Expert Opinion on Biological Therapy

ISSN: 1471-2598 (Print) 1744-7682 (Online) Journal homepage: http://www.tandfonline.com/loi/iebt20

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To cite this article: Zsófia Majláth, Nóra Török, József Toldi & László Vécsei (2016): Promising therapeutic agents for the treatment of Parkinson's disease, Expert Opinion on Biological Therapy, DOI: 10.1517/14712598.2016.1164687

To link to this article: http://dx.doi.org/10.1517/14712598.2016.1164687

Accepted author version posted online: 09 Mar 2016.

Publisher: Taylor & Francis

Journal: *Expert Opinion on Biological Therapy*

DOI: 10.1517/14712598.2016.1164687

Promising therapeutic agents for the treatment of Parkinson's disease

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Review

Promising therapeutic agents for the treatment of Parkinson's disease

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Abstract

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

Areas covered: This review focuses on promising novel therapies that are at present under investigation in Phase I or Phase II trials. Special emphasis is placed on gene therapies: vectors, the utilized gene constructs and the side-effects. Moreover, the main risk factors of the gene therapy (the insertional mutagenesis, the uncontrolled overproduction of the expressed protein and the autoimmune and inflammatory responses) are described.

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

Gene therapy appears to be promising candidate for the management of motor complications in advanced stages of Parkinson's disease. In addition to dopamine replacement therapy, this field may also offer a solution for neurogenesis and neuroprotection.

Highlights box:

- To date, only an *in vivo* gene therapy approach has been utilized in Parkinson's disease (PD).
- The safest viral vector is the adeno-associated viral type 2 (AAV2) vector, but its limitation is the restriction of the size of the delivered gene constructs; only lentiviral vectors can deliver larger gene constructs.
- The main risk factors of the gene therapy are the insertional mutagenesis, the uncontrolled overproduction of the expressed protein and the autoimmune and inflammatory responses of the patients.
- Most of the gene therapy trials in PD are focused on dopamine replacement, though the dopaminergic system is not the only affected neurotransmitter system in PD. Neurogenesis and neuroprotection should be promising new foci of this research field.
- Clinical evidence as to the efficacy of the calcium channel blocker isradipine and the metabotropic glutamate receptor 5 antagonists has not yet been proven.
- Adenosine A_{2A} antagonists show promising results in the management of motor complications in advanced stages of PD.

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

1. Introduction

Parkinson's disease (PD) is 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 problems in the aging societies, its social significance is tremendous. A meta-analysis of a worldwide dataset revealed an increasing prevalence of PD with age: 41/100,000 among those between 40 and 49 years; 428/100,000 among those between 60 and 69 years; and 1903/100,000 among those above 80 years of age[2].

Protein aggregation, mitochondrial disturbances, oxidative stress, glutamate excitotoxicity, alterations of the tryptophan metabolism, immunological mechanisms, and genetic predisposition have all been suggested 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 in walking). In addition to these, however, non-motor symptoms are also characteristic of the disorder (i.e. dementia, depression, sleep disorder, and 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, prove to be a milestone in the treatment of PD. However, the drug can evoke side-effects, which include motor fluctuations, L-DOPA-induced dyskinesia (LID) and non-motor fluctuations. In advanced stages of PD, motor complications may appear, where the therapeutic window of L-DOPA becomes narrow: low plasma L-DOPA levels lead to an end-of dose worsening of the symptoms (wearing-off), whereas high levels may induce worsening of the dyskinesia. These motor complications are more frequent after L-DOPA treatment than after DA agonists. Both L-DOPA and DA agonists may also promote the development of other non-motor complications, such as cognitive dysfunction and neuropsychiatric symptoms (i.e. compulsive behavior and impulse control disorders (ICDs)) [15, 16]. ICDs include gambling, pathological shopping, compulsive eating and hypersexuality. Interestingly, levodopa addicition may also develop, which has the same features as other medication addiction: compulsive drug intake, persistent use of the drug and withdrawal symptoms [17]. Risk factors of developing ICDs are male sex, younger age at the onset of PD, and a prior history of any addiction or depression, and the trigger factor is dopaminergic therapy, especially DA agonists. The benefits of dopaminergic treatment may therefore be overshadowed by these troublesome side-effects aand by the appearance of symptoms that do not respond to dopaminergic treatments (i.e. autonomic symptoms, gait and balance problems, and cognitive impairment)[18].

An interesting issue is the placebo effect during clinical trials, which can be particularly high in PD patients. The placebo effect means that simply because of the expectation of a therapeutic benefit, an intervention or drug gives the sensation of being more efficacious. Positron emission tomography (PET) studies have confirmed an endogenous striatal DA release induced by placebo, which correlated with the perceived beneficial effect of a placebo [19]. DA release is closely connected with a reward mechanisms in the brain, but PET studies have also confirmed that DA release in the ventral striatum is associated with the expectation of a reward and not the actual reward itself [20]. These findings promote the understanding of difficulties for drug development in PD. These issues, together with the lack of available neuroprotective agents 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, but so far only a small number have reached the market [21, 22].

In this review, we focus on summarizing the results of recent phase I and II clinical trials with new potential therapeutic agents in PD. Gene therapy is at the focus of the article because it is a novel approach in the therapeutic palette. Besides gene therapy, novel drug candidates for drug development are also evaluated, which are currently undergoing Phase I or Phase II clinical trials.

2. Novel therapeutic possibilities in PD

2.1. Gene therapy

In the past decade, a new player has appeared in the therapeutic palette, the aim of which is to fill the above-mentioned therapeutic gaps, i.e. 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[18].

Clinical gene therapy approaches can be divided into two categories (Figure 1.). The first option is *ex vivo* gene therapy, in which the patients receive 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 have applied the *in vivo* method with the use of viral vectors; however, *ex vivo* gene therapeutic strategies may also play a role in the treatment of PD in the future[23-25]. It is of note that 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[26].

Figure 1.

A number of risk factors can be inherited as regards *in vivo* gene therapy too, including the induction of insertional mutagenesis and autoimmune and inflammatory responses in the patients. In the case of insertion mutagenesis, the introduced gene is integrated 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 mitigated by the use

of certain viral vectors and by a careful control of immune and inflammatory 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; in the present work, therefore, the review of these techniques is limited to short descriptions. These methods include electroporation, the gene gun, the 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 approaches allow an easier access to the central nervous system (CNS), as they solve the problem of passing 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, 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 an immune response. At present, the most widely applied vectors are the lentiviral and the adeno-associated viral (AAV) vectors. In the case of AAV, the majority of the virus genome is removed, which results in a decreased risk of secondary immune reactions and insertion mutagenesis. As 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. It is noteworthy that these constructs integrate into the host genome, which on the one hand may evoke insertional mutagenesis, whereas on the other hand this approach enables a 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. Neither of these two viruses can pass through the BBB, and this form of treatment therefore requires craniotomy.

The aims of gene therapy in PD are to increase the extent of DA production or the number of dopaminergic nerve terminals. However, PD affects not only the dopaminergic, but also other

neurotransmitter systems, such as the noradrenergic, serotoninergic, glutamatergic and cholinergic systems. Gene therapy is mainly focused on the dopaminergic system, but a more general repair approach might be more effective. This is one of the reasons why gene therapy has so far had only limited results. Altogether eight PD gene therapies have so far been conducted in phase I or phase II clinical trials (Table 1.). All of them involve the use of AAV or lentiviral vectors.

2.1.1 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[27].

The gene used was 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[28] and that GABAergic drugs injected into the region of the subthalamic nucleus (STN) could attenuate the disease symptoms[29]. In PD, the activity of the STN is increased, mainly due to a decrease in GABAergic inhibition from the globus pallidus[30-32]. In line with these findings, encouraging results emerged from preclinical experiments on rats[33] and macaques[34] 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 patient underwent surgery, and there were no dropouts or lost patients. 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. ^{18}Fluorodeoxyglucose positron emission tomography (^{18}F-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[35]. In contrast, the activity of the cognition-related network did not change after gene transfer, which suggests that the modulation of the abnormal network activity underlies the clinical benefit of the AAV-GAD gene therapy in PD[35].

After this successful Phase I study, a double-blind, Phase II, randomized-controlled trial was conducted in seven centers in the USA, which was a bilateral delivery trial with AAV2-GAD[36]. All the 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 for sham surgery and 22 for AAV2-GAD therapy; out of these, eventually 21 and 16 patients were assessed, respectively. The sham group received a bilateral intradural injection of sterile saline. The endpoint of the trial was at 6 months after surgery. A 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 the UPDRS scores as compared with the sham group the 6-months 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 suggested the use of individualized subject selection based on a predetermined network criterion, which may limit the need for sham interventions in future clinical trials[37].

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 the 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 the direct injection of GDNF was proven to ameliorate nigrostriatal dopaminergic cell death and to promote dopaminergic axonal sprouting[38-41]. 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[42, 43]. Human ICV administration therapeutical studies provided rather promising results[44, 45], which could further facilitate the initiation of viral vector-mediated delivery of GDNF genes in the clinical practice[46].

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[47]. Twelve PD patients were treated in a Phase I trial with bilateral intraputaminal injection of NTN with 2 dose levels without serious adverse events[48]. The therapy was safe and well-tolerated, and after 1 year, a significant clinical improvement (36%) was reached in OFF-medication motor UPDRS scores. At the same time, the ^{18}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[49]. Unfortunately, there were serious adverse events in 13 of 38 patients from the treated group and in 4 of 20 from the sham surgery cohort. Three patients from the first group (one glioblastoma, one esophageal adenocarcinoma and one adenocarcinoma of the prostate) and two from the second (parotid gland tumor, apocrine gland adenocarcinoma) group developed tumors. The quantitatative PCR assays were negative for AAV2-NTN on each occasion. In the case of the glioblastoma the deeper investigation revealed that it had been present on MRI before the study entry. For these reason, the tumors were not thought to be related to the AAV2-NTN treatment, albeit this possibility cannot be completely discounted. Two patients from the treated group died (one from a myocardial infarction at 47 days and one from a pulmonary embolism at 91 days postoperatively), but these deaths were not adjudged to be related to the treatment[49]. 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[50].

On the basis of 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[18]. Even though this trial could not confirm the efficacy of the treatment at the primary endpoint, significant improvements were achieved in certain secondary endpoints. The therapy was safe and well tolerated.

Recently published results failed to show better efficacy as compared with sham surgery in a double-blind, randomized AAV2-NTN treatment bilaterally in the substantia nigra and the putamen (NTC00985517)[51]. There were no significant differences between the two groups in the primary and most of the secondary endpoints. No clinically relevant adverse events occurred in response to the treatment; only two patients suffered cerebral hemorrhages with transient symptoms. The therapy was safe and well tolerated.

The post-mortem assessment of four patients after putaminal NTN treatment revealed modest improvement in the patients's brain even four years after the therapy[52]. This was evidence of the long-term, stable and persistently targeted gene-transfer-mediated neutrophic factor expression, but these neurons represented only a very small proportion of the total neuronal population. These results may help in the design of the treatment protocols in future therapies.

A new study is currently recruiting 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 be effective only in relatively mild PD, which can be an explanation for the negative results of the clinical trials to date[18, 53].

2.1.3 AAV2-AADC

Another gene therapeutic 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 as a result of the loss of nigrostriatal neurons, thereby reducing the level of endogenous DA. In consequence of the reduced DA levels, the patients require higher doses

of L-DOPA[47]. *In vivo* gene therapy through use of the AAV2-AADC construct can enhance DA synthesis and may ameliorate the efficacy of the applied L-DOPA treatment. The therapeutic benefit may be the reduction of the utilized dose of L-DOPA, which can lead to 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[54, 55]; lower doses of L-DOPA were therefore sufficient and behavioral improvement could be achieved without the 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[56]. The results showed a modest improvement; nevertheless, the absence of a 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 attempts with higher doses in future trials.

In the next trial, 10 patients with moderately advanced PD received bilateral intraputaminal treatment[57]. Five of them received low-dose and five of them 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 too, only the surgical intervention showing a possible association with increased risks of intracranial hemorrhage and headache. Asymptomatic hemorrhage (in 2 subjects), small subdural/subarachnoid hemorrhage (in one patient), intracerebral hemorrhage associated with a venous infarct (in one subject) and a symptomatic hemorrhagic infarct occurred. The hemorrhages took place along the trajectory of the catheter, but far from the site of infusion, and were presumably side-effects of the surgical procedure. The most common adverse events were the self-limited headache and discomfort at the operation site, but they were short-lived. No related adverse events occurred during the AAV2-AADC therapy. The measured total and motor rating scales improved in both treatment groups. The 6-month ^{18}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 amount of dopaminergic medication necessary was reduced in 8 patients (5 in the high-dose and 3 in 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 analyze the magnetic resonance imaging (MRI) and PET data from the above-mentioned Phase I trial retrospectively. Moreover, the study correlated the data with a similar non-human primate dataset to improve future PD gene therapy trials in preparation for the initiation of the Phase II trial[58]**.** 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. In view of the joint analysis 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 Lenti–TH–AADC–GCH

Lenti–TH–AADC–GCH (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[18]. These vectors were able to increase DA concentrations, and a three-gened lentivirus vector was therefore later developed to transduce genes for all three enzymes [Lenti–TH–AADC–GCH]. This was able to increase extracellular striatal DA concentrations in animal models of PD [59]. 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 with an MPTP macaque model treated with a striatal injection of the tricinstronic lentiviral vector indicated that this treatment was safe and effective without evoking dyskinesias[59].

The first Phase I/II open-label trial with a 12-month follow-up demonstrated the safety and efficacy of this therapy after bilateral injection into the putamen[60]. Fifteen patients received three doses of the drug, three of them low-dose ($1.9*10^7$ transducing units (TU)), six of them mid-dose ($4.0*10^7$ TU), and six of them 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. A significant improvement in mean UPDRS motor scores OFF medication could be detected as compared with the baseline in every patient at 6 months and after one year (NCT00627588)[60]. This safety, tolerability and efficacy trial has been prolonged for 10 years in order to provide further data concerning this therapy (NCT01856439). Furthermore, preparations have been started to optimize the effective drug dose for a randomized, placebo-controlled human clinical trial[18].

To summarize these results, the above clinical trials have shown that these therapies are generally safe and well tolerated, suggesting that this method could be 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 in both the dopaminergic and the glutamatergic neurotransmission. On the basis of the findings of animal models, metabotropic and ionotropic glutamate receptor antagonists have been suggested to be able to alleviate LID[61]. Mavoglurant (AFQ056) is a selective metabotropic glutamate receptor 5 antagonist, the beneficial effects of which on LID were first revealed in primates[62]. 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[63]. Another clinical trial also confirmed its efficacy and safety in 2013; however, two subsequent clinical trials (NCT01385592 and NCT01491529) failed to prove its efficacy and the investigations of mavoglurant have therefore been discontinued[64]. The current approaches to the investigation of dyskinesia are not consequent, and numerous different dyskinesia scales are available. These scales measure changes relating to the applied treatment in different ways and their sensitivity shows high variability, therefore further testing is recommended to evaluate their clinical properties and validity[65, 66]. The evaluation of novel drugs for the treatment of dyskinesia depends extensively on the

choice of rating scales. Another important issue is the relatively high placebo effect in PD patients, and placebo-associated improvements may confound the interpretation of the results. The impact of the placebo effect on the outcome must be considered in the study design[67]. Moreover, although no dyskinesia rating scales are free from placebo effects, the differences between these scales, and especially their objective and subjective factors, may seriously influence the evaluation of improvement[65].

Dipraglurant (ADX48621), another metabotropic glutamate 5 receptor antagonist, 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 producer has already announced the initiation of a Phase II trial, and a PET-imaging study is also currently ongoing to assess the metabotropic glutamate receptor 5 occupancy of dipraglurant[68, 69].

2.2.2. Calcium-channel blockers

Non-selective calcium-channel blockers, such as flunarizine and cinnarizine and in rare cases other calcium channel blockers such as amlodipine have been reported to cause drug-induced parkinsonism[70, 71]. However, more recent epidemiological studies did not confirm any association or indicated that the use of calcium-channel blockers as antihypertensive therapy was associated with a reduced risk of developing PD[72-75]. Isradipine is a dihydropyridine calcium-channel blocker and is an approved drug for the treatment of hypertension. However, it has recently been suggested to have a disease-modifying potential in PD patients. The first data implying its protective role came from mouse models of PD[76, 77]. 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[78, 79].

A pilot study evaluated the safety and tolerability of isradipine in PD patients in 2010 (NCT00753636), which confirmed that isradipine up to 10 mg was well tolerated and caused only minor side-effects, the most frequent ones being dizziness and leg edema[80]. Furthermore, in that study, isradipine had no effect on the blood pressure or motor function of PD patients. These results have been confirmed by a Phase II trial, which established that the maximum tolerated dose of isradipine was 10 mg[81]. While the current data did not confirm any immediate symptomatic benefit in PD patients, on the basis of 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 (NCT02168842).

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 the 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 early monotherapy for previously untreated PD patients, but they may also hold promise for PD patients with motor fluctuations or dyskinesia[82]. Another important benefit of A_{2A} antagonists might be the better side-effect profile as compared with DA agonists. 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 monotherapy and in combination with L-DOPA. As monotherapy, istradefylline did not improve the motor symptoms of PD patients[83, 84]. However, as adjunctive therapy to L-DOPA, istradefylline produced more promising results. Several studies revealed an improvement in UPDRS motor scores, though some of them did not lead to any motor improvement[83, 85, 86]. On the other hand, a more consistent finding was the reduction of the OFF-time and the prolonged effect of L-DOPA[83, 85, 87-89]. Istradefylline was generally well tolerated, the most commonly reported adverse events being nausea, dizziness, and the prolongation of dyskinesia during the ON-time. Interestingly, the severity of dyskinesia did not worsen, only its duration increasing, 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 the UPRDS motor scores by 20%. The beneficial effects were particularly pronounced in relation to the amelioration of

bradykinesia[90]. 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 achieved statistical significance in all outcome measures: a reduction of the OFF-time, an increase of the ON-time, and improvements in both motor and non-motor UPDRS scores[91]. 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 the investigations therefore now focus on a next-generation compound, V8144[92].

3. Conclusions

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 is that they contribute to the solution of major safety hurdles that previously suppressed CNS-related gene therapy. The main remaining tasks include the development of more predictive animal models, the optimization of clinical trial design and patient selection, the development of better delivery approaches and finally the establishment of the appropriate dose. Metabotropic glutamate receptor 5 antagonists are under investigation for the therapy of LID, but so far only limited results are available. The calcium-channel blocker isradipine has been suggested to be neuroprotective, though to date only the safety has been confirmed in PD patients. Adenosine A_{2A} antagonists are of promise for the management of motor complications in advanced PD patients.

4. Expert opinion

The therapeutic management of PD patients often poses 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, and 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 with the aim of the solution of the problem of this therapeutic gap. Over the last 10 years, several new techniques have appeared in the palette of clinical trials.

One of them was gene therapy. Like other therapeutic modalities, gene therapy approaches have both advantages and disadvantages. The most important advantages include the fact that these approaches may exert both symptomatic and disease-modifying effects, and that, through the application of genome-integrating lentiviral vectors, long-term gene expression can be achieved. The symptomatic approach has concentrated on increasing DA 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[18, 93]. The main disadvantages of all therapies directed at replacing DA are unlikely to solve the burden of non-dopaminergic problems in PD. The disease-modifying approach has focused on halting the disease progression, restoring the neuronal function and increasing the dopaminerg nerve terminals (GDNF and NTN)[18]. However, the use of this therapy involves several inherited risks and side-effects. Some of these side-effects are attributed to craniotomy such as headache and hemorrhage. No serious adverse events relating to the virus or the carried gene(s) have occurred in the clinical trials performed so far. The currently applied viral vectors are unable to penetrate the BBB, and efforts are therefore needed to develop 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 attain better efficiency in gene therapy[94]. To summarize the results detailed above, gene therapy that targets the striatum, STN and substantia nigra can be safe and well tolerated in PD patients, but significant challenges remain to be solved in the future. The most important questions are how we can control and modulate gene expression, and determine the optimal target, dose and patient populations. 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, but the currently available data are limited, and larger cohorts of patients 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 tests on isradipine in advanced stages too in order to permit conclusions on this potential side-effect. Nevertheless, isradipine seems to be generally well tolerated, and hence efficacy studies are awaited to

prove its disease-modifying property. Importantly, longer durations of trials are needed to assess the disease-modifying and neuroprotective capacity of the drug.

After the first promising results, the metabotropic glutamate receptor 5 antagonist mavoglurant failed to prove its efficacy in the treatment of LID. However, clinical trials have 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 initial data were reassuring. Importantly, antagonists of metabotropic glutamate receptor 5 were well tolerated and safe; future investigations are therefore definitely warranted.

Adenosine A_{2A} antagonists are promising novel candidates for drug development, and 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. Although 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 has so far produced more conclusive results, and importantly, it did not worsen dyskinesia in the ON-time. This drug was also able to improve the non-motor UPDRS scores. The currently available therapies are often unable to manage motor complications in advanced PD patients; A_{2A} antagonists are therefore promising candidates and are likely to reach the market in the next decade.

5. Conflicts of interest

The authours declare that they have no conflicts of interest.

6. Acknowledgements

This work was supported by the project TÁMOP-4.2.6.3.1., by the Hungarian Brain Research Program (NAP, Grant No. KTIA_13_NAP-A-III/9. and KTIA_13_NAP-A-II/17.) and by the MTA-SZTE Neuroscience Research Group of the Hungarian Academy of Sciences and the University of Szeged.

For the linguistic corrections, we are grateful to David Durham, Oxford, UK and Levente Szalárdy MD.

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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	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-surgery: No Randomized: No	No	1	-Significant improvements in motor UPDRS scores -Safe and well tolerated	00195143	[27, 35]
GAD	AAV2	b. STN	44	-25≤ UPDRS motor score (OFF state)	$1*10^{12}$ vg/ml	6 month Double-blind: Yes Sham-surgery: 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	[36]
GDNF	AAV2	b. putamen	Ongoing	Advanced	$0.7*10^{12}$ vg/patient	Ongoing Double-blind: No Sham-surgery: No Randomized: No	NA	1	NA	01621581	[46]
Neurturin	AAV2	b. putamen	12	-Moderat	Low $1.3*10^{11}$	1-year Double-	No	1	-Significant improvements in motor	00252850	[48]

(CERE-120)				e to severe, -H and Y stage 3 or 4, -30≤ UPDRS motor score (OFF state)	vg/patient High $5.4*10^{11}$ vg/patient	blind: No Sham-surgery: No Randomized: No			UPDRS scores -Safe and well tolerated		
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-surgery: 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	[49]
Neurturin (CERE-120)	AAV2	b. putamen, STN	6	- Moderately advanced, - H and Y stage 2 or 3	Low $4.0*10^{11}$ vg/patient High $5.4*10^{11}$ vg/patient	2 years Double-blind: No Sham-surgery: No Randomized: No	No	1	-Safe and well tolerated	00985517	[50]

				-34≤ UPDRS motor score (OFF state)							
Neurturin (CERE-120)	AAV2	b. putamen, STN	51/47	good response to L-Dopa -stable doses of antiparkinsonian drugs for at least 6 weeks -mean H and Y stage 2.5 (0.51) -the mean values of UPDRS motor scores (OFF state) larger than 35 in both	Substantia nigra $2.0*10^{11}$ vg/patient Putamen $1.0*10^{12}$ vg/patient	2-year Double-blind: Yes Sham-surgery: Yes Randomized: Yes	No clinically adverse events occurred	1	Safe and well tolerated	00985517	[51]

				groups							
AADC	AAV2	b. putamen	5	- Moderate to advanced	Low	6 month Double-blind: No Sham-surgery: No Randomized: No	No	1	- Safe and well tolerated - Modest improvement (interpretation dificulties: no control, non-blinded analysis) -PET: evidence of sustained gene expression	NA	[56]
AADC	AAV2	b. putamen	10	- Moderately advanced	Low $9*10^{10}$ vg/patient High $3*10^{11}$ vg/patient	6 month Double-blind: No Sham-surgery: No Randomized: No	No serious 1 symptomatic and 2 asymptomatic intracranial hemorrhages, headache	1	-Safe and well tolerated -The necessary amount of dopaminergic medication was reduced in 8 patients -FMT PET: 30% increase in 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	00229736	[57]
ProSavin® (Lenti–TH–AADC	lenti-virus	b. putamen	15	-H and Y stage 3 or 4 in (OFF state)	Low $1.9*10^{7}$ TU Medium $4.0*10^{7}$ TU	1-year, prolonged for 10 years Double-	No serious 51 mild, 3 moderate	1/2	-Safe and well tolerated -Significant improvement in mean UPDRS part III motor scores OFF medication	00627588; 01856439	[60]

–GCH)				-UPDRS (OFF medication) between 20 and 60;	High $1*10^8$ TU	blind: No Sham-surgery: No Randomized: No			-Improvement in motor behavior was observed in all patients.		

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

Table 2. Summary of the main clinical trials on metabotropic glutamate receptor 5 antagonists, calcium channel blockers and adenosine A_{2A} antagonists

Drug name	n	Clinical state	Dose	Study features	Adverse events	Study phase	Outcome	Ref.
Mavoglurant (AFQ056)	15	moderate to severe LID	25-150mg twice daily	16 day RCT placebo-controlled, double-blind (NCT00582673)	mostly mild: most common dizziness 4 serious: worsening of dyskinesia, hyperkinesia	2	significant improvements in dyskinesia (Lang-Fahn Activities of Daily Living Dyskinesia Scale and UPDRS scores)	63
Mavoglurant (AFQ056)	14	severe LID	25-150mg twice daily	16 day RCT placebo-controlled, double-blind (NCT00888004)	mostly mild 2 serious: psychosis, worsening of dyskinesia	2	significant improvements in dyskinesia (modified Abnormal Involuntary Movement Scale and UPDRS scores)	63
Dipraglurant (ADX48621)	NA	moderate to severe LID	50mg once daily – to 100mg three times daily	4 weeks placebo-controlled, RCT, double-blind (NCT01336088)	nausea, dizziness, dyskinesia	2	moderate efficacy (Abnormal Involuntary Movement Scale and UPDRS scores)	68, 69
Isradipine	31	PD stage 2	5-20mg	1 year non-randomized,	only minor: dizziness, leg edema	2	up to 10mg well tolerated	80

				open-label safety study (NCT00753636)				
Istradefylline	15	PD moderate to advanced stage	40-80mg	6 week, placebo-controlled, safety and efficacy study	no important	2	alone no efficacy, in combination with levodopa potentiated antiparkinsonian effect with less dyskinesia (UPDRS scores)	[83]
Istradefylline	176	PD patients Hoehn-Yahr stages 1-2.5	40mg	12 week, double blind, placebo controlled (NCT00199433 (6002-US-051))	similar as placebo	2	no efficacy	[84]
Istradefylline	83	levodopa-treated PD patients with both motor fluctuations and peak-dose dyskinesias	20-40mg	12-week, double-blind, randomized, placebo-controlled, exploratory study (6002-US-001)	most common: nausea	2	recuction of OFF state, no change of dyskinesia severity	[85]
Istradefylline	363	PD patients with motor complications	20-40mg	12-week double blind placebo controlled RCT	most common: dyskinesia	2	reduced the daily OFF time compared with placebo	[86]

				(NCT00455507 (6002-0608))				
Istradefylline	196	PD patients with wearing-off motor fluctuations	40mg	12-week double-blind, multicenter, placebo controlled RCT (NCT00456586 (6002-US-005))	generally mild	2	significantly reduced OFF time	[88]
Istradefylline	395	PD patients with motor complications	20-60mg	12-week double blind, placebo-controlled RCT (NCT00456794 (6002-US-006))	dyskinesia, nausea, dizziness, and hallucinations	2	significantly reduced OFF time	[89]
Tozadenant	30	patients with mild to moderate PD	20-60mg	7 days, randomized, double-blind, placebo controlled, study (NCT00605553)	dizziness, nausea, dyskinesia, and insomnia	2	improve UPRDS motor scores by 20%	[90]
Tozadenant	420	PD patients with motor fluctuations	60-240mg	international, multicentre, randomised, double-blind, placebo-	dizziness, nausea, dyskinesia	2	120mg and 180mg well tolerated and significantly reduced OFF time	[91]

				controlled, parallel-group, dose-finding clinical trial (NCT01283594)				

Figure legends

Figure 1. Gene therapy approaches.

I. *Ex vivo* gene therapy: with 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 the form of a plasmid DNA. 2. The genetically modified DNA is transferred by cell-specific direct tissue injection (or in the 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).

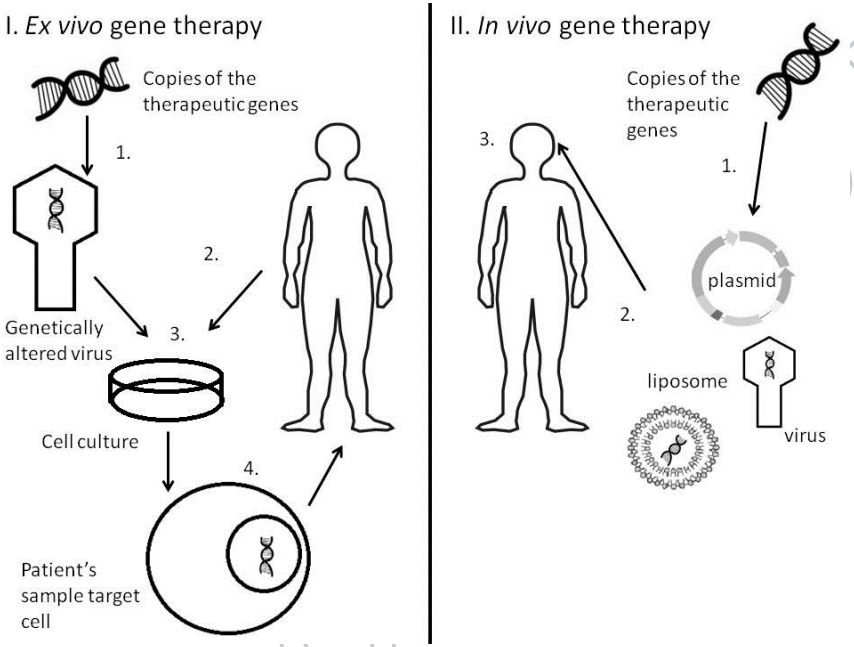
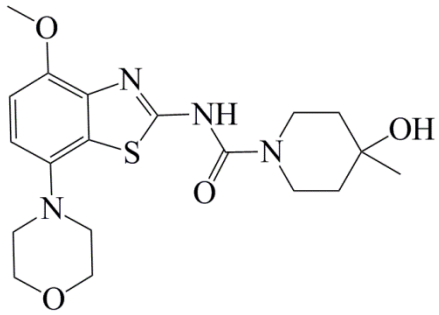
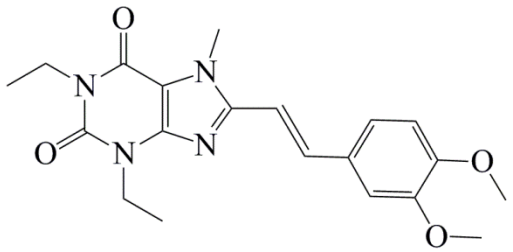


Figure 2. Adenosine A_{2A} antagonists

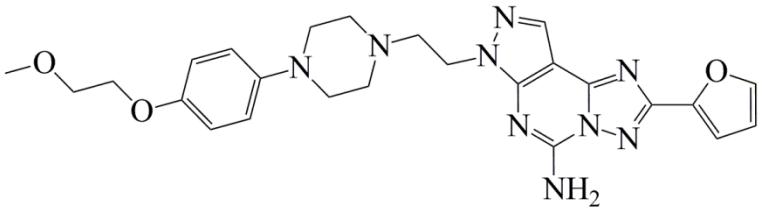
This figure displays the chemical structures of adenosine A_{2A} antagonists.



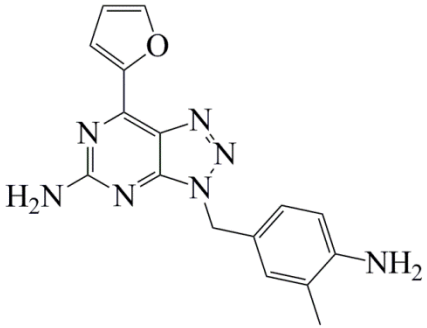
Tozadenant (SYN115)



Istradefylline



Preladenant



Vipadenant