EXPERT OPINION

Two new adenosine receptor antagonists for the treatment of Parkinson's disease: istradefylline versus tozadenant

Santiago Perez-Lloret & Marcelo Merello[†]
[†]*Raul Carrea Institute for Neurological Research, Buenos Aires, Argentina*

Introduction: Adenosine A2A receptors are localized in the brain, mainly within the caudate and putamen nuclei of the basal ganglia. Their activation leads to stimulation of the 'indirect' pathway. Conversely, administration of A2A receptor antagonists leads to inhibition of this pathway, which was translated into reduced hypomotility in several animal models of parkinsonism.

Areas covered: In this review, the effects of two A2A receptor antagonists, istradefylline and tozadenant, on parkinsonian symptoms in animal and humans will be discussed.

Expert opinion: Animal studies have shown potent antiparkinsonian effects for several A2A receptor antagonists, including istradefylline. In clinical trials, istradefylline reduced OFF time when administered with levodopa, but results are inconclusive. Results with tozadenant are scarce. Modification of thalamic blood flow compatible with reduced inhibition was noted in one small trial, followed by a significant reduction in OFF time in a larger one. Therefore, both drugs show promising efficacy for the reduction of OFF time in levodopa-treated Parkinson's disease patients, but further research is needed in order to obtain definitive conclusions.

Keywords: A2A receptor antagonists, adenosine, motor fluctuations, wearing-off

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1. Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by cardinal motor and non-motor symptoms, both contributing to worsen patient handicap and disability levels [1-4]. Dopaminergic medications have dramatically improved PD management [3,5-7], but major unmet needs remain, mainly linked to a lack of efficacious neuroprotective treatment. Few, if any, effective treatments exist currently for most non-motor symptoms, and dopaminergic medications available induce many adverse reactions (neuropsychiatric and motor complications such as OFF problems and levodopa-induced dyskinesias), limiting their use. Novel antiparkinsonian medications are therefore necessary to address such needs.

Recent results suggest that two new adenosine receptor A2A antagonists, istradefylline and tozadenant, might be effective for the treatment of PD symptoms [8,9]. In this review, the role of adenosine neurotransmission in PD symptoms and the potential efficacy and safety of A2A antagonists for the indication proposed will be discussed.

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Article highlights.

- Activation of A2A receptors in putamen nuclei leads to reduction in movement frequency and intensity through activation of the 'indirect' pathway.
- A2A receptor antagonist, istradefylline, restores hypomotility and reduces disability in various animal models of parkinsonism, both as monotherapy and with low levodopa doses.
- In humans, no effects were observed during monotherapy, while OFF time was reduced in many clinical trials, although not in all of them.
- Results with tozadenant, another A2A receptor antagonist, are scarce but suggest a similar effect on OFF time.
- Safety profile is good, with non-troublesome dyskinesia, nausea, constipation and dizziness as the most common adverse events described.

This box summarizes key points contained in the article.

2. Adenosine neurotransmission

The endogenous neuromodulator adenosine plays an important role in regulating a number of physiologic functions within the nervous system, including the behavior of basal ganglia circuits [9,10]. In this section, the major characteristics of adenosine neurotransmission, including adenosine receptor pharmacology and principal effects induced on basal ganglia circuits, will be reviewed.

2.1 Adenosine physiology and metabolism

Adenosine is composed of adenine, a purine base, and ribose. It is a byproduct of cell energy metabolism that also serves as a neurotransmitter [9], and is generated by rapid degradation of AMP formed after ATP dephosphorylation during energy-consuming cell processes. The plasma membrane monoamine transporter assures a balance between intra- and extracellular concentrations of adenosine. However, increased intracellular adenosine levels can result from two different sources [11]. On one hand, through increased energy consumption by cells along the aforementioned metabolic pathway, or alternatively extracellular adenosine can be generated through rapid hydrolysis of ATP, released either as a neurotransmitter or during cell death [12,13].

2.2 Adenosine receptors and physiological effects

Adenosine binds to four G-protein–coupled 7-TMS–type receptors: A1, A2A, A2B and A3 [14]. A1 and A3 are coupled to Gi proteins, whereas A2 activates Gs proteins. The former decrease cAMP levels and increase potassium conductance, while Gs increase cAMP and induce Na currents. Binding affinity intensity follows the order: A1 = A2A = A3 > A2B [15]. A1 receptors are highly expressed in cortex, cerebellum and hippocampus, with lower levels in other brain locations [16]. A3 receptors follow a similar expression pattern but are present in smaller numbers in all regions, as are A2B receptors. Finally, A2A receptors are expressed mostly in the basal ganglia and olfactory tubercle [16].

Receptor number appears to be critically important for effect [17,18]. Adenosine only acts as a neurotransmitter in physiological conditions where receptors are very abundant, or under pathological conditions where high concentrations of the compound exist [19].

The main role of adenosine is linked to tissue protection and repair [20], but it can also exert other functions. Vascular beds are rich in A2A receptors, where adenosine induces vasodilation [21]. It also increases respiratory rate and stimulates angiogenesis after hypoxia [21] and is a potent inhibitor of the immune system [22]. Effects of A2A receptor activation in the brain will be reviewed in the following section.

2.3 Adenosine effects on basal ganglia circuits

Within the striatum, A2A receptors are found mostly, but not exclusively, in dendrites, dendritic spines, axons and axonal terminals of GABAergic neurons along the 'indirect' pathway [23,24]. Studies in postmortem human brain have shown that A2A receptor expression is concentrated in caudate and putamen nuclei, with higher levels in the latter compared to caudate and accumbens nuclei [25]. It should be mentioned that the putamen is involved primarily in motor circuits of the basal ganglia [26]. A2A receptors are also expressed in cholinergic terminals, where they induce acetylcholine release [27], and presynaptically at cortical glutamatergic terminals contacting spiny neurons of the 'direct' pathway [28,29].

Neurophysiology studies suggest that A2A receptor activation can modulate cortical excitatory input to spiny neurons [30]. Such a control is exerted primarily by at least two reciprocal antagonistic interactions between A2A and dopamine D2 receptors [31]. In one, A2A and D2 receptors form heteromers, which allow the former to counteract D2-mediated inhibitory input to the 'indirect' pathway [32,33]. This interaction accounts for suppressant effects of A2A receptor agonists on locomotor activity [31]. However, A2A or D2 receptors can also exist as 'homomers' (i.e., a complex formed by several units of a single type of receptor, either A2A or D2). Under normal conditions, D2 homomer activation leads to spiny neuron discharge inhibition. However, in the presence of dopamine depletion, A2A homomers become active, further suppressing locomotor activity [31]. Accordingly, A2A antagonists reduce spiny neuron activation and hence GABA release [34,35]. Interestingly, caffeine, a nonspecific adenosine receptor antagonist, loses its motor stimulant properties in the A2A receptor knockout (KO) mouse model [16].

A2A receptors may also form heteromers with glutamatergic mGlu5 receptors [16]. Such receptors modulate cortical excitatory input and have been implicated in the genesis of dyskinesias [36]. mGlu5 receptor antagonists have been shown to reduce dyskinesia intensity in animal PD model and in very early human studies [37]. Different studies suggest that A2A activation is needed for mGlu5 receptor activation, and vice versa [38].

Finally, A2A/A1 receptor heteromers are also found presynaptically in glutamatergic terminals of spiny neurons giving rise to the 'direct' pathway [28,29]. Activation of these receptors leads to inhibition of glutamate release.

According to the classic model describing basal ganglia function, dopamine depletion would cause depression of the 'direct' pathway and overactivation of the 'indirect' pathway [26]. By acting on A2A receptor homomers in spiny neurons of the 'indirect' pathway, A2A receptor antagonists may reduce pathway activation and thus contribute to restore balance [39]. Results in animal PD models with istradefylline, as will be discussed in later sections, agree with these predictions [9]. Interestingly, A2A antagonists have been shown to enhance dopamine release after administration of levodopa, possibly by an indirect mechanism involving the stimulation of the tyrosine hydroxylase activity within striatal dopaminergic terminals [40]. Additionally, these agents potentiate D1 agonist or levodopa effects on immediate early gene expression [41], which may further help restore the imbalance between 'direct' and 'indirect' pathways generated by dopamine depletion. Interestingly, increased receptor expression has been observed in postmortem studies of PD patients with dyskinesia [42]. These results suggest that overexpression may be somehow related to dyskinesia development. In line with these results, allosteric blocking of mGlu5 receptor activation by A2A antagonists may contribute to dyskinesia control. Furthermore, inhibition of glutamate release along the 'direct' pathway may further alleviate dyskinesia. A summary of A2A antagonist action sites is offered in **Figure 1**.

Finally, A2A receptor antagonists may also exert neuroprotective effects [16]. Consumption of caffeine, a potent nonselective adenosine receptor antagonist, is known to reduce lifetime cumulative risk of developing PD both in humans and in PD animal models [16,43,44]. Neuroprotection has also been observed in the 6-hydroxydopamine (6-OHDA) rat model, after pretreatment with 8-(3-chlorostyryl)caffeine, which is more selective for the A2A receptor [45]. These results suggest that A2A antagonists may have potential neuroprotective effects in PD.

3. Istradefylline

Non-xantinic A2A receptor antagonists were developed from a very unusual source [46]. Mefloquine, a potent antimalarial agent, is infrequently associated with some severe neuropsychiatric side effects, such as disturbed sleep, heightened anxiety, panic attacks, depression, psychosis and seizures. These initial findings prompted research for potential neurological effects of the drug. Results showed that (-)-(R,S)-enantiomer of mefloquine behaved as a potent and moderately selective A2A antagonist. Although the compound was not found to be effective in *in vivo* animal models due to its lipophilic structure, which makes activation of membrane receptors less likely, it gave substance to a chemical development program that resulted in the synthesis of several antagonists, including istradefylline [47-49].

Istradefylline was approved in March 2013 in Japan, for adjunctive treatment use in PD patients experiencing wearing-off fluctuations [4,50]. A 'New Drug Application' was also filed in the US, but the FDA rejected approval and requested additional data. Ongoing istradefylline development programs for other indications including major depressive disorders and restless leg syndrome have currently been halted.

3.1 Pharmacodynamics and results in PD animal models

Istradefylline binding profile studies have been conducted using stably expressed receptors in human cell lines [46]. Binding affinity for the A2A receptor was established at 35.9 ± 4.76 standard error of the mean. Binding affinities for A1, A2B or A3 receptors were 79-, 50- and > 84 times higher, respectively. In healthy subjects, 90% receptor occupancy lasting for 14 days was noted, with doses of 5 mg/day [25]. In rats and humans, studies using positron emission tomography detected istradefylline in areas where A2A receptors are abundant (i.e., basal ganglia), as well as elsewhere (cerebellum and thalamus) [25,51]. *In vitro* studies suggest that istradefylline does not display affinity for a variety of receptors including, among others, adrenergic, dopaminergic, gabaergic, cholinergic, serotoninergic and opiergic receptors [52], nor does it induce significant inhibition of the monoaminooxidase enzyme.

Istradefylline's effects on parkinsonian symptoms have been tested in various animal models, including mice models of catalepsy induced by CGS 21680, haloperidol or reserpine, and in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-lesioned rats [53]. Istradefylline significantly ameliorated catalepsy induced by drugs in a dose-dependent fashion. Similarly, it also reverted hypomotility induced by MPTP administration. Interestingly, a combination of subthreshold doses of istradefylline and levodopa also markedly improved drug-induced catalepsy.

The effects of istradefylline were also investigated on rotational behavior induced by apomorphine or L-DOPA in rats with unilateral 6-OHDA lesions [54]. Istradefylline increased contralateral rotation induced by apomorphine and levodopa. In all cases, the effect was dose-dependent and the response to apomorphine was not only increased, but also extended by 25%. Interestingly, istradefylline effect vanished with higher levodopa doses, probably indicating that synergic action of these drugs has a maximum limit.

In common marmosets treated with MPTP, istradefylline produced dose-related increases in motor activity when administered as monotherapy [55,56]. Authors observed that movement patterns resulting from istradefylline administration resembled physiological ones, to a greater extent than those observed after levodopa or dopamine agonist administration. Disability scores also improved considerably, although not as much as with levodopa treatment. Effects were sustained and reversible, and all effects were blocked by an A2A receptor agonist, thus confirming the istradefylline's effect [55,56].

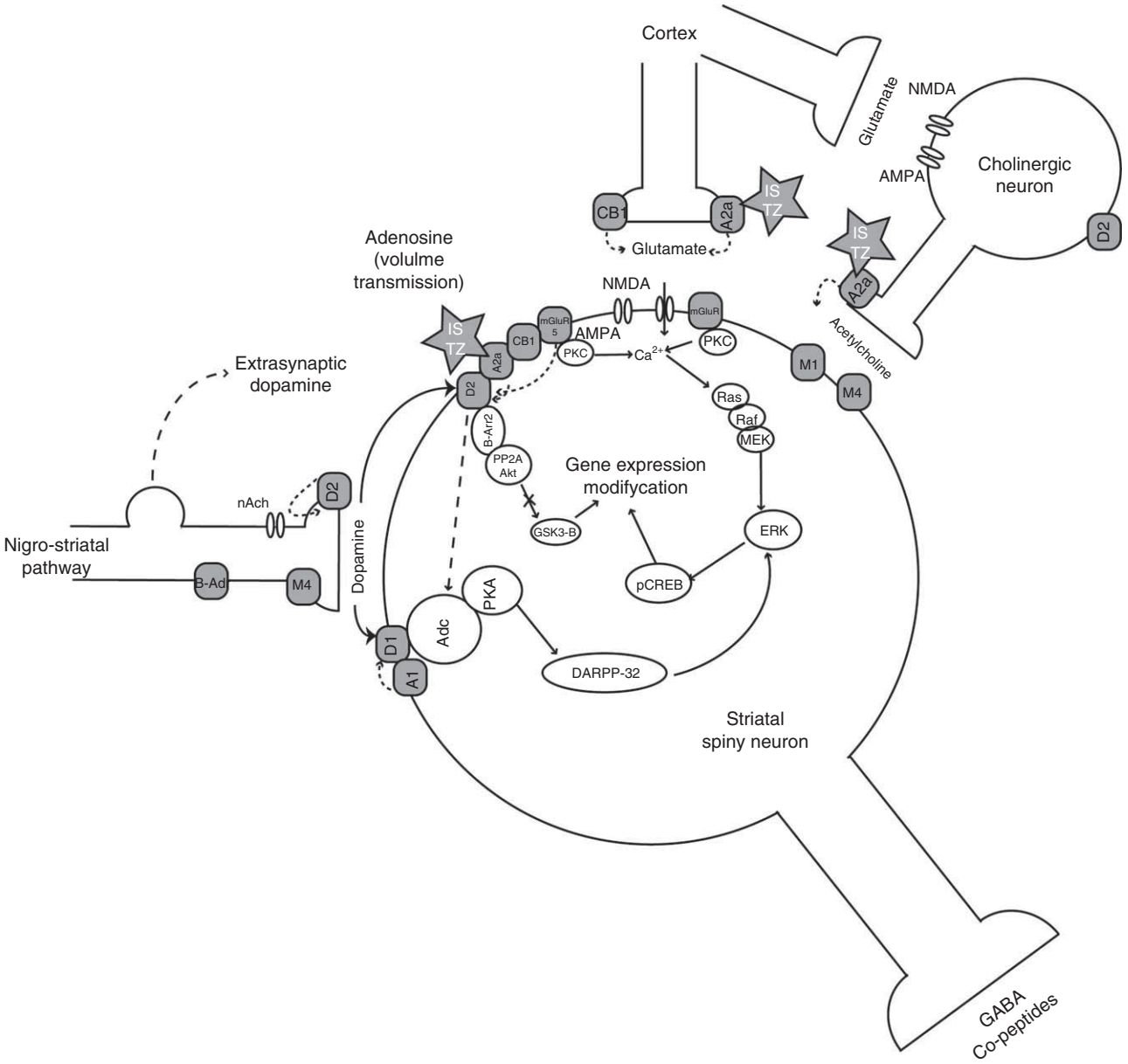

Figure 1. Schematic representation of a striatal spiny neuron depicting A2A receptor antagonist action sites.
Star symbols indicate istradefylline (IS) and tozadenant (TZ) action sites.
Receptors: A1/A2A: Adenosinergic; AMPA/NMDA: Glutamatergic ionic; B-adr: Beta-adrenergic; CB: Cannabinoidergic; D1/D2: Dopaminergic; M: Muscarinic; mGlu: Glutamatergic metabotropic; nAch: Nicotinic.

Potential tremolytic effects of istradefylline were tested in pimozide-induced tremulous jaw in rats [57]. Chronic treatment with 1.0 mg/kg of pimozide induced tremulous jaw movements and increased ventrolateral striatal c-fos expression, both of which were reduced by co-administration of istradefylline. Interestingly, tropicamide, a muscarinic receptor antagonist, also counteracted tremor, but had no effect on c-fos expression. These results suggest that tremolytic effects of istradefylline may be mediated through inhibition of acetylcholine release by striatal interneurons, although this is probably not the only mechanism.

Potential effects of istradefylline on levodopa-induced motor complications (i.e., motor fluctuations and dyskinesias) were studied in rat and monkey PD models [58]. In rats unilaterally lesioned with 6-OHDA, istradefylline prevented shortening of motor response produced by chronic levodopa administration. Moreover, levodopa-induced hyperphosphorylation at S845 residues on AMPA receptor GluR1 subunits, which is a marker of enhanced glutamatergic neurotransmittion, is thought to contribute to levodopa-induced motor complications [59,60], and was reduced by istradefylline administration. In primates, the drug prevented development of dyskinesias observed after

Table 1. Results with istradefylline in animal models of PD.

Animal species	PD model	Istradefylline effects	Suggested mechanism	Ref.
Rodent	Antipsychotic/reserpine-induced catalepsy and MPTP	Reverted hypomotility as monotherapy and with LD	Reduced activation of the 'indirect' pathway	[53]
Rodent	6-OHDA treatment	Increased apomorphine-induced rotational behavior	Reduced activation of the 'indirect' pathway	[54]
Monkey	MPTP treatment	Increased motility, reduced disability	Reduced activation of the 'indirect' pathway	[55,56]
Rodent	Antipsychotic-induced jaw tremor	Reduced tremor	Inhibition of acetylcholine release	[57]
Rodent	6-OHDA treatments	Reduced LD effect weaning	Reduced activation of the 'indirect' pathway	[58,61]
Monkey	MPTP	Prevented dyskinesia development	Reduced activation of the 'direct' pathway	[58]

6-OHDA: 6-Hydroxydopamine; MPTP: 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; LD: Levodopa; PD: Parkinson's disease.

chronic apomorphine treatment. These results suggest that istradefylline could be useful to treat wearing-off fluctuations without exacerbating dyskinesia severity. Similarly, istradefylline monotherapy did not induce dyskinesias in 6-OHDA-lesioned rats, with or without levodopa pre-sensitization [61]. Interestingly, some results suggest that pre-sensitization with levodopa requires A2A receptor activation, as KO mice do not display this phenomenon [62]. These and other results would indicate that A2A plays a role in levodopa control of 'direct' pathway effects.

A summary of animal studies is offered in **Table 1**.

3.2 Human pharmacokinetics

In rats, istradefylline showed moderate bioavailability after oral administration but good penetration across the blood–brain barrier [63].

In humans, oral istradefylline pharmacokinetics corresponded to a dose-dependent bi-compartmental model, reflecting accumulation in unknown sites [64,65]. Plasmatic half-life was 64 – 69 h [50]. Non-smoker patients with hepatic disease showed increased plasma levels and longer plasma half-life [50].

Drug–drug interactions were also observed with CYP 3A4 inhibitors [64], such as grapefruit juice, protease inhibitors used for HIV infection treatment, macrolide antibiotics, azole antifungals (ketoconazole, itraconazole, etc.), calcium-channel blockers and fluoxetine (weak), and smoking was shown to impair istradefylline metabolism. Finally, because istradefylline is a cytochrome 3A4 and p-glycoprotein inhibitor, caution is recommended in relation to simultaneous use of other drugs sharing the same metabolic pathway [66].

Istradefylline did not seem to affect levodopa/carbidopa pharmacokinetics [50].

3.3 PD treatment efficacy

A summary of clinical trials with istradefylline is offered in **Table 2**. A first proof-of-concept study was conducted in 15 patients with advanced PD [67]. Drug effects were tested as monotherapy and during levodopa infusion. Istradefylline (40 or 80 mg/day) showed no antiparkinsonian effect when administered as monotherapy, but when administered at 80 mg potentiated by 36% ($p < 0.02$) the magnitude of motor response to a low dose of levodopa as measured by the Unified PD rating Scale motor score, it caused less dyskinesia than that induced by an optimal levodopa dose.

Accordingly, a subsequent Phase IIb, 12-week, randomized, double-blind, placebo-controlled trial, conducted in 176 patients with early PD, showed no effect after 40 mg of istradefylline as monotherapy [68].

On the other hand, an exploratory Phase IIa, 12-week, placebo-controlled RCT conducted in 83 advanced PD patients showed a 1.5-h reduction in daily OFF time ($p < 0.05$) as assessed by home diaries, at the cost of an increase in ON time with non-troublesome dyskinesias [69]. Three other Phase IIb, 12-week, parallel placebo-controlled RCTs were conducted in advanced PD patients. Different doses (20, 40 or 60 mg/day) were used depending on the trial [70,71]. Overall, a consistent but relatively modest reduction in time spent OFF (< 1 h) was observed, with reports of increased non-troublesome dyskinesia.

Five large 12-week, parallel placebo-controlled Phase III RCTs have since been conducted in 200 – 600 patients with advanced PD [72-75], the results of one of which remain unpublished (NCT00199394). Studies by Hauser as well as by Mizuno and colleagues confirmed Phase II findings [72,73,75]. In the study conducted by Pourcher and colleagues, however, three istradefylline doses (10, 20 and 40 mg/day) were compared to placebo with negative results regarding OFF time, although significant effects on unified PD rating scale (UPDRS) III scores were noted for patients in ON state receiving the highest dose [74]. Results from another three-arm parallel study, where istradefylline 40 mg/day was compared to placebo and to entacapone, have yet to be reported (NCT00199394). A summary of human and animal studies with istradefylline is offered in **Table 3**.

Results from a recently published meta-analysis including some of the clinical trials with istradefylline [76] appear to

Table 2. Clinical trials with istradefylline or tozadenant.

Author/Year	Patient number	Design	Duration	Drug daily dose (mg)	Comparator	Main outcome
Istradefylline						
Bara-Jimenez 2003	15 Moderate-advanced PD	RCT (LD infusion)	6 w	40, 80	Placebo	UPDRS III, AIMS
Hauser 2003	83 Advanced PD	RCT	12 w	20, 40	Placebo	OFF time
Hauser 2008	231 Advanced PD	RCT	12 w	20	Placebo	OFF time
LeWitt 2008	196 Advanced PD	RCT	12 w	40	Placebo	OFF time
Stacy 2008	395 Advanced PD	RCT	12 w	20, 60	Placebo	OFF time
Fernandez 2010	176 Early PD	RCT	12 w	40	Placebo	UPDRS III
Mizuno 2010	363 Advanced PD	RCT	12 w	20, 40	Placebo	OFF time
Mizuno 2013	373 Advanced PD	RCT	12 w	20, 40	Placebo	OFF time
Pourcher 2011	610 Advanced PD	RCT	12 w	10, 20, 40	Placebo	OFF time
Unpublished NCT00199394	405 Advanced PD	RCT	16 w	40	Placebo Entacapone	OFF time
Factor 2010	977 Advanced PD	OL	52 w	10, 60	-	OFF time
Tozadenant						
Black 2010	21 Moderate-advanced PD	RCT	1 w	20 or 50	Placebo	Cerebral blood flow
Hauser 2013	337 Advanced PD	RCT	12 w	60, 120, 180, 240	Placebo	OFF time

AIMS: Abnormal involuntary movement scale; LD: Levodopa; OL: Open-label uncontrolled trial; PD: Parkinson's disease; RCT: Randomized, double-blind, controlled trial; UPDRS: Unified PD rating scale; w: Weeks.

Table 3. Summary of Clinical Development Programs on istradefylline.

	Antiparkinsonian effect		Dyskinesias	Dose employed
	Monotherapy	Adjunct to L-DOPA		
Animals	++	++	0/-	0.5 – 10 mg/kg
Early Phase II (LD infusion)	0	+ (only with low LD doses)	+	40 – 80 mg
Late Phase II	0	+	+	20 – 60 mg
Phase III	NE	I (3 out of 5 studies positive)	+	10 – 40 mg

0: No effect; +: Mild effect; ++: Strong effect; -: Reduction; I: Inconclusive; LD: Levodopa; NE: Not evaluated.

confirm a significant effect for 40 mg/day of istradefylline over placebo in relation to time spent in OFF state, a finding not observed for the 20 mg/day dose. Similarly, significant reduction in UPDRS motor score in ON state was noted, but only for the 40 mg/day dose. Results should however be interpreted with caution, as unpublished data for istradefylline were logically not included in the meta-analysis.

A report on the follow-up of istradefylline studies in the US suggests that benefits might be maintained for up to 52 weeks [77].

A double-blind, placebo-controlled, randomized Phase III study is being conducted in 95 sites in 8 countries (NCT01968031) [78]. Patients will be randomized to istradefylline 20 mg/day, 40 mg/day or placebo. Principal outcome is the change from baseline in OFF hours per day.

3.4 Safety

Istradefylline was well tolerated in Phase II and III studies [50]. Treatment-emergent adverse events (TEAEs) affected about 60 – 80% of patients, with serious ones observed in <5% of cases. Nausea, constipation and dizziness appear to be frequent TEAEs. Dyskinesia was significantly more common with 40 mg/day istradefylline, but not with 20 mg/day. Long-term tolerability was also reported to be adequate [77].

Istradefylline potential for inducing psychosis was studied in a rodent model, by registering prepulse inhibition (PPI) of startle [79]. PPI deficits are associated with schizophrenia and are disrupted by dopamine agonists in rats and in humans [80,81]. In this study, pramipexole, pergolide and apomorphine significantly disrupted PPI in rats and mice, while istradefylline had a marginal effect in mice but not in rats. In one clinical trial, dose-dependent hallucinations were more frequent in istradefylline-treated patients [74]. These results were not observed in other trials.

3.5 Other results with istradefylline

The effects of istradefylline on altered cognition, which is a common feature of PD [82], were studied in the 6-OHDA

rat model [83]. Cognitive performance was evaluated using object recognition as well as delayed alternation tasks. Istradefylline significantly improved cognition and increased dopamine levels in the prefrontal cortex. These results have not been replicated in humans.

Istradefylline effect in animal models of depression, another common feature of PD, has also been studied [84]. Rodents were subjected to forced swimming and tail suspension tests. Istradefylline significantly decreased immobility time in both tests. Interestingly, drug effects were potentiated by antidepressants and blocked by corticosterone, which suggests that the effect is mediated by hypothalamic–pituitary–adrenal axis modulation.

Psychomotor slowing, anergia and fatigue are characteristics of depression, dependent in turn on nucleus accumbens function [85]. In rats, behavioral activation can be tested by letting the rats choose between responding to a fixed-ratio 5 lever-pressing schedule for a highly preferred food (i.e., high-carbohydrate pellets) versus approaching and consuming a less-preferred rodent chow. In a recent study, istradefylline was able to reverse the alteration in choice behavior induced by D1 and D2 antagonists [86].

4. Tozadenant

Tozadenant (SYN115) is another A2A receptor antagonist, for which published evidence is much scarcer. Regrettably, no studies in PD animal models have been published so far.

Pharmacodynamic responses to tozadenant administration in humans were evaluated by cerebral blood flow (CBF) imaging, which has been proposed as a tool to accelerate pharmaceutical dose finding, but is not yet widely applied [87]. With this objective in mind, a randomized, double-blind, placebo-controlled, crossover study was conducted on 21 levodopa-treated PD patients. Patients received either placebo or tozadenant, 20 or 50 mg/day for 7 days. On the study day (day 8), CBF scans were obtained before and during levodopa infusion. Tozadenant produced significant decrease in thalamic CBF, consistent with reduced activity, following reduced pallidothalamic inhibition, probably via the 'indirect' pathway. Decreased CBF was also observed in other cortical regions connected with alertness. These results also coincided with decreased self-reported sleepiness.

Clinical effects of tozadenant were studied in 337 PD patients with wearing-off by means of a double-blind, placebo-controlled, 12-week trial [88]. Primary outcome measure was change in hours per day spent in the OFF state between baseline and week 12. Tozadenant doses tested were 60, 120, 180 or 240 mg/day. Mean placebo-corrected changes from baseline during OFF time with 120 or 180 mg/day of tozadenant were -1.2 and -1.1 h, respectively (both $p < 0.05$). Troublesome dyskinesias did not increase with tozadenant. UPDRS motor scores in ON state were also significantly reduced with 120 or 180 mg/day. The most common adverse events were dyskinesia, nausea, dizziness, constipation, PD worsening, insomnia and falls. A summary of clinical trials with tozadenant is offered in **Table 2**. The drug has entered into Phase III development, with patient enrollment for the next trial probably beginning by 2015 [89].

5. Conclusion

Adenosine A2A receptors are localized mainly in the caudate and putamen nuclei. Their activation leads to reductions in movement frequency and intensity, by activation of the 'indirect' pathway. Conversely, A2A receptor antagonists cause increased movement and behavioral activation. Animal studies have shown a significant antiparkinsonian effect for some A2A receptor antagonists, including istradefylline. The effect was observed both with monotherapy and after combined administration with low doses of levodopa. Istradefylline did not exacerbate levodopa-induced dyskinesia. These results prompted exploration of effects in humans in several clinical trials, with varying results. Antiparkinsonian effects observed with istradefylline monotherapy could not be replicated in PD patients. Conversely, as adjuvant to levodopa, istradefylline significantly reduced OFF time in many trials, but not in all of them, which was probably the reason the FDA issued a letter of non-approval. Regrettably, not all clinical trials with istradefylline have been published. Indeed, one key trial comparing istradefylline to placebo and entacapone remains unpublished, therefore precluding full evaluation of the effects of this drug. Istradefylline was well tolerated but increased dyskinesias at higher doses.

Evidence with tozadenant is scarce, with one positive clinical trial recently presented as an abstract, which warrants near publication. Therefore, one may conclude that both drugs appear to be promising alternatives for the treatment of PD and deserve further exploration.

6. Expert opinion

Currently, the main alternatives for the treatment of levodopa wearing-off phenomena are to increase the dosing frequency of levodopa, and/or the coadyuvant use of oral dopamine agonists, monoamino-oxidase B inhibitors or catechol-o-methyl enzyme inhibitors [63]. Continuous infusion of duodopa or apomorphine can also be tried before the indication of deep brain stimulation. One of the greatest limitations to such therapies is commonly observed adverse neuropsychiatric reactions, especially with dopamine agonists [7]. Istradefylline presents as an interesting therapeutic alternative, since in addition to the clinical effects on OFF time, it may offer a better neuropsychiatric profile, while retaining the effects on mood and cognition observed with dopamine agonists. Effects of istradefylline on sleep have not been characterized, but a psycho-stimulant effect similar to caffeine might be expected [90], making the drug an interesting alternative for patients with daytime sleepiness.

Nevertheless, limitations to treatment with istradefylline must also be mentioned. First, even though animal models suggest that the drug has a low potential for inducing dyskinesia, clinical trials have shown this adverse reaction even in patients on low doses, which was generally rated as non-troublesome by patients. The reason behind the discrepancies observed between animal and human studies remains unclear. Effects of A2A receptor antagonists are not restricted to the brain. Indeed, cardiovascular, respiratory and immune functions are affected by these drugs. Up to the now, clinical trials have not shown important adverse reactions in these organ systems. Nonetheless, investigators and physicians should remain attentive. This is especially relevant in light of the great drug–drug interaction potential via blockage of CYP 3A4 or p-glycoproteins. These are common pharmacological metabolic pathways, making drug–drug interactions leading to increased drug toxicity more likely. Therefore, careful risk/benefit analysis is advisable before proposing istradefylline treatment to PD patients with wearing-off fluctuations caution also probably extended for tozadenant use.

Declaration of interest

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

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Affiliation

Santiago Perez-Lloret[1,2] MD PhD & Marcelo Merello[†1] MD PhD
[†]Author for correspondence
[1]Raul Carrea Institute for Neurological Research, Movement Disorders Section, Montañeses 2325 (1425), Buenos Aires, Argentina
Tel: +54 11 57773200;
Fax: +54 11 57773200;
E-mail: mmerello@fleni.org.ar
[2]Catholic University, Clinical Pharmacology & Epidemiology Laboratory, Buenos Aires, Argentina