Promising therapeutic agents for the treatment of Parkinson's disease

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Abstract

Introduction: Therapeutic management of Parkinson's disease is not yet fully resolved, with motor fluctuations and levodopa-induced dyskinesia representing special therapeutic challanges. Furthermore, no disease-modifying therapies are currently available.

Areas covered: The review focuses on promising novel therapies that are at present under investigation in Phase I and Phase II trials. Special emphasis will be placed on gene therapies, adenosine A_{2A} antagonists, metabotropic glutamate receptor 5 antagonists, and calcium channel blockers.

Expert opinion: Gene therapies represent a promising field in the therapeutic palette. In order to mitigate the side effects of this therapy, the developments are focusing on the applied vectors.

Isradipine has been suggested to have neuroprotective properties; however, clinical evidence is still eagerly awaited. Though the development of metabotropic glutamate receptor 5 antagonist mavoglurant has recently been discontinued due to moderated efficacy, investigations on dipraglurant are still ongoing. Adenosine A_{2A} antagonists appear to be promising agents in the management of motor complications in advanced stages of Parkinson's disease.

Keywords: Parkinson's disease, levodopa-induced dyskinesia, adenosine A_{2A} antagonist, gene therapy, metabotropic glutamate receptors

1. Introduction

Parkinson's disease (PD) represent the second most prevalent chronic progressive neurodegenerative disease among the elderly[1]. Its neuropathological hallmarks include the preferential degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNPc) and the presence of intraneuronal inclusions consisting primarily of a-synuclein, the Lewy bodies. As the disease affects millions of families worldwide and causes serious problem in the aging societies, its social significance is remarkable. A meta-analysis of a worldwide dataset revealed an increasing prevalence of PD with age: 41/100,000 between 40 and 49 years; 428/100,000 between 60 and 69 years; and 1903/100,000 above 80 years of age[2].

Protein aggregation, mitochondrial disturbances, oxidative stress, glutamate excitotoxicity, alterations of the tryptophan metabolism, immunological mechanisms, and genetic predisposition are all proposed to play significant roles in the etiopathology of the disease[3-13].

The diagnosis is based on the identification of the classical motor symptoms (tremor, rigidity, hypokinesia and difficulty walking). In addition to these, however, non-motor symptoms are also characteristic of the disorder (i.e., dementia, depression, sleep disorder, as well as emotional, cognitive, and behavioral problems) [14].

The introduction of long-term dopamine (DA) replacement therapy with 3,4-dihydroxy-L-phenylalanine (L-DOPA), the precursor of DA, represents a milestone in the treatment of PD. However, the drug can evoke side effects, which include L-DOPA-induced dyskinesia (LID) and non-motor fluctuations with cognitive dysfunction and

neuropsychiatric symptoms (i.e., compulsive behaviors and impulse control disorders) [15, 16]. Therefore, the benefits of L-DOPA treatment may be overshadowed by these troublesome side effects as well as by the appearence of symptoms that are not responsive to dopaminergic treatments (i.e., autonomic symptoms, gait and balance problems, and cognitive impairment)[17]. These issues together with the lack of neuroprotective agents available represent the driving force behind the search for new therapeutic possibilities. In the last decade, several novel drugs have been developed and tested in PD, however, so far only a small number of them have reached the market [18, 19].

In this review, we focused on summarizing the results of recent phase I and II clinical trials with new potential therapeutic agents in PD.

2. Novel therapeutic possibilities in PD

2.1. Gene therapy

In the past decade, a new player appeared in the therapeutic palette, aiming to fill the above mentioned therapeutic gaps, that is gene therapy. Gene therapy refers to the application of a gene or genetic material (including DNA and RNA) as an agent to modulate cellular/biological functions with the aim of treating a disease[17].

Clinical gene therapy approaches can be divided into two categories (Figure 1.). The first option is *ex vivo* gene therapy, in which the patients recieve genetically modified cells that express a desired protein or proteins. The second possibility is *in vivo* gene therapy, in which the genetic information is directly inserted into the patient's own cells. To date, all human clinical trials applied the *in vivo* method by the use of viral vectors; however, *ex vivo* gene therapeutic strategies may as well play a role in the treatment of PD in the future[20-22]. Of note, specific risks exist in association with the use of gene therapeutic approaches. Indeed, the uncontrolled overproduction of the expressed protein can cause undesirable effects. Appropriate selection of the gene promoter, a region that controls the gene expression, might be a solution for this probem[23].

Figure 1.

A number of risk factors inheritedly exist as regards the *in vivo* gene therapy as well, including the induction of insertional mutagenesis as well as autoimmune and inflammatory responses in the patients. In the case of insertion mutagenesis, the introduced gene integrates into the host genome at a site that promotes oncogenesis. The use of viral vectors can keep the risks of insertion mutagenesis at a low level. Further potential risks of harm include the autoimmune and inflammatory responses of the body of the patients. This risk can be also mitigated by the use of certain viral vectors and by a careful control of immune and inflamatory responses during the therapy.

Viral and non-viral vectors are both available for the delivery of genetic material into the host cells. Non-viral techniques are mainly tested in preclinical models; therefore, in this present work, the review of these techniques is limited to short descriptions. These methods include electroporation, gene gun, intranasal injection of the genetic material, and liposomes coated with polyethyleneglycol (PEG). The electroporation techniques enhance the permeability of the membranes after the injection of the genetic material by applying controlled electric fields. The gene gun method represents a direct gene delivery into tissues or cells by injecting gold particles coated with DNA, which can penetrate into the nucleus. The other two approches allow an easier access to the central nervous system (CNS), as they solve the problem of getting across the blood-brain barrier (BBB). The first solution is the direct intranasal injection of the genetic material, whereas the second option is transferring the genetic material via liposomes (coated with PEG), which are stable in blood and, after modifications, they can be actively transported into the CNS.

The first vectors used for gene therapy were of adenoviral and herpes simplex viral types; however, they were replaced by two vectors that are less toxic and less prone to produce immune response. At present, the most widely applied vectors are the lentiviral and the adeno-associated viral (AAV) vectors. In case of AAV, the majority of the virus genome is removed, which results in a decreased risks of secondary immune reactions and insertion mutagenesis. Because the majority of the virus genome is removed, the viral genome remains episomic and is not integrated into the host genome, thereby reducing the risk of insertional mutagenesis. Due to these advantages of the AAV vectors, this is the most commonly used type of vectors for gene therapy. Nonetheless, it has a main limitation, which is the restriction of the size of the delivered gene constructs. On the other hand, lentiviral vectors can deliver larger gene constructs. Of note, these constructs integrate into the host genome, which on the one hand may evoke insertional mutagenesis, whereas on the other hand, this approach enables longer

gene expression as a benefit of the integration. Nevertheless, it should be noted that the target neurons are mainly in their postmitotic stage, which may limit the risk of insertional mutagenesis. One of the main problems to be solved as regards viral gene therapy is the penetration of the agents across the BBB. Notably, neither of these two viruses can pass through the BBB; therefore, this form of treatment requires craniotomy.

The aims of gene therapy in PD are to increase the extent of DA production or the number dopaminergic nerve terminals. Altogether eight PD gene therapies have so far been conducted in phase I or phase II clinical trials (Table 1.). All of them used AAV or lentiviral vectors.

2.1.1 ~~AAV2-GAD~~Adeno-associated viral type 2- glutamic acid decarboxylase (AAV2-GAD)

The first human *in vivo* gene therapy study with the aim of treating neurodegenerative disorders was a safety and tolerability study with the AAV2-GAD construct in PD[24].

The used gene was the glutamic acid decarboxylase (GAD), which encodes the rate-limiting enzyme for the synthesis of gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter within the brain. Earlier studies revealed that the CSF level of GABA is significantly decreased in PD[25] and that GABAergic drugs injected into the region of the subthalamic nucleus (STN) could attenuate the disease symptoms[26]. In PD, the activity of the STN is increased, mainly due to a decrease in GABAergic inhibition from the globus pallidus[27-29]. In line with these, encouraging results emerged from preclinical experiments on rats[30] and macaques[31] with AAV2-GAD therapy.

In a human clinical trial, 11 male and 1 female PD patients between 25 and 70 years of age and with a Hoehn and Yahr stage of 3 or greater were enrolled, all of them presenting with intolerable motor complications due to L-DOPA. Four patients received low-dose, four received medium-dose, and four received high-dose AAV2–GAD injections, which were injected unilaterally into the subthalamic nucleus (STN) region of the clinically less affected side. Each patients underwent surgery, and there were no dropouts or patients lost. No treatment-related adverse events or immune responses were reported during the one year of follow-up. Significant improvements were measured in the motor Unified Parkinson's Disease Rating Scale (UPDRS) scores after 3 months, predominantly on the side of the body contralateral to the surgery, and this effect persisted for the duration of the trial. 18F-

fluorodeoxyglucose positron emission tomography (18F-FDG-PET) was used to assess the changes in regional metabolism and network activity after the treatment. The above results were associated with increases in metabolism in the premotor cortex of the operated hemispheres, suggesting that the therapy changed the activity of the motor cortico-striato-pallido-thalamo-cortical circuit, which ameliorated the motor function ~~as well as preserved the cognitive functioning in these patients~~[32]. Contrary the activity of the cognition-related network did not change after gene transfer, which suggests that the modulation of abnormal network activity underlies the clinical benefit of the AAV-GAD gene therapy in PD[32].

After this successful Phase I study, a double-blind, Phase II, randomised-controlled trial was conducted in seven centers in the USA, which was a bilateral delivery trial with AAV2-GAD[33]. All patients enrolled had a progressive, L-DOPA-responsive, advanced PD, with a UPDRS motor score of 25 or above, and with an age betwen 30 and 75 years. The utilized dose was the highest applied in the Phase I trial ($1*10^{12}$ vg/ml). 23 patients were randomly selected to sham surgery and 22 to AAV2-GAD therapy; out of these, eventually 21 and 16 patients were examined, respectively. The sham group received bilateral intradural injection of sterile saline. The endpoint of the trial was at 6 months after surgery. Significant difference was observable in the UPDRS scores, with 8.1-(23%) and 4.7-point (13%) decreases in the AAV2-GAD and the sham group, respectively. The AAV2-GAD group achieved a significantly greater improvement from baseline in UPDRS scores as compared with the sham group the 6-mont duration of the study. The reported mild and moderate adverse events were probably related to surgery, presenting in headache and nausea.

These results support the rationale for further development of bilateral injection therapy with AAV2-GAD into the subthalamic nucleus for PD, and suggest promising opportunities for gene therapy in other neurological disorders.

It could be noticed from the above results that not only the treatment group but also the sham-treated group achieved a certain extent of amelioration. This placebo or sham effect represents a major obstacle in the development of therapies in PD. A recent study suggest the use of individualized subject selection based on a predetermined network criterion, which may limit the need for sham interventions in future clinical trials[34].

2.1.2 AAV2-Glial-derived Neurotrophic Factor

Gene therapy can be applied to halt the disease progression and to restore neuronal function. To achieve these goals, neurotrophic factors can be used to promote normal cell function and to enhance the survival of damaged nigral dopaminergic neurons.

The most extensively studied neurotrophic factor in PD is the glial cell line-derived neurotrophic factor (GDNF). Its safety and efficacy have been demonstared at the preclinical level in PD animal model studies, in which direct injection of GDNF was proven to ameliorate nigrostriatal dopaminergic cell death and to promote dopaminergic axonal sprouting[35-38]. Moreover, in primate models of PD, findings on the effects of GDNF treatment with the use of different viral vectors suggested that this form of therapy may mediate plasticity in the DA-depleted brain and ameliorate the lesion-induced behavioral deficits[39, 40].

Human ICV administration therapeutical studies provided rather promising results[41, 42], which could further facilitate the initiation of viral vector-mediated delivery of GDNF genes in the clinical practice[43].

The most extensively examined GDNF family member, Neurturin (NTN) (CERE-120), showed efficacy and safety both in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced primate and 6-hydroxydopamine (6-OHDA)-induced rodent models of the disease[44].

Twelve PD patients were treated in a Phase I trial with bilateral intraputaminal injection of NTN with 2 dose levels without serious adverse events[45]. The therapy was safe and well-tolerated, and after 1 year, significant clinical improvement (36%) was reached in OFF-medication motor UPDRS scores. At the same time, the 18F Fluorodopa PET imaging did not indicate significant increases in the number of dopaminergic nerve terminals.

In 2010, 58 PD patients were enrolled in a randomized, double-blind, sham surgery-controlled clinical trial[46]**. Unfortunately, there were serious adverse events in 13 of 38 patients from the treated group and 4 of 20 from the sham surgery cohort. Three patients from the first (one glioblastoma, one oesophageal adenocarcinoma and one adenocarcinoma of the prostate) and two from the second** (parotid gland tumor, apocrine gland adenocarcinoma) **group developed tumors. The quan**titatative PCR assays were negative for AAV2-neurturin in each occasion. In case of the glioblastoma the deeper investigation revealed that it had been present on MRI before the study entry. For these reasons the tumours were not thought to be related to the AAV2-neurturin treatment, albeit this possibility cannot be completely foreclosed. Two patients from the treated group died (one myocardial infarction

at 47 days and one pulmonary embolism at 91 days postoperatively), but these deaths were not judged to be related to the treatment[46].

~~Moreover,~~The patients who received NTN treatment did not reach significant improvement in OFF-state motor UPDRS scores at the end of the first year. However, the study raised the possibility that benefit might be achieved by additional targeting of the substantia nigra and by the use of longer term follow-up periods in future studies.

In a two-year safety trial of bilateral therapy of CERE-120 injected into the SN and putamen suggested that the procedures were safe (Class IV evidence) and well tolerated, with no serious adverse events reported[47].

Based on these observations, a Phase IIb, double-blind, sham surgery-controlled trial investigated the efficacy of combined intraputaminal and intranigral gene delivery of CERE-120 in PD patients[17]. Even though this trial could not confirm the efficacy of the treatment at the primary endpoint, there were significant improvements achieved in certain secondary endpoints. Nevertheless, the therapy was safe and well tolerated.

A recent published results failed to show better efficacy compare to sham surgery in a double-blind, randomized AAV2-Neururin treatment bilaterally in the substantia nigra and the putamen (NTC00985517)[48]. There were no significant difference between the two groups in the primary and most in the secondary endpoints. No clinically relevant adverse events occurred to the treatment; only two patients had cerebral hemorrhages with transient symptoms. The therapy was safe and well-tolerated.

Post-mortem assessment of four patients after putaminal neurturin treatment revealed modest improvement in the patients's brain even four year after the therapy[49]. It was an evidence of the long-term, stable and persistently targeted gene-transfer-mediated neutrophic factor expression, but these neurons represented a very small proportion of the total neuronal population. These results may help to design the treatment protocols in the future therapies.

A new study is currently recruiting its participants for an open-label dose-escalation study of AAV2–GDNF delivery in advanced PD patients to analyze the safety, tolerability, and efficacy of bilateral treatment into the putamen in 4 doses (NCT01621581).

The summary of recent studies suggests that this therapeutic approach may only be effective in relatively mild PD, which can be an explanation for the negative results of the clinical trials to date[17, 50].

2.1.3 **AAV2-AADC**

Another gene therapeutical opportunity in PD is to improve the efficiency of L-DOPA conversion to DA. The aromatic L-amino acid decarboxylase (AADC) gene encodes an enzyme that transforms both endogenous and pharmacologically administered L-DOPA to DA, which suggests a promising opportunity. In advanced PD, the activity of AADC is reduced due to the loss of nigrostriatal neurons, thereby reducing the level of endogenous DA. Due to reduced DA levels, the patients require higher doses of L-DOPA[44]. *In vivo* gene therapy by the use of AAV2-AADC construct can enhance DA synthesis and may ameliorate the efficacy of the applied L-DOPA treatment. The terapeutic benefit might be the reduction of the utilized dose of L-DOPA, which could result in an alleviation in the associated side effects.

Earlier preclinical studies with primate models of PD resulted in robust gene expression changes lasting for more than seven years[51, 52]; therefore, lower doses of L-DOPA were sufficient and behavioral improvement could be reached without side effects typically associated with higher doses of L-DOPA.

Five moderate-to-advanced PD patients were enrolled in the first human Phase I safety trial with bilateral injection of a low dose of AAV2-AADC vector into the putamen[53]. The results showed a modest improvement; nevertheless, the absence of control group and the non-blinded analysis made the interpretation difficult. These initial data demonstrated the safety and tolerability of the therapy at low-dose, and prompted to try higher doses in future trials to come.

In the next trial, 10 patients with moderately advanced PD received bilateral intraputaminal treatment[54]. Five of them received low-dose and five of them received high-dose therapy, and the standardized clinical rating scales were used to measure the clinical state at baseline and at 6 months**.** The therapy was well tolerated in these cohorts as well, only the surgical intervention showed possible association with increased risks of intracranial hemorrhage and headache. Asymptomatic hemorrhage (at 2 subjects), small subdural/subarachnoid hemorrhage (at one patient), intracerebral hemorrhage associated with venous infarct (at one subject) and symptomatic hemorrhagic infarct had occurred. The hemorrhages happened along the trajectory of the catheter, but far from the place of infusion, presumably there were side effects of the surgical procedure. The most common

adverse event were the self-limited headache and discomfort at the operation site, but they were short-lived. No related adverse events occurred along the AAV2-AADC therapy. The measured total and motor rating scales improved in both treatment groups. The 6-month ^{18}F fluoro-L-m-tyrosine (FMT) PET results showed greater improvement in the higher-dose as compared with the lower-dose cohort (75% vs 30%). The necessary amount of dopaminergic medication was reduced in 8 patients (5 from the high-dose and 3 from the low-dose group). These results provided class IV evidence for the improvement of the mean scores in the UPDRS by approximately 30% in both the ON and OFF states.

A subsequent study aimed to retrospectively analyze the magnetic resonance imaging (MRI) and PET data from theabove mentioned Phase I trial. Morover, the study correlated the data with similar non-human primate dataset to improve future PD gene therapy trials in preparation for the initiation of the Phase II trial[55]**.** Ten PD patients treated with bilateral MRI-guided putaminal infusions of AAV2-AADC were enrolled and three normal adult non-human primates received similar infusions into their thalamus. Based on the joint alanysis of the MRI, PET, and AADC immunohistochemistry results, the authors presented recommendations for future protocols with the use of T2-weighted MRI, as this modality appeared to allow visualization of a significant part of the distribution volume of the AADC therapy.

2.1.4 **ProSavin®**

ProSavin® therapy includes 3 different genes that are involved in the production of endogenous DA synthesis. Tyrosine hydroxylase (TH) and guanosine triphosphate cyclohydrolase (GCH) are responsible for catalyzing the conversion of dietary tyrosine to L-DOPA, which can then be further metabolized to DA via AADC. The aim of this approach is not only to increase the DA level in the striatum (via increased AADC activity), but also to further increase the availability of endogenous L-DOPA.

Preclinical studies in 6-OHDA-induced rodent and MPTP-induced primate PD models provided promising results with the intrastriatal transduction of three AAV vectors, which separately carried the three genes[17]. These vectors were able to increase dopamine concentrations, so later a three gened lentivirus vector was developed to transduce genes for all three enzymes [Lenti–TH–AADC–GCH (ProSavin®)]. This was able to increase extracellular striatal DA concentrations in animal models of PD[56]. The advantage of this

technique is that it may be suitable for providing long-term gene expression and thereby less pulsatile DA delivery in the striatum. This benefit could presumably reduce the risk of L-DOPA-associated side effects (i.e. dyskinesia and hallucination). The first results in an MPTP macaque model treated with striatal injection of the tricinstronic lentiviral vector demonstrated that this treatment was safe and effective without evoking dyskinesias[57].

The first Phase I/II open-label trial with a 12-month follow-up demonstrated the safety and efficacy of ProSavin® after bilateral injection into the putamen[56]. Fifteen patients received three doses of ProSavin®, three of them received low-dose ($1.9*10^7$ transducing units (TU)), six of them received mid-dose ($4.0*10^7$ TU), and six of them received high-dose ($1*10^8$ TU) treatment. After the first year of follow-up, 54 mild or moderate adverse events were reported, and no serious adverse events occured. Significant improvement could be detected in mean UPDRS motor scores OFF medication compared with baseline in every patients at 6 months and after one year (NCT00627588)[56]. This safety, tolerability and efficacy trial has been prolonged for 10 years in order to provide further data about ProSavin® therapy (NCT01856439). Furthermore, preparations have been started to optimize the effective drug dose for a randomized, placebo-controlled human clinical trial[17].

Summarizing these results, the above clinical trials have shown that these therapies are generally safe and well tolerated, suggesting that this method could be an applicable treatment for PD in the near future.

2.2. Other therapeutic possibilities:

2.2.1. Metabotropic glutamate receptor 5 antagonists

The development of LID, an important complication of L-DOPA substitution, has a severe impact on the quality of life of PD patients. The pathomechanism of LID has been associated with alterations of both dopaminergic and glutamatergic neurotransmission. Metabotropic and ionotropic glutamate receptor antagonists have been suggested to be able to alleviate LID based on the findings of animal models[58]. Mavoglurant (AFQ056) is a selective metabotropic glutamate receptor 5 antagonist, the beneficial effects of which on LID were first shown in primates[59]. Two randomized, double-blind, placebo-controlled Phase II studies evaluated the efficacy of mavoglurant in PD patients with moderate-to-severe LID. The two studies assessed LID by the use of two different scales: the Lang-Fahn Activities of Daily Living

Dyskinesia Scale and the modified Abnormal Involuntary Movement Scale. Although these studies involved only 29 patients, both of them clearly confirmed the efficacy of mavoglurant. Dyskinesia significantly improved without any influence on the antiparkinsonian effect of L-DOPA[60]. Another clinical trial conducted by **Stocchi** et al. also confirmed its efficacy and safety in 2013(Nincs az endnote-ban ilyen szerző, sőt utána is két cikket említesz, de csak egy hivatkozás van a mondat végén); however, two following clinical trials (NCT01385592 and NCT01491529) have failed to prove its efficacy and the investigations of mavolgurant have therefore been discontinued[61].

Dipraglurant (ADX48621), another metabotropic glutamate 5 receptor antagnoist, has so far been investigated in a Phase IIa study. The primary outcomes were safety and tolerability, and the study involved PD patients with moderate-to-severe LID. The results showed a moderate efficacy in reducing LID, and the drug was generally well tolerated. The main adverse events reported were nausea, dizziness, and dyskinesia. The company has already announced the initiation of a Phase II trial, and a PET-imaging study is also currently ongoing to assess metabotropic glutamate receptor 5 occupancy of dipraglurant[62, 63].

2.2.2. **Calcium channel blockers**

Isradipine is a member of dihydropiridyne calcium channel blockers and is an approved drug for treating hypertension. However, it has recently been suggested to have a disease-modifying potential in PD patients. The first data suggesting its protective role came from mouse models of PD[64, 65]. Later, epidemiological studies also indicated that the use of calcium channel blockers as antihypertensive therapy was associated with a reduced risk of developing PD[66, 67]. The possible background and the importance of Cav1.3-containing L-type calcium channels in the regulation of DA receptor responses in the substantia nigra have been described only recently[68, 69].

A pilot study evaluated the safety and tolerability of isradipine in PD patients in 2010, which confirmed that isradipine up to 10 mg was was well tolerated and caused only minor side effects, the most frequent ones being dizziness and leg edema[70]. Furthermore, in this study, isradipine had no effect on blood pressure or motor function of PD patients. These results have been confirmed by a Phase II trial, which established the maximum tolerated dose of isradipine to be 10 mg[71]. Whilst the current data did not confirm any immediate symptomatic benefit in PD patients, based on the promising preclinical results and the good tolerability,

isradipine warrants further investigation to assess its possible neuroprotective capacity. A Phase III study is currently ongoing to assess the efficacy of isradipine in PD.

2.2.3. **Adenosine A2A receptor antagonists**

Adenosine A_{2A} receptors have been implicated in the pathomechanism of PD, as they may take part in the modulation of glutamatergic and GABA-ergic neurotransmission and may also influence striatal DA receptors. This mode of action may influence the activation of the indirect striatopallidal pathway. Adenosine A_{2A} antagonists (Figure 2.). have been tested as an early monotherapy for previously untreated PD patients, but they may also hold promise for PD patients with motor fluctuations or dyskinesia [72]. Several A_{2A} antagonists have already been developed, such as istradefylline, tozadenant, vipadenant and preladenant.

Figure 2.

Istradefylline is the first A_{2A} antagonist that has been approved for marketing in Japan, although in the United States, the Food and Drug Administration (FDA) rejected its approval. The drug has been tested both as a monotherapy and in combination with L-DOPA. As a monotherapy, istradefylline did not improve motor symptoms of PD patients[73, 74]. However, as an adjunctive therapy to L-DOPA, istradefylline produced more promising results. Several studies revealed an improvement in UPDRS motor scores; however, some of them did not prove any motor improvement[73, 75, 76]. On the other hand, a more consistent finding was the reduction of the OFF-time and the prolonged effect of L-DOPA[73, 75, 77-79]. Istradefylline was generally well tolerated, the most commonly reported adverse events were nausea, dizziness, and the prolongation of dyskinesia during the ON-time. Interestingly, the severity of dyskinesia did not worsen, only its duration increased, which was mostly considered by the patients to be well tolerable. As the FDA did not approve the use of istradefylline and considered the available evidence to be insufficient, further investigations are on their way to assess the efficacy of this novel drug.

Tozadenant is another very promising A_{2A} receptor antagonist, which has already successfully completed two Phase II trials. In the first trial, 20 and 6 mg daily doses of tozadenant were assessed, and the drug was confirmed to improve UPRDS motors scores by 20%. The

beneficial effects were particularly pronounced in relation to the amelioration of bradykinesia[80]. The effect was dose-dependent. In the other Phase II trial, four doses of tozadenant were investigated, ranging between 60 mg and 240 mg. This study was of 12 weeks duration and all doses were administered in combination with L-DOPA. The results reached statistical significance in all outcome measures: reduction of OFF-time, increase of ON-time, and improvements in both motor and non-motor UPDRS scores[81]. The reported adverse events were very similar to those of istradefylline: dizziness, nausea, dyskinesia, and insomnia.

Preladenant and vipadenant displayed promising efficacy in Phase II trials; however, the research on both drugs has been discontinued. Preladenant failed in Phase III trials, while vipadenant was associated with safety issues, and therefore the investigations now focus on a next-generation compound, V8144[82].

3. Conclusion

The first evidence from the PD gene therapy trials showed that these approaches are safe and well tolerated, but none of the studies have indicated sufficiently robust clinical efficacy. The most important advantage of these studies that they contribute to the solution of major safety hurdles that previously suppressed CNS-related gene therapy. The main remainig tasks include the development of more predictive animal models, optimization of clinical trial design and patient selection, development of better delivery approaches and finally the establishment of the appropriate dose. Metabotropic glutamate receptor 5 antagonists are investigated for the therapy of LID, so far, only limited results are available. The calcium channel blocker isradipine has been suggested to be neuroprotective, currently only the safety is confirmed in PD patients. Adenosin A_{2A} antagonists show promise for the management of motor complications in advanced PD patients.

4. Expert opinion

4.

Therapeutic management of PD patients often represents a challenge for neurologists. While the gold standard remains L-DOPA substitution, long-term therapy may induce motor complications such as dyskinesia, and the non-physiological stimulation of DA receptors may

also result in motor fluctuations. The therapy of these complications, as well as that of non-motor symptoms remain to be solved. Another important therapeutic gap is the lack of disease-modifying agents, as currently no proved neuroprotective drug is available. A number of novel approaches exist aiming to solve the problem of this terapeutic gap. Over the last 10 years, several new techniques appeared in the palette of clinical trials. One of them was the gene therapy. Like other therapetic modalities, gene therapy approaches have both advantages and disadvantages. The most important advantages include that these approaches may exert both symptomatic and disease-modifying effects, and that, by the application of genome-integrating lentiviral vectors, long-term gene expression can be reached. The symptomatic approach have concentrated on increasing of the dopamine production (AADC, TH, GCH)/enhancing the efficiency of the levodopa conversion to dopamine (AADC) and normalizing the basal ganglia circuitry (GAD) by modulation of the neuronal phenotype[17, 83]. The main disadvantages of all therapies directed at replacing dopamine are unlikely to solve the burden of non-dopaminergic problems in PD. The disease modifying approach have focused on halt the disease progression, restore neuronal function and increasing dopaminerg nerve terminals (GDNF, Neurturin)[17]. However, the use of this therapy carries several inherited risks and side effects. Some of these side effects are attributed to craniotomy such as headache and hemorrhage. No serious adverse events related to the virus or the carried gene(s) occurred in the clinical trials so far. ~~Indeed, T~~the currently applied viral vectors are unable to penetrate the BBB; therefore, effort needs to be put in the development of gene therapeutic approaches that will not require surgery in the future. Immunogenicity and carcinogenicity are also among the main risks of the therapy; however, certain approaches already exist to decrease these risks. Other disadvantages of viral vectors include their poor specificity to the target cells, the limited size of the genes that can be transduced, and the high expenses of the approach. Therefore, other approaches (non-viral vectors, nanocarriers etc.) may be potential alternatives to viral vectors to reach better efficiency in gene therapy[84]. Summarizing the above detailed results, gene therapy that targets the striatum, STN, and substantia nigra can be safe and well tolerated in PD patients; however, significant challenges remains to be solved in the future. The most important questions are how we can control and modulate gene expression, and how to determine the optimal target, dose, and patient population. The answers to these questions require further clinical investigations.

The calcium channel inhibitor isradipine has been suggested to have neuroprotective properties, but strong clinical evidence is still eagerly awaited. Clinical studies suggest that

isradipine is well tolerated; however, the currently available data is limited, and larger cohorts of patient are needed to draw conclusions. The risk of orthostatic hypotension, which is a frequent symptom in PD patients, is an important issue; however, only patients in very early stages of PD have so far been involved in the trials, which necessitates isradipine be tested in advanced stages as well to permit conclusions on this potential side effect. Nevertheless, isradipine seems to be generally well tolerated, hence efficacy studies are awaited to prove its disease-modifying property.

After the first promising results, the metabotropic glutamate receptor 5 antagonist mavoglurant failed to prove efficacy in the treatment of LID. However, clinical trials confirmed that targeting metabotropic glutamate receptors 5 may still be a rational approach to manage LID. Dipraglurant is currently investigated in clinical trials, but the first data were reassuring. Improtantly, antagonists of metabotropic glutamate receptor 5 were well tolerated and safe; therefore, future investigations are definitely warranted.

Adenosine A_{2A} antagonists are promising novel candidates for drug development, especially for the management of motor complications in advanced stages of PD. Istradefylline is already marketed in Japan; however, the FDA considered the available evidence inconclusive for approval. While the prolongation of the ON-time seems to be confirmed, the different trials yielded mixed results as concerns the motor symptoms. Another important aspect is the presence of dyskinesia, as it has been reported to be prolonged by istradefylline. Though most patients considered the dyskinesia non-troublesome, further investigations are justified to assess the global effect of istradefylline on motor functions and the quality of life of PD patients. Tozadenant have so far produced more conclusive results, and importantly, it did not worsen dyskinesia in the ON-time. This drug was also able to improve non-motor UPDRS scores. The currently available therapies are often unable to manage motor complications in advanced PD patients; therefore, A_{2A} antagonists are promising candidates and are likely to reach the market in the next decade.

5. Conflict of interest

The authours declare that they have no conflicts of interest.

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1. Table Summary of clinical gene therapy trials in Parkinson's disease

Gene	Vector	Target	n	Clinical state	Dose	Study features~~duration~~	Adverse events	Study phase	Outcome	NTC-number	Ref.
GAD	AAV2	u. STN	12	-H and Y stage 3 or greater	Low $1*10^{11}$ vg/ml Medium $3*10^{11}$ vg/ml High $1*10^{12}$ vg/ml	1 year Double-blind: No Sham-suregry: No Randomized: No	No	1	-Significant improvements in motor UPDRS scores -Safe and well tolerated	00195143	24, 32
GAD	AAV2	b. STN	44	-25≤ UPDRS motor score (OFF state)	$1*10^{12}$ vg/ml	6 months Double-blind: Yes Sham-suregry: Yes Randomized: Yes	1 serious, not attributed to the treatment Mild or moderate: headache, nausea	2	-Significant improvements in motor UPDRS scores -Safe and well tolerated	00643890	33
GDNF	AAV2	b. puta	Ongoing	Advanced	$0.7*10^{12}$ vg/patie	O~~o~~ngoing	NA	1	NA	01	43

		men			nt	Double-blind: No Sham-suregry: No Randomized: No				6215811	
Neurturin (CERE-120)	AAV2	b. putamen	12	-Moderate to severe, -H and Y stage 3 or 4, -30≤ UPDRS motor score (OFF state)	Low $1.3*10^{11}$ vg/patient High $5.4*10^{11}$ vg/patient	1 year Double-blind: No Sham-suregry: No Randomized: No	No	1	-Significant improvements in motor UPDRS scores -Safe and well tolerated	00252850	[45]
Neurturin (CERE-120)	AAV2	b. putamen	58	-Advanced PD, -30≤ UPDRS motor score (OFF state)	$5.4*10^{11}$ vg/patient	1 year Double-blind: Yes Sham-suregry: Yes Randomized: Yes	Serious adverse events: 13 of 38 in the neurturin group (3 tumors) 4 of 20 in the sham surgery controls (2 tumors)	2	-No significant improvements in motor UPDRS scores	00400634	[46]
Neurturin (CERE	AAV2	b. putamen	6	-Moderately advanced, - H and Y stage	Low $4.0*10^{11}$ vg/patie	2 years Double-blind: No	No	1	-Safe and well tolerated	009	[47]

-120)		, STN		2 or 3 -34≤ UPDRS motor score (OFF state)	nt High $5.4*10^{11}$ vg/patient	Sham-suregry: No Randomized: No				8 5 5 1 7	
Neurturin (CERE-120)	AAV2	b. putamen , STN	51/47	-good-response to L-Dopa -stable doses of antiparkinsoinan drugs for at least 6 weeks -mean H and Y stage 2.5 (0.51) -the mean value of UPDRS motor score (OFF state) were bigger than 35 in both groups	Substantia nigra $2.0*10^{11}$ vg/patient Putamen $1.0*10^{12}$ vg/patient	2 years Double-blind: Yes Sham-suregry: Yes Randomized: Yes	No clinically adverse events occured	1	Safe and well tolerated	0 0 9 8 5 5 1 7	[48]
AADC	AAV2	b. putamen	5	-Moderate to advanced	Low	6 months Double-blind: No Sham-suregry: No Randomized: No	No	1	- Safe and well tolerated - Modest improvement (interpretation dificulties: no control, non-blinded analysis) -PET: evidence of sustained gene expression	N A	[53]
AADC	AAV2	b. putamen	10	-Moderately advanced	Low $9*10^{10}$ vg/patie	6 months Double-blind: No	No serious 1	1	-Safe and well tolerated -The necessary amount of dopaminergic	0 0 2	[54]

					nt High $3*10^{11}$ vg/patient	Sham-suregry: No Randomized: No	symptomatic and 2 asymptomatic intracranial hemorrhages, headache		medication was reduced in 8 patients -FMT PET: 30% increase of putaminal uptake in the low-dose cohort, 75% increase in the high-dose cohort -Total and motor rating scales improved in both cohorts -Motor diaries also showed increased ON-time and reduced OFF-time without increased ON-time dyskinesia	29736	
ProSavin® (Lenti–TH–AADC–GCH)	lenti-virus	b. putamen	15	-H and Y stage 3 or 4 in (OFF state) -UPDRS (OFF medication) between 20 and 60;	Low $1.9*10^{7}$ TU Medium $4.0*10^{7}$ TU High $1*10^{8}$ TU	1 year, prolonged for 10 years Double-blind: No Sham-suregry: No Randomized: No	No serious 51 mild, three moderate	1/2	-Safe and well tolerated -Significant improvement in mean UPDRS part III motor scores OFF medication -Improvement in motor behavior was observed in all patients.	00627588-;-018564	[56]

										[3 9]	

u.:unilateral, b.: bilateral, H and Y: Hoehn and Yahr stage, TU: transducing units.

Figure legends

Figure 1. Gene therapy approaches.

I. *Ex vivo* gene therapy: In case of this approach, patients receive genetically modified cells that express a desired protein or proteins. The genetic modifications of the patient's target cells are performed outside the body, in a cell culture.

1.:Copies of the therapeutic gene(s) integrate into the viral DNA. 2.: The target cells of the patient are removed and grown in a cell culture. 3.: The cultured cells are transfected with the genetically modified virus. 4.: These transfected cells are reintroduced into the patient's body, where they express the necessary protein(s).

II. *In vivo* gene therapy: In this case, the genetic information is directly inserted into the patient's own cells.

1.: The therapeutic gene(s) can be inserted into viral DNA, coated in a liposome or created in form of a plasmid DNA. 2.:The genetically modified DNA is transferred by cell-specific direct tissue injection (or in case of a plasmid vector by dermal vaccination). 3.: Inside the patient's body, the inserted DNA is incorporated into the cells of the targeted tissue and starts to produce the encoded protein(s).

Figure 2. Adenosin A_{2A} antagonists

This figure displays the chemical structures of adenosin A_{2A} antagonists.