.

Cell Transplantation Therapy

In 1985, implantation of autologous adrenal medulla cells into striatum of PD patients was tested in a clinical trial to produce a local catecholamine source [51]. However, this method was abandoned because of lack of efficacy and adverse effects [52]. After 1985, some studies were done in this area but next studies showed no significant changes compared with sham-operated controls and some side effects such as dyskinesia were observed [53]. Therefore, the studies on PD treatment with cell therapy were stopped for a decade but at the moment, clinical cell therapy for PD has already entered a new phase. The main cause of Parkinson's disease underlying motor symptoms is the destruction of the nigrostriatal dopaminergic system [51, 52]. Hence, transplantation of neuronal stem cells into the brain of Parkinson's patients to produce dopamine-producing cells can be a good idea to treat PD. (i) Human embryonic stem (ES) cells, (ii) human induced pluripotent stem (iPS) cells, and (iii) human fetal mesencephalic tissue are three types of cells that can be used for this purpose [54, 55].

Human Embryonic Stem (ES) Cells

Human embryonic stem cells (hESCs) that are obtained from the inner cell mass of the blastocyst have some properties such as their unlimited self-renewal capacity and the potential to differentiate into specialized cells of the three germ layers [56]. Hence, hESCs are optimal cell source for cell-replacement therapies [56]. Some studies about cell therapy are summarized below. By manipulating several growth factors in human embryonic stem (ES) cells such as FGF8, FGF-2b, and SHH, researchers have been able to produce dopaminergic neurons from rodent embryonic stem cells for transplantation with a density of 160,000 viable cells per milliliter into the striata of animal models of PD. This strategy has not only been associated with the improvement of behavioral defects and graft survival in experimental models of PD, but also contained potentially tumourigenic and mitotic undifferentiated neuroepithelial cells [55, 57]. Kriks and co-workers described a protocol which efficiently ((for differentiated hESCs, 2 3 1 ml deposits of 75,000 cells/ml (total of 150,000 cells) were transplanted for d10, or for d16: 2 3 2 ml deposits of 75,000 cells/ml (total of 300,000 cells)) converts human ES cells to dopaminergic neurons and by inhibition SMAD signaling and high levels of Sonic Hedgehog floor plate cells are derived in vitro and by activation of Wnt signaling dopaminergic precursors are produced and the cells then were grafted to the intrastriatal rodents, resulting in a large number of dopaminergic neurons with a long survival and no tumor [58]. Human ES cells were able to produce a large number of dopaminergic neurons. Other advantages include long-term survival and improved behavioral deficits [58]. Additionally, by overexpressing nuclear receptor related 1 protein (NURR1), which is a transcription factor related to development of dopaminergic neurons in ES cells, it is possible to produce more dopamine for transplant into the brains of PD animals [59–62]. Conversion of human ES cells to dopaminergic neurons through alteration of signaling pathways in some animal models (rat, rodent, mice, monkey) has been efficiently described by Kriks and colleagues in 2011 [63]. When researchers implanted porcine-derived DA-producing cells in the brain of a PD patient, they observed an adequate clinical improvement, which suggests that dopamine-producing xenografts can survive in the human brain [64]. The authors also grafted human fetal-derived dopaminergic tissues into the striatum of the PD patients and observed an increasing level of dopamine in brain of PD patients [64]. Another study has been done by Grealish et al. and observed that grafts of human embryonic stem cell-derived dopaminergic neurons implanted in a rat for Parkinson's disease have the capacity for long-term survival and axonal growth similar to that of human fetal mesencephalic dopaminergic neuron [65]. Stem-cell therapy with ES cells has many disadvantages such as purity of the material injected and risk of brain tumors and

Table 2 Natural products with anti-PD properties

Class	Name of products
Flavonoids	Baicalein, quercetin, puerarin, daidzein, genistein, hyperoside, naringin, and curcumin
Polyphenols	Epigallocatechin gallate (EGCG), resveratrol, danshensu, salvianolic acid B, eleutheroside B, magnolol, fraxetin, and esculin
Coumarins	Umbelliferone
Quinones	2-methoxy-6-acetyl-7-methyljuglone(MAM), thymoquinone, and alaternin
Triterpenoid saponins	Astragaloside
Steroidal saponins	Ginsenoside Rb1, ginsenoside Rd., ginsenoside Re, ginsenoside Rg1, notoginsenoside Rg1, and panaxatriol saponin
Alkaloids	Ligustrazine, nicotine, isorhynchophylline (IsoRhy), and L-stepholidine
Terpenoids	Triptolide, ginkgolide B, catalpol, paeoniflorin, isoborneol, and 10-O-trans-p-Coumaroylcatalpol (OCC)

this technology has some unsolved issues such as immune reactions, ethical problems, and for a useful treatment for PD in clinic requires more studies. Also, potency of the generated dopaminergic neurons after transplantation in animal models, finding suitable patient for clinical trials, control of cell growth, and phenotype instability are another challenges [54, 66].

Cell Reprograming

Sir John Gurdon in the early 1960s for the first time showed that cell reprograming has multipotency of somatic cells by producing tadpoles from nuclei isolated from the frog intestine [67]. A few decades later, this topic became very novel, when it was linked to the cloning of Dolly the sheep by Shinya Yamanaka and causing a huge revolution in this area [68, 69].

Reprograming cells, also called iPSCs cells, generated by reprogramming fibroblasts through a pluripotent stage can also be used to produce dopamine in the brain and is based on transforming an existing cell transcription program to match multiple cells that can later be split into specific cells. Therefore, a one somatic cell type can be directly reprogrammed into another specialized cell, such as neurons, which are called induced neurons (iNs) [70, 71]. Few, but promising, studies have been done in iPS cells area. Recently, Hallett et al. reported that dopaminergic neurons derived from autologous iPS cells, which were transmitted to the stratum of a non-human primate, have survived for 2 years and lead to motor progression in monkey [72]. In this technology, there are some advantages and disadvantages that are presented here. Patient-specific cells can be acquired from skin biopsies without immune reactions and ethical issues associated with human embryonic stem cells [73–75]. Also, iPSCs seem to carry a differentiation potential similar to embryonic stem cells (ESCs), and therefore can be used for producing authentic and functional dopamine neurons [63, 76]. Another benefit of iPSCs is the ability to generate from any individual for transplantation which eliminates the problem of rejection by the antigens of the HLA system because previous studies have shown that MHC matching enhances cell viability and function of iPSC-derived dopamine neurons in non-human primates [76, 77]. iNs have another advantage that direct reprogramming avoids the pluripotent stage and directly produces postmitotic cells and as a result, the risk of proliferating cells which could develop tumors become minimized [78].

One of the most potential problem in this method is to set up reprograming. Also, the safety issue about this method are tumorigenesis and increased risk for susceptibility to the pathological process [66]. These findings provide evidences for supporting further clinical translation of iPS cell-derived dopaminergic neurons for transplantation in Parkinson's disease but before such cells can be used as a source of therapeutic neurons in clinics, it is necessary to solve their problems of efficiency and safety in preclinical researches and it is needed to ensure proper reprograming and not showing any disease-related pathology.

Human Fetal Mesencephalic Tissue

Studies about transplantation of human fetal mesencephalic tissue started three decades ago. These studies provided some valuable insights for PD therapy. Many studies have shown that fetal dopaminergic neurons can survive and grow after an intrastriatal transplant in the PD patient's brain and obtained some clinical benefits in allogeneic human fetal ventral mesencephalic (FVM) tissue transplantation in PD patients [54, 79]. These cells survive, and by creating a synaptic relationship, increase dopamine within the host cell.

Allografts of human fetal ventral mesencephalic (VM) tissue is currently the most effective cell for cell therapy in Parkinson's patients. These cells contain developing midbrain dopamine and their precursors [80]. Some successful open-lab trials exist in this area that reported improved motor symptoms in some PD patients [81–83]. Also, F–DOPA uptake improved and these trials showed long-term graft survival by postmortem analysis for over a decade and it was seen that the transplanted tissue become functionally integrated into the host circuitry although some placebo-controlled studies showed only single profit the first endpoint [84–86]. In some patients, dyskinesia was observed even in the absence of L-dopa drugs, although the long-term graft survival evaluation in some populations confirmed the open-label trial findings [87, 88]. Tissue preparation, patient selection, primary endpoints, immunosuppression procedures, and general trial design are some reasons for variable results [89–91]. Also, high quality dopamine-cells have been used for clinical or preclinical testing as an option therapy for Parkinson's disease, but transplantation of human fetal mesencephalic tissues has not yet become a cure for PD treatment [54]. The main challenge for this type of cell therapy is ethical issues raised by the use of fetal tissue. Immunological and inflammatory response of the host, poor standardization of the cell material processing and tissue dissection, and safety and efficacy of stem cell therapy in this strategy are another issues and must be solved [54, 89–91].

Immunological and Anti-Inflammatory Therapy in PD

In the brain of PD patient, the presence of activated microglia has been shown which suggests an immunological and inflammatory mechanism. Besides, there is some evidence for the role of gut microbiota and gastrointestinal tract in the immunological pathogenesis of PD by breaking down the food

and producing short-chain fatty acids that modulate the brain's microglia and promotion of synuclein pathology [92, 93]. However, although there is no consensus on whether inflammatory changes occur in primary or secondary patients, there is evidence that the inflammatory changes represent immune reactions to α-synuclein [94, 95]. Immunogenic epitopes in α-synuclein can produce antibodies against α-synuclein and might be a good therapeutic target for immunization [96, 97]. Previously. Sulzer and colleagues found that in patient with PD T cells identify certain peptides derived from α-synuclein as antigenic epitopes that they are located near the C-terminus and N-terminus of α-synuclein [96]. A number of studies have shown that about 40% of Parkinson patient had reactivity against α-synuclein, but it is not clear whether the immune system of patients with PD leads to nerve damage or immune cells that are activated against α-synuclein are deleterious or the accumulation of α-synuclein causes immunogenic proteins. These results, despite the charm, need to be repeated in a larger group of patients with Parkinson's disease, especially those at an early stage of Parkinson's disease that are not treated with any drug. Also, in cases where it is not yet clear whether the active immune cells against α-synuclein in the brain or blood of Parkinson's patients are harmful or beneficial and maybe aggregation α-synuclein is a reason for immunologic protein [98, 99]. Also, a series of evidence has been obtained with genetic methods such as genome-wide association studies that confirmed the role of autoimmune mechanisms in pathogenesis of Parkinson's disease. These studies found strong association between HLA-major histocompatibility complex locus and PD. For example, a study that were done on 138,511 individuals of European ancestry a study conducted on the ancestors of Europeans identified 17 novel loci with an overlap between PD loci and some autoimmune diseases [100]. Hence, by vaccination or by monoclonal therapy, antigenic epitopes of α-synuclein can be a good option for target therapy [101–103]. For example, 9E4 is a monoclonal antibody against C-terminus epitope of α-synuclein, which is in preclinical phase and can reduce accumulation α-synuclein and improves motor and behavioral deficits in mouse models. PRX002/RG7935 (Prothena/Roche) and MEDI1341 (MedImmune/Astra Zeneca) are another antibodies with the ability to target C-terminus [103, 104]. Some preclinical studies for these antibodies are present. For PRX002/RG7935. a > 400-fold higher avidity/affinity of PRX002 for aggregated versus monomeric α-synuclein and penetration into the CNS in preclinical studies has been confirmed and in a phase IA, a dose of up to 30 mg/kg is safe [102, 104, 105]. In a phase IB, 80 patients received PRX002/RG7935 in different doses (0.3, 1.0, 3.0, 10, 30, or 60 mg/kg) for a 3-month period and followed up for 24 week. In this study, PRX002/RG7935 was well tolerated and no harmful reaction was observed except in 7% of patients with some side effects such as headache, diarrhea, and peripheral edema [106]. Also, BIIB054 (Biogen) and chBIIB054 (Biogen) are another monoclonal antibodies against N-terminus region of α-synuclein. There are no other published studies on this monoclonal antibodies but in a mouse model of expressing preformed fibrils, they reduced motor impairment to more than 50% [105, 107–110]. Vaccination against α-synuclein is a therapeutic strategy in Parkinson's disease and other synucleinopathies and some companies are working in this field. Previous studies have shown that active vaccination with short peptides (AFFITOPEs®) could reduce behavioral and pathological deficits in two transgenic mouse model of synucleinopathies [111]. However, the use of monoclonal antibodies can be effectively used for Parkinson's purposeful treatment and is a promising strategy to favorably modify the progression of Parkinson's disease but more research is needed to further improve the efficacy and safety of these strategies before these methods can be considered in patients with PD. In sum, diagnostic and advanced biomarkers are strongly needed to use these drugs, which can then be used in the early phase of the disease or even in the progressive phase.

Gene Therapy for Parkinson Disease

Gene therapy was first described in 1972 as a mean to "replace bad DNA with good DNA and can be used for diseases treatment at DNA level [112]. The gene therapy targets for PD can be classified as disease and non-disease modifying. Some disease modifying targets with the ability to slowing the progression of Parkinson's disease are including neurturin, GDNF, BDNF, PDGF. CDNF, VEGF-A, and non-disease modifying that are symptomatic and target dopamine or GABA synthesis [113–117]. For these reasons, gene therapy can be a promising strategy for PD treatment. In recent years, several gene therapy methods for PD have entered the clinical trials [9, 118, 119]. Some gene therapy methods for PD include (I) AADC-TH-GCH gene therapy, (ii) viral vectors-mediated gene delivery, (iii) RNA interference-based therapy, and (iv) CRISPR-Cas9 gene editing system [9, 120]. These gene therapy strategies are discussed below. Also, some pre-clinical and clinical studies are summarized.

AADC-TH-GCH Gene Therapy System

In AADC-TH-GCH gene therapy system, aromatic amino acid decarboxylase (AADC), tyrosine hydroxylase (TH), and guanosine triphosphate cyclohydrolase (GTC) are three required enzymes for the conversion of tyrosine to levodopa and subsequently synthesize of dopamine from L-DOPA [121]. In Parkinson's disease, the elimination of dopamine neurons in nigrostriatal leads to impairment in the production and release of dopamine in the striatum [122]. Therefore,

production of a source for dopamine in the striatum can be a good option for this shortage and this idea can be realized by expressing the genes that produce the enzymes necessary for the synthesis of dopamine in non-degenerating striatal medium spiny neurons (MSNs) [123–125]. Some clinical studies have assessed the efficacy and safety of bilateral AAVDDC delivery to the putamen in PD patients. The dopa decarboxylase (DDC) transmission by viral vector was well tolerated and motor complications were generally improved and a follow-up study showed sustained expression of this gene for 4 years [125–127]. Another study was a phase I/II trial and showed efficacy and safety lentivirus vectors for intraputaminal delivery of DDC, TH, and GCH-1 (also known as ProSavin) in moderate to advance for PD patients and ProSavin improved off-medication part III motor Unified PD Rating Scale scores for up to 12 months in all PD patients [128]. Using a lentivirus, which carries three genes TH, GCH, and AADC into the bilateral putamen by evaluating two dose levels, previous report observed that all patients treated in the second dose level of gene therapy have shown improvement in motor function until 34% in comparison to patient's pretreatment motor function. Therefore, delivering of three genes through gene therapy can lead to an increasing level of dopamine in PD patients [129, 130]. There is a challenge that whether medium spiny neurons are the right candidates to be targeted because striatal medium spiny neurons lack the ability to store and release DA in vesicles. On the other hand, continuous and unregulated expression can lead to continuous high cytosolic and extracellular DA levels and can be harmful [131–133]. Altogether, previous studies support this strategy for PD treatment but follow-up of randomized placebo-controlled clinical studies is necessary to support these findings. Safety of viral vectors is another problem that needs to be addressed.

Viral Vector-Mediated Gene Delivery System

In viral vector-mediated gene delivery system, several viral vectors such as recombinant adeno-associated virus (rAAV), lentivirus, non-lentivirus vectors, and adenovirus can carry target genes for injection into the brain of PD patients. This technique is currently being used in animal models and it has been observed that these viral vectors, by integration into host cell genome and downstream of the promoters, are capable of expressing these genes and thus producing dopamine [9, 118]. For example, it was reported that the use of AAV2 for delivery of genes GDNF, BDNF, and NGF can increase dopamine level in striatum and substantia nigra pars compacta because one of the primary reasons for neuronal cell death in Parkinson's disease is lack of neuronal growth factors, such as BDNF, NGF, GDNF, and FGF-2b [134, 135]. Animals that receive expressing GDNF vectors showed a stable and long-term level of GDNF [136]. Also, in 2009, Eberling JL and co-workers showed that when an AAV2 vector containing GDNF were injected into putamen a monkey treated with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; MPTP lesioning consisted of one or two right intracarotid artery infusions of 2.0–4.0 mg of MPTP-HCl followed by additional intravenous administrations of 0.2 to 0.5 mg=kg doses of MPTP-HCl), an enhancement of the locomotor activities and increased the DA-terminals were seen [137]. A placebo-controlled double blind phase II trial was started with 51 patients that were divided into sham ($n = 24$) and surgery ($n = 27$) groups by administering 150 μl and 30 μl glutamic acid decarboxylase gene (GABA) into putamen and substantia nigra, respectively, using an AAV vector. Their results showed an increasing GABA level which can help to balance the firing of the neurons in the PD brain and thereby cause normalization of the inhibitory signal [138]. The problems with using this method are stimulating the immune system that viruses may cause unwanted illness or mutations in genome of host cells [136].

RNA Interference

RNA interference is a natural cellular process that regulates the expression of genes and prevents the entry of viruses and other transposable elements into the genome [139, 140]. RNA interference-based therapy is a powerful approach for gene silencing through administration of small interfering RNAs (siRNAs) which can prevent the expression of genes related to PD such as *SNCA*, *Parkin*, and *PINK* genes [141]. A study conducted by Helmschrodt and co-workers showed that siRNA against α-synuclein (SNCA) was complexed with PEI F25-LMW and injected into the lateral ventricle of a mice overexpressing human wild-type SNCA. In this study, they observed 5 days after injection of 0.75 μg PEI/siRNA, mRNA expression of SNCA in the striatum was decreased until 65% and reduction of SNCA protein until ~ 50%. This study supported the efficacy and safety of PEI nanoparticle-mediated delivery of siRNA to the brain for therapeutic PD. Also, side effects and toxicity symptoms in mice were not observed and they suggested the efficacy and safety of PEI nanoparticle-mediated delivery of siRNA to the brain for PD therapeutic intervention [142]. Recently, treatment of PC12 cells with polyethylene glycol-polyethyleneimine (PEG/PEI) siSNCA complex was reported to significantly decrease SNCA-mRNA expression and prevent MPTP-induced apoptosis. Concentration was 100 mM PEG-PEI/siSNCA and prevented these morphological changes in injured PC12 cells, suggesting a protective effect of PEG-PEI/siSNCA against nuclear damage [143]. Another study used the RNA interference technique help to form a specific biomarker to detect sporadic form of Parkinson's disease and for investigation of the expression changes of *SCNA* gene, where they used skin fibroblast from patients and knock-down *PINK1* gene [144]. This research showed that the expression changes detected from the two cell lines had the potential as a biomarker and

this can be a non-invasive way to help physician to diagnose the PD [144]. This technique opens some new possibilities for genetic screening and it is very efficient and with a great potential. Also, RNAi can target some genes that are connected in sequence. Similarly, other methods of RNAi have some issues such as variable and incomplete knock-down, and resistance to RNAi in some genes. Also, Knocking down protein effectively is very difficult because they have long half-lives [145, 146].

CRISPR-Cas9 Gene Editing System

CRISPR-Cas9 is a gene editing system which has the ability to add, modify or degrade certain sequences of the nucleic acids at the genome level [147–149]. Hence this technique can use for patients with PD. By using this technique Basu and co-workers produced a cell line that can expresses SNCA tagged with a nano-Luc luciferase reporter [150]. Titration of cell counts was between 2500 to 50,000 copies and produced a linear increase in luminescence activity. Their research showed an endogenous monitoring of SNCA transcription, which has the ability to produce an efficient drug screening tool for therapeutic intervention options in Parkinson disease [150]. CRISPR/Cas9 systems challenges include (i) off-target effect, (ii) delivery efficiency of Cas9 into cells or tissue, and (iii) ethical concern related to the use of CRISPR technology in humans [151]. Despite the fact that these therapies appear to be very appealing and promising further studies are needed for their safe applications.

Alternative Types of Gene Therapy

Optogenetics and chemogenetics can be used as alternatives for treating symptoms of PD and in comparison with DBS or ablation they are considered as a more specific intervention [113]. Optogenetics is an emerging technology that uses targeted expression of opsins or G protein coupled receptors that allows precise control of the neuronal activity of specific transfected cell populations [152].

Optogenetics studies of PD have so far been aimed at deconstructing the parkinsonian circuitry and mapping the effects of DBS, although several studies showed optogenetic stimulation to enhance motor behavior in rodents with parkinsonian disorder. Therefore, optogenetic inhibition of the M1 motor cortex or subthalamic nucleus could become a feasible alternative to DBS as this potentially avoids the need for implantation of electrodes or optic cables [153, 154].

Similar to optogenetics, chemogenetics has been widely used to examine neural circuits. Chemogenetics has also the potential to serve as a therapeutic method by itself [155]. In a study by Pienaar and co-workers [156], a rat model with parkinsonian phenotype was treated with the irreversible ubiquitin-proteasome inhibitor lactacystin. In this study, transgenic rats showed an improvement in motor behavior when treated with the otherwise pharmacologically inert clozapine-N-oxide (CNO), potentially suggesting pedunculopontine nucleus (PPN) as a future target for PD therapy using chemogenetics [155]. Opto- and chemogenetics, as compared to gene therapy, may have fewer side effects.

Future Perspectives

If dopaminergic grafts using cell therapy can relieve motor symptoms for more than a decade, cellular therapies for PD can be introduced into the clinic and several therapeutic options exist for PD patients who are at advanced stages of the disease [54]. There are some issues for successful clinical application of cell transplantation in PD patients including limitation on the ability of neurons to produce dopamine, safety, and selecting suitable patients for the first clinical trials with dopaminergic neurons derived from stem cells or reprogrammed cells [54]. Generally, since the last three decades, many efforts have been made in the field of cell therapy, and various methods have been used. Human ES and iPS cell-derived dopaminergic neurons are two types of these strategies that are now being moved towards clinical application if scientists can solve some biosecurity aspects of these cells. The main advantages of these cells are generation of somatic stem cells that are safe against immune rejection. Indeed, some aspects that need to be improved are development of surgical methods for cell transplantation, considering inflammatory and immunological processes on the progression of PD and the implanted cells, patient selection for clinical trials and monitoring, selection types of cells for transplantation, and planning clinical trials.

Immunotherapy against PD is based on targeting α-synuclein and can be very effective but there are a number of potential issues or problems in this area. First, interfering with the physiological function of α-synuclein can be solved with generation of conformation-specific antibodies for the "pathogenic" types of α-synuclein. Second, it is difficult to delivery antibodies to the brain and this problem can be solved with engineering antibodies that can penetrate from blood-brain barrier. Finally, it is necessary to validate efficacy of immunotherapy in non-human primate models. Also, for passive immunization, toxicity and efficacy humanized antibodies have to be tested in non-human primates [157].

Regarding the gene therapy method, it is necessary to consider several points before using it, including identification of the exact genes used, appropriate selection of new vectors, development of safe gene markers, and precise expression of the gene in the central nervous system [9]. As a result, while gene therapy has not yet provided real treatments for PD, there is increasing evidence that this treatment can be an important

way to treat the future of PD. Editing the genome with CRISPR-CAS9 technology may be another future way of personalized gene therapy for known mutations that lead to PD.

Conclusions

Due to a series of problems and limitations with current drugs in terms of side effects, efficacy, and also cost, new therapeutic strategies have recently been introduced for PD treatment. These novel strategies include gene therapy, immunotherapy, and cell transplantation therapy that are still under investigation and mostly tested in animal models. Although there are a number of unresolved issues such as safety and efficacy about these new therapies, there is a great hope that in the near future, we will see the presence of these methods in the clinics for PD treatment. Another challenge in this field is lack of suitable animal models to reproduce behavioral defects and neuropathological changes in humans. This limitation can be addressed by some strategies such as combination of two or more animal models. For example, transgenic model and application of new techniques for gene editing such as CRISP-Cas9 that are able to correct the mutated genes involved in PD have been introduced. Overall, further translational experiments and robust evidence from randomized controlled trials are warranted to verify the therapeutic impact of novel strategies.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

References

1. De Lau LM, Breteler MM (2006) Epidemiology of Parkinson's disease. Lancet Neurol 5(6):525–535
2. Marin B, Couratier P, Lannuzel A, Logroscino G (2018) Other neurocognitive disorders in tropical health (amyotrophic lateral sclerosis and Parkinson's disease). In: Neuroepidemiology in tropical health. Elsevier, pp. 167–183
3. Chin-Chan M, Navarro-Yepes J, Quintanilla-Vega B (2015) Environmental pollutants as risk factors for neurodegenerative disorders: Alzheimer and Parkinson diseases. Front Cell Neurosci 9:124
4. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M (1998) α-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with Lewy bodies. Proc Natl Acad Sci 95(11):6469–6473
5. Obeso J, Stamelou M, Goetz C, Poewe W, Lang A, Weintraub D, Burn D, Halliday G et al (2017) Past, present, and future of Parkinson's disease: a special essay on the 200th anniversary of the Shaking Palsy. Mov Disord 32(9):1264–1310
6. Bertram L, Tanzi RE (2005) The genetic epidemiology of neurodegenerative disease. J Clin Invest 115(6):1449–1457
7. Davie CA (2008) A review of Parkinson's disease. Br Med Bull 86(1):109–127
8. Hauser RA, Lew MF, Hurtig HI, Ondo WG, Wojcieszek J, Fitzer-Attas CJ, Group TOlS (2009) Long-term outcome of early versus delayed rasagiline treatment in early Parkinson's disease. Mov Disord 24(4):564–573
9. Maiti P, Manna J, Dunbar GL (2017) Current understanding of the molecular mechanisms in Parkinson's disease: Targets for potential treatments. Translational Neurodegeneration 6(1):28
10. Pont-Sunyer C, Hotter A, Gaig C, Seppi K, Compta Y, Katzenschlager R, Mas N, Hofeneder D et al (2015) The onset of nonmotor symptoms in Parkinson's disease (the ONSET PD study). Mov Disord 30(2):229–237
11. Weintraub D, Simuni T, Caspell-Garcia C, Coffey C, Lasch S, Siderowf A, Aarsland D, Barone P et al (2015) Cognitive performance and neuropsychiatric symptoms in early, untreated Parkinson's disease. Mov Disord 30(7):919–927
12. Sveinbjornsdottir S (2016) The clinical symptoms of Parkinson's disease. J Neurochem 139(S1):318–324
13. Alam M, Schmidt W (2002) Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. Behav Brain Res 136(1):317–324
14. Hoang QQ (2014) Pathway for Parkinson disease. Proc Natl Acad Sci 111(7):2402–2403
15. Gibb W, Lees A (1988) The relevance of the Lewy body to the pathogenesis of idiopathic Parkinson's disease. J Neurol Neurosurg Psychiatry 51(6):745–752
16. Seidel K, Mahlke J, Siswanto S, Krüger R, Heinsen H, Auburger G, Bouzrou M, Grinberg LT et al (2015) The brainstem pathologies of Parkinson's disease and dementia with Lewy bodies. Brain Pathol 25(2):121–135
17. Goedert M, Spillantini MG, Del Tredici K, Braak H (2013) 100 years of Lewy pathology. Nat Rev Neurol 9(1):13–24
18. Oertel W, Schulz JB (2016) Current and experimental treatments of Parkinson disease: a guide for neuroscientists. J Neurochem 139(S1):325–337
19. Dauer W, Przedborski S (2003) Parkinson's disease: mechanisms and models. Neuron 39(6):889–909
20. Jenner P (1998) Oxidative mechanisms in nigral cell death in Parkinson's disease. Mov Disord 13:24–34
21. Yang YX, Wood NW, Latchman DS (2009) Molecular basis of Parkinson's disease. Neuroreport 20(2):150–156
22. Jain S, Wood NW, Healy DG (2005) Molecular genetic pathways in Parkinson's disease: a review. Clin Sci 109(4):355–364
23. Warner TT, Schapira AH (2003) Genetic and environmental factors in the cause of Parkinson's disease. Ann Neurol 53(S3):S16–S25
24. Delamarre A, Meissner WG (2017) Epidemiology, environmental risk factors and genetics of Parkinson's disease. Presse Med 46(2): 175–181
25. Kalinderi K, Bostantjopoulou S, Fidani L (2016) The genetic background of Parkinson's disease: current progress and future prospects. Acta Neurol Scand 134(5):314–326
26. Tambasco N, Nigro P, Romoli M, Prontera P, Simoni S, Calabresi P (2016) A53T in a parkinsonian family: a clinical update of the SNCA phenotypes. J Neural Transm 123(11):1301–1307
27. Mastrangelo L (2017) The genetics of Parkinson disease. In: Adv Genet, vol 98. Elsevier, pp 43–62
28. Reichmann H (2016) Modern treatment in Parkinson's disease, a personal approach. J Neural Transm 123(1):73–80
29. Peschanski M, Defer G, N'guyen J, Ricolfi F, Monfort J, Remy P, Geny C, Samson Y et al (1994) Bilateral motor improvement and alteration of L-dopa effect in two patients with Parkinson's disease following intrastriatal transplantation of foetal ventral mesencephalon. Brain 117(3):487–499

30. Goetz CG, Poewe W, Rascol O, Sampaio C (2005) Evidence-based medical review update: pharmacological and surgical treatments of Parkinson's disease: 2001 to 2004. Mov Disord 20(5): 523–539
31. Connolly BS, Lang AE (2014) Pharmacological treatment of Parkinson disease: a review. Jama 311(16):1670–1683
32. Chan AK, McGovern RA, Brown LT, Sheehy JP, Zacharia BE, Mikell CB, Bruce SS, Ford B et al (2014) Disparities in access to deep brain stimulation surgery for Parkinson disease: interaction between African American race and Medicaid use. JAMA Neurol 71(3):291–299
33. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag A-E, Lang AE (2017) Parkinson disease. Nature reviews. Disease Primers 3:17013
34. Pinna A, Bonaventura J, Farré D, Sánchez M, Simola N, Mallol J, Lluís C, Costa G et al (2014) L-DOPA disrupts adenosine A2A–cannabinoid CB1–dopamine D2 receptor heteromer cross-talk in the striatum of hemiparkinsonian rats: biochemical and behavioral studies. Exp Neurol 253:180–191
35. Cools R, Barker RA, Sahakian BJ, Robbins TW (2003) L-Dopa medication remediates cognitive inflexibility, but increases impulsivity in patients with Parkinson's disease. Neuropsychologia 41(11):1431–1441
36. Hornykiewicz O (1975) The mechanisms of action of L-dopa in Parkinson's disease. In: Minireviews of the neurosciences from life sciences. Elsevier, pp. 421–431
37. Wade LA, Katzman R (1975) 3-0-Methyldopa uptake and inhibition of L-dopa at the blood-brain barrier. Life Sci 17(1):131–136
38. Godwin-Austen R, Frears C, Tomlinson E, Kok H (1969) Effects of L-dopa in Parkinson's disease. Lancet 294(7613):165–168
39. Cenci MA (2014) Presynaptic mechanisms of L-DOPA-induced dyskinesia: the findings, the debate, and the therapeutic implications. Front Neurol 5:242
40. Mazo NA, Echeverria V, Cabezas R, Avila-Rodriguez M, Tarasov VV, Yarla NS, Aliev G, Barreto GE (2017) Medicinal plants as protective strategies against Parkinson's disease. Curr Pharm Des 23(28):4180–4188. https://doi.org/10.2174/1381612823666170316142803
41. Santos G, Giraldez-Alvarez LD, Avila-Rodriguez M, Capani F, Galembeck E, Neto AG, Barreto GE, Andrade B (2016) SUR1 receptor interaction with hesperidin and linarin predicts possible mechanisms of action of valeriana officinalis in Parkinson. Front Aging Neurosci 8:97. https://doi.org/10.3389/fnagi.2016.00097
42. Jurado-Coronel JC, Avila-Rodriguez M, Echeverria V, Hidalgo OA, Gonzalez J, Aliev G, Barreto GE (2016) Implication of green tea as a possible therapeutic approach for Parkinson disease. CNS Neurol Disord Drug Targets 15(3):292–300
43. Sutachan JJ, Casas Z, Albarracin SL, Stab BR 2nd, Samudio I, Gonzalez J, Morales L, Barreto GE (2012) Cellular and molecular mechanisms of antioxidants in Parkinson's disease. Nutr Neurosci 15(3):120–126. https://doi.org/10.1179/1476830511Y.0000000033
44. Albarracin SL, Stab B, Casas Z, Sutachan JJ, Samudio I, Gonzalez J, Gonzalo L, Capani F et al (2012) Effects of natural antioxidants in neurodegenerative disease. Nutr Neurosci 15(1):1–9. https://doi.org/10.1179/1476830511Y.0000000028
45. de Oliveria DM, Barreto G, De Andrade DV, Saraceno E, Aon-Bertolino L, Capani F, Dos Santos El Bacha R, Giraldez LD (2009) Cytoprotective effect of *Valeriana officinalis* extract on an in vitro experimental model of Parkinson disease. Neurochem Res 34(2):215–220. https://doi.org/10.1007/s11064-008-9749-y
46. Valverde GDAD, Madureira de Oliveria D, Barreto G, Bertolino LA, Saraceno E, Capani F, Giraldez LD (2008) Effects of the extract of Anemopaegma mirandum (Catuaba) on rotenone-induced apoptosis in human neuroblastomas SH-SY5Y cells. Brain Res 1198:188–196. https://doi.org/10.1016/j.brainres.2008.01.006
47. Solayman M, Islam M, Alam F, Ibrahim Khalil M, Amjad Kamal M, Hua Gan S (2017) Natural products combating neurodegeneration: Parkinson's disease. Curr Drug Metab 18(1):50–61
48. Ríos J-L, Onteniente M, Picazo D, Montesinos M-C (2016) Medicinal plants and natural products as potential sources for antiparkinson drugs. Planta Med 82(11/12):942–951
49. Zhang H, Bai L, He J, Zhong L, Duan X, Ouyang L, Zhu Y, Zhang Y et al (2017) Recent advances in discovery and development of natural products as source for anti-Parkinson's disease lead compounds. Eur J Med Chem 141:257–272
50. Areiza-Mazo N, Robles J, Zamudio-Rodriguez JA, Giraldez L, Echeverria V, Barrera-Bailon B, Aliev G, Sahebkar A et al (2018) Extracts of Physalis peruviana protect astrocytic cells under oxidative stress with rotenone. Front Chem 6:276. https://doi.org/10.3389/fchem.2018.00276
51. Backlund E-O, Granberg P-O, Hamberger B, Knutsson E, Mårtensson A, Sedvall G, Seiger Å, Olson L (1985) Transplantation of adrenal medullary tissue to striatum in parkinsonism: first clinical trials. J Neurosurg 62(2):169–173
52. Lindvall O, Backlund EO, Farde L, Sedvall G, Freedman R, Hoffer B, Nobin A, Seiger Å et al (1987) Transplantation in Parkinson's disease: two cases of adrenal medullary grafts to the putamen. Ann Neurol 22(4):457–468
53. Freed CR, Greene PE, Breeze RE, Tsai W-Y, DuMouchel W, Kao R, Dillon S, Winfield H et al (2001) Transplantation of embryonic dopamine neurons for severe Parkinson's disease. N Engl J Med 344(10):710–719
54. Lindvall O (2015) Treatment of Parkinson's disease using cell transplantation. Philos Trans R Soc B 370(1680):20140370
55. Kim J-H, Auerbach JM, Rodríguez-Gómez JA, Velasco I, Gavin D, Lumelsky N, Lee S-H, Nguyen J et al (2002) Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease. Nature 418(6893):50–56
56. Thomson JA, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM (1998) Embryonic stem cell lines derived from human blastocysts. Science 282(5391):1145–1147
57. Kim H-J (2011) Stem cell potential in Parkinson's disease and molecular factors for the generation of dopamine neurons. Biochim Biophys Acta (BBA) – Mol Basis Dis 1812(1):1–11
58. Kirkeby A, Grealish S, Wolf DA, Nelander J, Wood J, Lundblad M, Lindvall O, Parmar M (2012) Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. Cell Rep 1(6):703–714
59. Fu M-H, Li C-L, Lin H-L, Chen P-C, Calkins MJ, Chang Y-F, Cheng P-H, Yang S-H (2015) Stem cell transplantation therapy in Parkinson's disease. SpringerPlus 4(1):597
60. Oh SM, Chang MY, Song JJ, Rhee YH, Joe EH, Lee HS, Yi SH, Lee SH (2015) Combined Nurr1 and Foxa2 roles in the therapy of Parkinson's disease. EMBO Mol Med e201404610
61. Roybon L, Christophersen NS, Brundin P, Li J-Y (2004) Stem cell therapy for Parkinson's disease: where do we stand? Cell Tissue Res 318(1):261–273
62. Zhang S-C, Li X-J, Johnson MA, Pankratz MT (2008) Human embryonic stem cells for brain repair? Philos Trans R Soc Lond B Biol Sci 363(1489):87–99
63. Kriks S, Shim J-W, Piao J, Ganat YM, Wakeman DR, Xie Z, Carrillo-Reid L, Auyeung G et al (2011) Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature 480(7378):547–551
64. Fitzpatrick KM, Raschke J, Emborg ME (2009) Cell-based therapies for Parkinson's disease: past, present, and future. Antioxid Redox Signal 11(9):2189–2208

65. Grealish S, Diguet E, Kirkeby A, Mattsson B, Heuer A, Bramoulle Y, Van Camp N, Perrier AL et al (2014) Human ESC-derived dopamine neurons show similar preclinical efficacy and potency to fetal neurons when grafted in a rat model of Parkinson's disease. Cell Stem Cell 15(5):653–665
66. Turner M, Leslie S, Martin NG, Peschanski M, Rao M, Taylor CJ, Trounson A, Turner D et al (2013) Toward the development of a global induced pluripotent stem cell library. Cell Stem Cell 13(4): 382–384
67. Gurdon JB (1962) The developmental capacity of nuclei taken from intestinal epithelium cells of feeding tadpoles. Development 10(4):622–640
68. Takahashi K, Okita K, Nakagawa M, Yamanaka S (2007) Induction of pluripotent stem cells from fibroblast cultures. Nat Protoc 2(12):3081–3089
69. Parmar M, Torper O, Drouin-Ouellet J (2018) Cell-based therapy for Parkinson's disease: a journey through decades towards the light side of the force. Eur J Neurosci
70. Masserdotti G, Gascón S, Götz M (2016) Direct neuronal reprogramming: learning from and for development. Development 143(14):2494–2510
71. Pfisterer U, Kirkeby A, Torper O, Wood J, Nelander J, Dufour A, Björklund A, Lindvall O et al (2011) Direct conversion of human fibroblasts to dopaminergic neurons. Proc Natl Acad Sci 108(25): 10343–10348
72. Hallett PJ, Deleidi M, Astradsson A, Smith GA, Cooper O, Osborn TM, Sundberg M, Moore MA et al (2015) Successful function of autologous iPSC-derived dopamine neurons following transplantation in a non-human primate model of Parkinson's disease. Cell Stem Cell 16(3):269–274
73. Doi D, Samata B, Katsukawa M, Kikuchi T, Morizane A, Ono Y, Sekiguchi K, Nakagawa M et al (2014) Isolation of human induced pluripotent stem cell-derived dopaminergic progenitors by cell sorting for successful transplantation. Stem Cell Rep 2(3): 337–350
74. Kikuchi T, Morizane A, Onoe H, Hayashi T, Kawasaki T, Saiki H, Miyamoto S, Takahashi J (2011) Survival of human induced pluripotent stem cell–derived midbrain dopaminergic neurons in the brain of a primate model of Parkinson's disease. J Parkinson's Disease 1(4):395–412
75. Sundberg M, Bogetofte H, Lawson T, Jansson J, Smith G, Astradsson A, Moore M, Osborn T et al (2013) Improved cell therapy protocols for Parkinson's disease based on differentiation efficiency and safety of hESC-, hiPSC-, and non-human primate iPSC-derived dopaminergic neurons. Stem Cells 31(8):1548–1562
76. Kikuchi T, Morizane A, Doi D, Magotani H, Onoe H, Hayashi T, Mizuma H, Takara S et al (2017) Human iPS cell-derived dopaminergic neurons function in a primate Parkinson's disease model. Nature 548(7669):592–596
77. Taylor CJ, Peacock S, Chaudhry AN, Bradley JA, Bolton EM (2012) Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. Cell Stem Cell 11(2):147–152
78. Marro S, Pang ZP, Yang N, Tsai M-C, Qu K, Chang HY, Südhof TC, Wernig M (2011) Direct lineage conversion of terminally differentiated hepatocytes to functional neurons. Cell Stem Cell 9(4):374–382
79. Lindvall O (2013) Developing dopaminergic cell therapy for Parkinson's disease—give up or move forward? Mov Disord 28(3):268–273
80. González C, Bonilla S, Isabel Flores A, Cano E, Liste I (2016) An update on human stem cell-based therapy in Parkinson's disease. Curr Stem Cell Res Ther 11(7):561–568
81. Barker RA, Barrett J, Mason SL, Björklund A (2013) Fetal dopaminergic transplantation trials and the future of neural grafting in Parkinson's disease. Lancet Neurol 12(1):84–91
82. Björklund A, Dunnett SB (2007) Dopamine neuron systems in the brain: an update. Trends Neurosci 30(5):194–202
83. Spencer DD, Robbins RJ, Naftolin F, Marek KL, Vollmer T, Leranth C, Roth RH, Price LH et al (1992) Unilateral transplantation of human fetal mesencephalic tissue into the caudate nucleus of patients with Parkinson's disease. N Engl J Med 327(22): 1541–1548
84. Lindvall O, Sawle G, Widner H, Rothwell JC, Björklund A, Brooks D, Brundin P, Frackowiak R et al (1994) Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease. Ann Neurol 35(2):172–180
85. Piccini P, Brooks DJ, Björklund A, Gunn RN, Grasby PM, Rimoldi O, Brundin P, Hagell P et al (1999) Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient. Nat Neurosci 2(12):1137–1140
86. Hallett PJ, Cooper O, Sadi D, Robertson H, Mendez I, Isacson O (2014) Long-term health of dopaminergic neuron transplants in Parkinson's disease patients. Cell Rep 7(6):1755–1761
87. Hagell P, Piccini P, Björklund A, Brundin P, Rehncrona S, Widner H, Crabb L, Pavese N et al (2002) Dyskinesias following neural transplantation in Parkinson's disease. Nat Neurosci 5(7):627–628
88. Freed CR, Zhou W, Breeze RE (2011) Dopamine cell transplantation for Parkinson's disease: the importance of controlled clinical trials. Neurotherapeutics 8(4):549–561
89. Carta M, Carlsson T, Muñoz A, Kirik D, Björklund A (2008) Serotonin–dopamine interaction in the induction and maintenance of L-DOPA-induced dyskinesias. Prog Brain Res 172:465–478
90. Politis M, Wu K, Loane C, Turkheimer F, Molloy S, Brooks D, Piccini P (2010) Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures. Neurology 75(21): 1920–1927
91. Rath A, Klein A, Papazoglou A, Pruszak J, Garcia J, Krause M, Maciaczyk J, Dunnett SB et al (2013) Survival and functional restoration of human fetal ventral mesencephalon following transplantation in a rat model of Parkinson's disease. Cell Transplant 22(7):1281–1293
92. Parashar A, Udayabanu M (2017) Gut microbiota: Implications in Parkinson's disease. Parkinsonism Relat Disord 38:1–7
93. Marizzoni M, Provasi S, Cattaneo A, Frisoni GB (2017) Microbiota and neurodegenerative diseases. Curr Opin Neurol 30(6):630–638
94. Aono H, Choudhury ME, Higaki H, Miyanishi K, Kigami Y, Fujita K, Ji A, Takahashi H et al (2017) Microglia may compensate for dopaminergic neuron loss in experimental Parkinsonism through selective elimination of glutamatergic synapses from the subthalamic nucleus. Glia 65(11):1833–1847
95. Blaylock RL (2017) Parkinson's disease: microglial/macrophage-induced immunoexcitotoxicity as a central mechanism of neurodegeneration. Surg Neurol Int 8:65
96. Sulzer D, Alcalay RN, Garretti F, Cote L, Kanter E, Agin-Liebes J, Liong C, McMurtrey C et al (2017) Erratum: T cells from patients with Parkinson's disease recognize α-synuclein peptides. Nature 549(7671):292
97. Lashuel HA, Overk CR, Oueslati A, Masliah E (2013) The many faces of α-synuclein: from structure and toxicity to therapeutic target. Nat Rev Neurosci 14(1):38–48
98. Holmans P, Moskvina V, Jones L, Sharma M, Consortium IPsDG, Vedernikov A, Buchel F, Sadd M et al (2012) A pathway-based analysis provides additional support for an immune-related genetic susceptibility to Parkinson's disease. Hum Mol Genet 22(5): 1039–1049
99. Jankovic J (2018) Immunologic treatment of Parkinson's disease. Future Med

100. Witoelar A, Jansen IE, Wang Y, Desikan RS, Gibbs JR, Blauwendraat C, Thompson WK, Hernandez DG et al (2017) Genome-wide pleiotropy between Parkinson disease and autoimmune diseases. JAMA Neurol 74(7):780–792
101. Li C, Guo Y, Xie W, Li X, Janokovic J, Le W (2010) Neuroprotection of pramipexole in UPS impairment induced animal model of Parkinson's disease. Neurochem Res 35(10):1546–1556
102. Dehay B, Decressac M, Bourdenx M, Guadagnino I, Fernagut PO, Tamburrino A, Bassil F, Meissner WG et al (2016) Targeting α-synuclein: therapeutic options. Mov Disord 31(6):882–888
103. Lawand NB, Saadé NE, El-Agnaf OM, Safieh-Garabedian B (2015) Targeting α-synuclein as a therapeutic strategy for Parkinson's disease. Expert Opin Ther Targets 19(10):1351–1360
104. Dehay B, Bourdenx M, Gorry P, Przedborski S, Vila M, Hunot S, Singleton A, Olanow CW et al (2015) Targeting α-synuclein for treatment of Parkinson's disease: mechanistic and therapeutic considerations. Lancet Neurol 14(8):855–866
105. Spencer B, Valera E, Rockenstein E, Overk C, Mante M, Adame A, Zago W, Seubert P et al (2017) Anti-α-synuclein immunotherapy reduces α-synuclein propagation in the axon and degeneration in a combined viral vector and transgenic model of synucleinopathy. Acta Neuropathol Commun 5(1):7
106. Jankovic J, Goodman I, Safirstein B, Schenk D, Kinney G, Koller M, Ness D, Griffith S et al (2018) Results from a phase 1b multiple ascending-dose study of PRX002/RG7935, an anti-alpha-synuclein monoclonal antibody, in patients with Parkinson's disease. Parkinsonism Relat Disord 46:e25
107. Games D, Valera E, Spencer B, Rockenstein E, Mante M, Adame A, Patrick C, Ubhi K et al (2014) Reducing C-terminal-truncated alpha-synuclein by immunotherapy attenuates neurodegeneration and propagation in Parkinson's disease-like models. J Neurosci 34(28):9441–9454
108. Sahin C, Lorenzen N, Lemminger L, Christiansen G, Møller IM, Vesterager LB, Pedersen LØ, Fog K et al (2017) Antibodies against the C-terminus of α-synuclein modulate its fibrillation. Biophys Chem 220:34–41
109. Weihofen A, Patel H, Huy C, Liu C, Combaluzier I, Mueller-Steiner S, Cavegn N, Strobel L, Kuznetsov G, Engber T (2017) Binding and functional characterization of human-derived anti-alpha-synuclein antibody BIIB054. Neurodegener Dis
110. Schenk DB, Koller M, Ness DK, Griffith SG, Grundman M, Zago W, Soto J, Atiee G et al (2017) First-in-human assessment of PRX002, an anti–α-synuclein monoclonal antibody, in healthy volunteers. Mov Disord 32(2):211–218
111. Mandler M, Valera E, Rockenstein E, Weninger H, Patrick C, Adame A, Santic R, Meindl S et al (2014) Next-generation active immunization approach for synucleinopathies: implications for Parkinson's disease clinical trials. Acta Neuropathol 127(6):861–879
112. Friedmann T, Roblin R (1972) Gene therapy for human genetic disease? Science 175(4025):949–955
113. Axelsen TM, Woldbye DP (2018) Gene therapy for Parkinson's disease, an update. J Parkinson's disease (Preprint) 1–21
114. Cabezas R, Baez-Jurado E, Hidalgo-Lanussa O, Echeverria V, Ashrad GM, Sahebkar A, Barreto GE (2018) Growth factors and neuroglobin in astrocyte protection against neurodegeneration and oxidative stress. Mol Neurobiol. https://doi.org/10.1007/s12035-018-1203-9
115. Cabezas R, Avila M, Gonzalez J, El-Bacha RS, Baez E, Garcia-Segura LM, Jurado Coronel JC, Capani F et al (2014) Astrocytic modulation of blood brain barrier: perspectives on Parkinson's disease. Front Cell Neurosci 8:211. https://doi.org/10.3389/fncel.2014.00211
116. Cabezas R, Avila-Rodriguez M, Vega-Vela NE, Echeverria V, Gonzalez J, Hidalgo OA, Santos AB, Aliev G et al (2016) Growth factors and astrocytes metabolism: possible roles for platelet derived growth factor. Med Chem 12(3):204–210
117. Cabezas R, El-Bacha RS, Gonzalez J, Barreto GE (2012) Mitochondrial functions in astrocytes: neuroprotective implications from oxidative damage by rotenone. Neurosci Res 74(2):80–90. https://doi.org/10.1016/j.neures.2012.07.008
118. Coune PG, Schneider BL, Aebischer P (2012) Parkinson's disease: gene therapies. Cold Spring Harb Perspect Med 2(4):a009431
119. Belin AC, Westerlund M (2008) Parkinson's disease: a genetic perspective. FEBS J 275(7):1377–1383
120. Bartus RT, Weinberg MS, Samulski RJ (2014) Parkinson's disease gene therapy: success by design meets failure by efficacy. Mol Ther 22(3):487–497
121. Meiser J, Weindl D, Hiller K (2013) Complexity of dopamine metabolism. Cell Commun Signal 11(1):34
122. Hutz MH, Rieder CR (2018) The future of pharmacogenetics in Parkinson's disease treatment. Future Med
123. Azzouz M, Martin-Rendon E, Barber RD, Mitrophanous KA, Carter EE, Rohll JB, Kingsman SM, Kingsman AJ et al (2002) 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 22(23):10302–10312
124. Forsayeth JR, Eberling JL, Sanftner LM, Zhen Z, Pivirotto P, Bringas J, Cunningham J, Bankiewicz KS (2006) A dose-ranging study of AAV-hAADC therapy in Parkinsonian monkeys. Mol Ther 14(4):571–577
125. Hadaczek P, Eberling JL, Pivirotto P, Bringas J, Forsayeth J, Bankiewicz KS (2010) Eight years of clinical improvement in MPTP-lesioned primates after gene therapy with AAV2-hAADC. Mol Ther 18(8):1458–1461
126. Eberling J, Jagust W, Christine C, Starr P, Larson P, Bankiewicz K, Aminoff M (2008) Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. Neurology 70(21):1980–1983
127. Mittermeyer G, Christine CW, Rosenbluth KH, Baker SL, Starr P, Larson P, Kaplan PL, Forsayeth J et al (2012) Long-term evaluation of a phase 1 study of AADC gene therapy for Parkinson's disease. Hum Gene Ther 23(4):377–381
128. Palfi S, Gurruchaga JM, Ralph GS, Lepetit H, Lavisse S, Buttery PC, Watts C, Miskin J et al (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(9923):1138–1146
129. Jarraya B, Boulet S, Ralph GS, Jan C, Bonvento G, Azzouz M, Miskin JE, Shin M et al (2009) Dopamine gene therapy for Parkinson's disease in a nonhuman primate without associated dyskinesia. Sci Transl Med 1(2):2ra4–2ra4
130. Muramatsu S-i (2010) The current status of gene therapy for Parkinson's disease. Ann Neurosci 17(2):92–95
131. Caudle WM, Colebrooke RE, Emson PC, Miller GW (2008) Altered vesicular dopamine storage in Parkinson's disease: a premature demise. Trends Neurosci 31(6):303–308
132. Chen L, Ding Y, Cagniard B, Van Laar AD, Mortimer A, Chi W, Hastings TG, Kang UJ et al (2008) Unregulated cytosolic dopamine causes neurodegeneration associated with oxidative stress in mice. J Neurosci 28(2):425–433
133. Man JH, Groenink L, Caiazzo M (2018) Cell reprogramming approaches in gene-and cell-based therapies for Parkinson's disease. J Control Release 286:114–124
134. Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK (2013) GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol Ther 138(2):155–175
135. d'Anglemont de Tassigny X, Pascual A, López-Barneo J (2015) GDNF-based therapies, GDNF-producing interneurons, and

trophic support of the dopaminergic nigrostriatal pathway. Implications for Parkinson's disease. Front Neuroanat 9:10
136. Kirik D, Cederfjäll E, Halliday G, Petersén Å (2017) Gene therapy for Parkinson's disease: disease modification by GDNF family of ligands. Neurobiol Dis 97:179–188
137. Eberling JL, Kells AP, Pivirotto P, Beyer J, Bringas J, Federoff HJ, Forsayeth J, Bankiewicz KS (2009) Functional effects of AAV2-GDNF on the dopaminergic nigrostriatal pathway in parkinsonian rhesus monkeys. Hum Gene Ther 20(5):511–518
138. Warren Olanow C, Bartus RT, Baumann TL, Factor S, Boulis N, Stacy M, Turner DA, Marks W et al (2015) Gene delivery of neurturin to putamen and substantia nigra in Parkinson disease: a double-blind, randomized, controlled trial. Ann Neurol 78(2): 248–257
139. Brantl S (2002) Antisense-RNA regulation and RNA interference. Biochim Biophys Acta Gene Struct Expr 1575(1–3):15–25
140. Agrawal N, Dasaradhi P, Mohmmed A, Malhotra P, Bhatnagar RK, Mukherjee SK (2003) RNA interference: biology, mechanism, and applications. Microbiol Mol Biol Rev 67(4):657–685
141. Häbig K, Walter M, Poths S, Riess O, Bonin M (2008) RNA interference of LRRK2–microarray expression analysis of a Parkinson's disease key player. Neurogenetics 9(2):83–94
142. Helmschrodt C, Höbel S, Schöniger S, Bauer A, Bonicelli J, Gringmuth M, Fietz SA, Aigner A et al (2017) Polyethylenimine nanoparticle-mediated siRNA delivery to reduce α-Synuclein expression in a model of Parkinson's disease. Mol Ther Nucleic Acids 9:57–68
143. Liu YY, Yang XY, Li Z, Liu ZL, Cheng D, Wang Y, Wen XJ, Hu JY et al (2014) Characterization of polyethylene glycol-polyethyleneimine as a vector for alpha-synuclein siRNA delivery to PC12 cells for Parkinson's disease. CNS Neurosci Ther 20(1): 76–85
144. Hoepken H-H, Gispert S, Azizov M, Klinkenberg M, Ricciardi F, Kurz A, Morales-Gordo B, Bonin M et al (2008) Parkinson patient fibroblasts show increased alpha-synuclein expression. Exp Neurol 212(2):307–313
145. Haussecker D (2014) Current issues of RNAi therapeutics delivery and development. J Control Release 195:49–54
146. McSwiggen J, Haeberli P, Chowrira B (2004) RNA interference mediated treatment of parkinson disease using short interfering nucleic acid (siNA). Google Patents
147. Ran FA, Hsu PD, Wright J, Agarwala V, Scott DA, Zhang F (2013) Genome engineering using the CRISPR-Cas9 system. Nat Protoc 8(11):2281–2308
148. Ran FA, Hsu PD, Lin C-Y, Gootenberg JS, Konermann S, Trevino AE, Scott DA, Inoue A et al (2013) Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. Cell 154(6):1380–1389
149. Sander JD, Joung JK (2014) CRISPR-Cas systems for editing, regulating and targeting genomes. Nat Biotechnol 32(4):347–355
150. Basu S, Adams L, Guhathakurta S, Kim Y-S (2017) A novel tool for monitoring endogenous alpha-synuclein transcription by NanoLuciferase tag insertion at the 3′ end using CRISPR-Cas9 genome editing technique. Sci Rep 7:45883
151. Cui Z, Renfu Q, Jinfu W (2018) Development and application of CRISPR/Cas9 technologies in genomic editing. Hum Mol Genet
152. Diester I, Kaufman MT, Mogri M, Pashaie R, Goo W, Yizhar O, Ramakrishnan C, Deisseroth K et al (2011) An optogenetic toolbox designed for primates. Nat Neurosci 14(3):387–397
153. Sanders TH, Jaeger D (2016) Optogenetic stimulation of cortico-subthalamic projections is sufficient to ameliorate bradykinesia in 6-ohda lesioned mice. Neurobiol Dis 95:225–237
154. Gradinaru V, Mogri M, Thompson KR, Henderson JM, Deisseroth K (2009) Optical deconstruction of parkinsonian neural circuitry. Science 324(5925):354–359
155. Pienaar IS, Gartside SE, Sharma P, De Paola V, Gretenkord S, Withers D, Elson JL, Dexter DT (2015) Pharmacogenetic stimulation of cholinergic pedunculopontine neurons reverses motor deficits in a rat model of Parkinson's disease. Mol Neurodegener 10(1):47
156. Pienaar IS, Harrison IF, Elson JL, Bury A, Woll P, Simon AK, Dexter DT (2015) An animal model mimicking pedunculopontine nucleus cholinergic degeneration in Parkinson's disease. Brain Struct Funct 220(1):479–500
157. Lee JS, Lee S-J (2016) Mechanism of anti-α-synuclein immunotherapy. J Mov Disord 9(1):14–19