Original Investigation | CLINICAL TRIAL

Preladenant as an Adjunctive Therapy With Levodopa in Parkinson Disease

Two Randomized Clinical Trials and Lessons Learned

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IMPORTANCE Preladenant is an adenosine 2A receptor antagonist that reduced "off" time in a placebo-controlled phase 2b trial in patients with Parkinson disease (PD). We sought to confirm its efficacy in phase 3 trials.

OBJECTIVE To evaluate preladenant as an adjunct to levodopa in patients with PD and motor fluctuations.

DESIGN, SETTING, AND PARTICIPANTS Two 12-week, phase 3, randomized, placebo-controlled, double-blind trials performed from July 15, 2010, to April 16, 2013. The setting included neurology clinics, clinical research centers, and hospitals in the Americas, the European Union, Eastern Europe, India, and South Africa. Participants included patients with moderate to severe PD taking levodopa who were experiencing motor fluctuations.

INTERVENTIONS In trial 1, a total of 778 eligible patients were randomized to the addition of preladenant (2 mg, 5 mg, or 10 mg twice daily), placebo, or rasagiline mesylate (1 mg/d) in a 1:1:1:1:1 ratio. In trial 2, a total of 476 eligible patients were randomized to the addition of preladenant (2 mg or 5 mg twice daily) or placebo in a 1:1:1 ratio.

MAIN OUTCOMES AND MEASURES The primary outcome measure was change in off time from baseline to week 12.

RESULTS In trial 1, neither preladenant nor rasagiline was superior to placebo in reducing off time from baseline to week 12. The differences vs placebo were −0.10 hour (95% CI, −0.69 to 0.46 hour) for preladenant 2 mg twice daily, −0.20 hour (95% CI, −0.75 to 0.41 hour) for preladenant 5 mg twice daily, −0.00 hour (95% CI, −0.62 to 0.53 hour) for preladenant 10 mg twice daily, and −0.30 hour (95% CI, −0.90 to 0.26 hour) for rasagiline mesylate 1 mg/d. In trial 2, preladenant was not superior to placebo in reducing off time from baseline to week 12. The differences vs placebo were −0.20 hour (95% CI, −0.72 to 0.35 hour) for preladenant 2 mg twice daily and −0.30 hour (95% CI, −0.86 to 0.21 hour) for preladenant 5 mg twice daily. Preladenant was well tolerated, with the most common adverse event that showed an increase over placebo in both trials being constipation (6%-8% for preladenant vs 1%-3% for placebo).

CONCLUSIONS AND RELEVANCE In these phase 3 trials, preladenant did not significantly reduce off time compared with placebo. That the active control rasagiline also failed to demonstrate a significant reduction in off time suggests that issues of study design or conduct may have affected these trials.

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The adenosine 2A (A_{2A}) receptor is a nondopaminergic target for the treatment of Parkinson disease (PD).[1,2] A_{2A} receptors are predominantly localized to striatopallidal medium spiny neurons, where they are colocalized with dopamine D_2 receptors.[3,4] The A_{2A} antagonists are thought to exert antiparkinsonian effects by reducing overactivity of striatopallidal output neurons in the indirect pathway.[5]

Caffeine is a nonspecific adenosine receptor antagonist that provides antiparkinsonian motor benefit and neuroprotective effects in animal models of PD.[6,7] Selective A_{2A} receptor antagonists, such as istradefylline and tozadenant, have been assessed for efficacy as adjuncts to levodopa in patients with PD and motor fluctuations. Tozadenant demonstrated efficacy in a phase 2 trial, and a phase 3 trial is under way.[8] Istradefylline demonstrated efficacy in phase 2 trials, but results in phase 3 trials were inconsistent.[9-14] Nonetheless, after a successful phase 3 trial in Japan, istradefylline was approved in that country,[12,14] and an international phase 3 trial is now ongoing (clinicaltrials.gov identifier NCT01968031).

Preladenant is a potent and selective A_{2A} antagonist.[15] In rodent and primate models of PD, preladenant improved motor function.[15,16] In a phase 2b trial evaluating preladenant as an adjunct to levodopa in patients with PD,[17] a dose response was demonstrated with preladenant (5 mg and 10 mg twice daily) providing a significant reduction in "off" time (time when medication has worn off and is no longer providing benefit with regard to mobility, slowness, and stiffness) compared with placebo, and preladenant was well tolerated. We report herein results from 2 phase 3 trials that evaluated preladenant as an adjunct to levodopa in patients with PD and motor fluctuations.

Methods

Overview

We conducted two 12-week, phase 3, randomized, placebo-controlled, double-blind trials in patients with PD and motor fluctuations. These trials were identical in design except for treatment arms. In trial 1, patients were randomized to the addition of preladenant (2 mg, 5 mg, or 10 mg twice daily), placebo, or rasagiline mesylate active control (1 mg/d). In trial 2, patients were randomized to the addition of preladenant (2 mg or 5 mg twice daily) or placebo. The study protocols can be found in Supplement 1 and Supplement 2.

Trial 1 was conducted at 121 sites in Eastern Europe, the European Union, India, Latin America, North America, and Turkey from July 15, 2010, to December 20, 2012. Trial 2 was conducted at 88 sites in Eastern Europe, Latin America, North America, and South Africa from March 14, 2011, to April 16, 2013. The trials were conducted in accord with principles of good clinical practice and were approved by appropriate institutional review boards and regulatory agencies. Written informed consent was obtained from each patient before participation.

Patients were randomized to treatment (**Figure 1**) using a computer-generated allocation schedule prepared by Merck & Co, Inc and implemented through an interactive voice response system. Investigators, site staff, patients, and monitoring staff remained masked to treatment allocation throughout the trials.

Patients

Both trials enrolled patients with moderate to severe PD who were experiencing motor fluctuations. Key inclusion criteria included diagnosis of PD based on the UK Parkinson's Disease Society Brain Bank criteria,[18] with Hoehn-Yahr stage between 2.5 and 4, and receipt of a stable, optimal treatment regimen, including levodopa, and experience of motor fluctuations with a minimum of 2 hours per day off time (per 3-day PD diary[19]). Stable dosages of dopamine agonists, entacapone, amantadine hydrochloride, and anticholinergics were permitted. Monoamine oxidase type B inhibitors were prohibited. Key exclusion criteria included hallucinations, prior surgery for PD, impulse control disorders, drug-induced or atypical parkinsonism, cognitive impairment (Montreal Cognitive Assessment[20] score <24 in trial 1 and <22 in trial 2), and untreated major depressive disorder (*Diagnostic and Statistical Manual of Mental Disorders* [Fourth Edition][21] criteria or a Beck Depression Inventory II[22] score ≥19), as well as other significant conditions that could interfere with assessments or participation (eg, psychotic disorder, stroke, and head injury).

Primary Outcomes and Efficacy and Safety Assessments

The primary outcome measure was change in off time from baseline to week 12. Patients used a PD diary[19] to denote their predominant status every half-hour over 24 hours to indicate whether they were off, on without dyskinesia, on with nontroublesome dyskinesia, on with troublesome dyskinesia, or asleep. Patients underwent PD diary training and concordance testing during screening. They then completed 3-day sets of diaries at baseline and weeks 2, 4, 8, and 12. The Unified Parkinson's Disease Rating Scale[23] (UPDRS) parts 1 through 4 were administered at baseline, day 1, and weeks 2, 4, 8, and 12. Safety was assessed by review of adverse events, laboratory values, vital signs, and electrocardiograms.

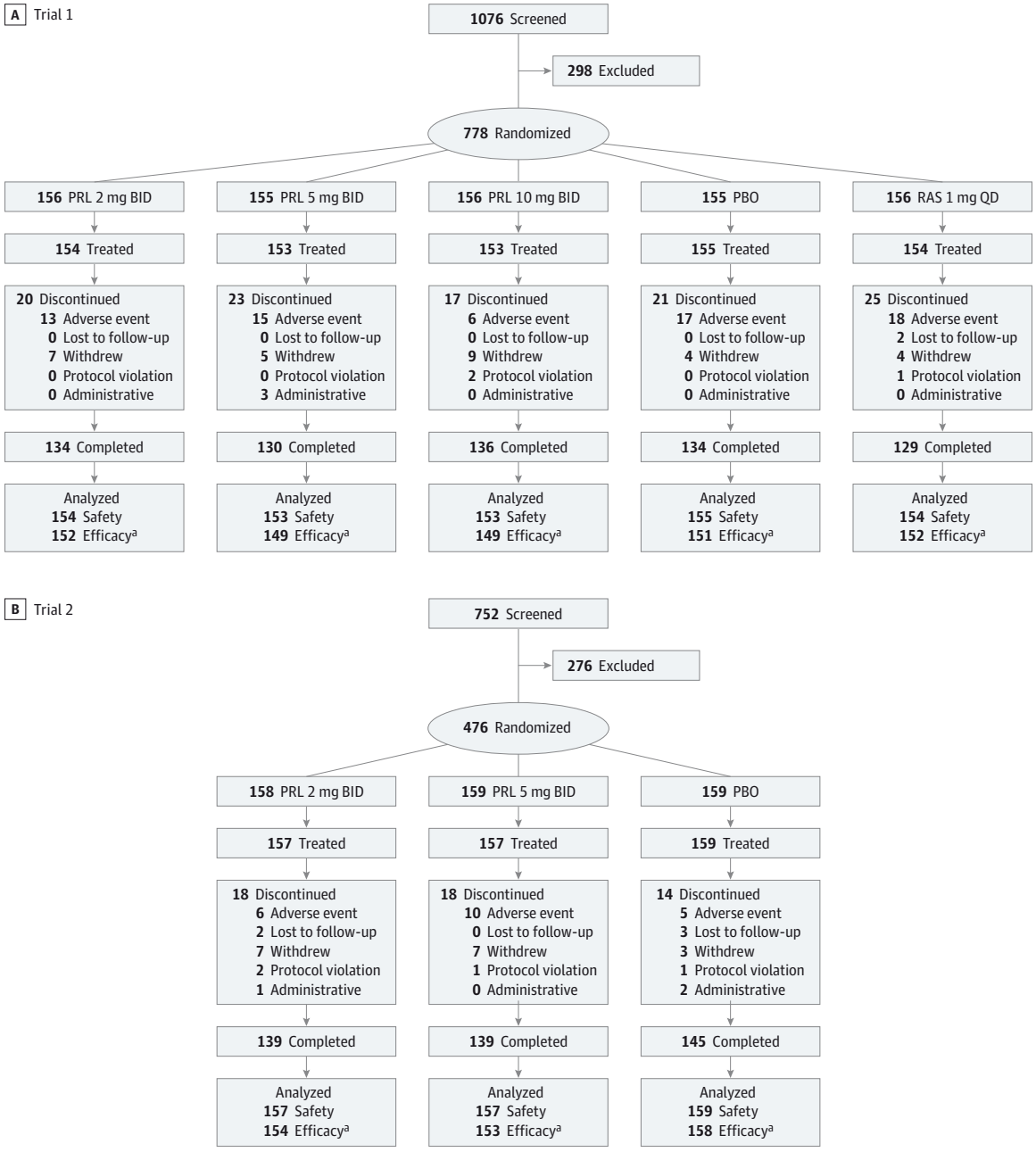
Statistical Analysis

The primary hypothesis in each trial was that at least 1 dosage of preladenant is superior to placebo as measured by change from baseline to week 12 in mean off time. The primary efficacy end point was analyzed using a constrained longitudinal data analysis approach with treatment, time, and treatment × time interaction as fixed effects and with patient as a random effect. The least-squares mean response and pairwise differences between preladenant dosages and placebo, along with 95% CIs, are reported. Using the same model, a comparison of rasagiline vs placebo was performed in trial 1. The efficacy population (full analysis set) consisted of all randomized patients with baseline data and postrandomization end point data after at least 1 dose of study medication.

Key secondary measures were the proportion of responders (≥30% reduction in mean off time from baseline to week 12) and change from baseline in mean on time without troublesome dyskinesia. Other secondary end points were analyzed but were uninformative and are not reported herein except for UPDRS part 3 scores.

Multiplicity was controlled through prespecified sequential testing procedures (eMethods in Supplement 3), whereby starting with the primary end point, the highest preladenant dosage was tested against placebo. If significant ($P \leq .05$), the next pre-

Figure 1. Participant Flow in Trial 1 and Trial 2



PRL indicates preladenant; PBO, placebo; and RAS, rasagiline.

[a] Fewer than the number treated because patients with no post baseline data were excluded from the efficacy analyses.

specified test of preladenant vs placebo was evaluated. Formal hypothesis testing stopped once a nonsignificant difference was encountered. However, nominal *P* values were still calculated.

Power

For each trial, the planned sample size was 150 patients per treatment group. This number provided at least 90% power to detect a difference between preladenant and placebo of 1 hour in change from baseline to week 12 in mean off time given an SD of 2.6 hours (as observed in the phase 2b study[17]) and a 2-sided α = .05.

Post Hoc Investigations (Trial 1 Only)

Once results of the studies were known, several post hoc investigations were undertaken. These post hoc analyses focused on trial 1 given that this trial included an active control arm (rasagiline) that failed to demonstrate efficacy. Investigations included an analysis of the integrity of the randomization process and treatment administration. In addition, a pharmacokinetic analysis evaluated whether expected plasma levels for randomized medications were achieved. The potential effect of caffeine consumption at baseline was evaluated by adding a caffeine term to the

Table 1. Baseline Characteristics of Treated Patients

Characteristic	Trial 1					Trial 2		
	Preladenant, 2 mg Twice Daily (n = 154)	Preladenant, 5 mg Twice Daily (n = 153)	Preladenant, 10 mg Twice Daily (n = 153)	Placebo (n = 155)	Rasagiline Mesylate, 1 mg/d (n = 154)	Preladenant, 2 mg Twice Daily (n = 157)	Preladenant, 5 mg Twice Daily (n = 157)	Placebo (n = 159)
Age, mean (SD), y	61.6 (9.3)	62.6 (8.5)	63.5 (8.4)	63.0 (8.4)	63.6 (9.0)	62.9 (9.0)	64.2 (8.7)	64.2 (8.9)
Male sex, No. (%)	97 (63.0)	78 (51.0)	92 (60.1)	78 (50.3)	95 (61.7)	108 (68.8)	86 (54.8)	95 (59.7)
PD duration, median (range), y	8.5 (1.2-25.2)	8.2 (2.0-23.6)	8.9 (1.7-25.2)	8.2 (1.3-40.8)	8.3 (1.1-22.5)	7.2 (1.1-25.3)	8.2 (1.6-20.7)	7.0 (1.0-22.0)
Levodopa dosage, median (range), mg/d	725 (50-2500)	625 (125-3500)	681 (100-2000)	650 (50-3600)	800 (100-2500)	600 (150-3300)	625 (100-2000)	625 (125-3000)
Hoehn-Yahr stage, No. (%)[a]								
2.5	68 (44.2)	70 (45.8)	69 (45.1)	63 (40.6)	63 (40.9)	89 (56.7)	76 (48.4)	79 (49.7)
3	72 (46.8)	73 (47.7)	76 (49.7)	78 (50.3)	77 (50.0)	66 (42.0)	74 (47.1)	68 (42.7)
4	14 (9.1)	10 (6.5)	7 (4.6)	13 (8.4)	13 (8.4)	2 (1.3)	7 (4.5)	10 (6.3)
Region, No. (%)[b]								
Eastern Europe	50 (32.1)	60 (38.7)	67 (42.9)	64 (41.3)	66 (42.3)	53 (33.5)	59 (37.1)	52 (32.7)
European Union	46 (29.5)	42 (27.1)	43 (27.6)	41 (26.5)	41 (26.3)	0	0	0
North America	26 (16.7)	26 (16.8)	28 (17.9)	20 (12.9)	23 (14.7)	63 (39.9)	56 (35.2)	66 (41.5)
Latin America	13 (8.3)	12 (7.7)	8 (5.1)	14 (9.0)	15 (9.6)	39 (24.7)	40 (25.2)	37 (23.2)
India	21 (13.5)	15 (9.7)	10 (6.4)	16 (10.3)	11 (7.1)	0	0	0
South Africa	0	0	0	0	0	3 (1.9)	4 (2.5)	4 (2.5)
PD medication, No. (%)								
Levodopa[c]	154 (100)	153 (100)	153 (100)	155 (100)	154 (100)	157 (100)	157 (100)	159 (100)
Dopamine agonist	93 (60.4)	115 (75.2)	113 (73.9)	102 (65.8)	112 (72.7)	93 (59.2)	92 (58.6)	95 (59.7)
Amantadine hydrochloride	42 (27.3)	46 (30.1)	40 (26.1)	50 (32.3)	53 (34.4)	45 (28.7)	40 (25.5)	46 (28.9)
COMT inhibitor[d]	20 (13.0)	18 (11.8)	13 (8.5)	11 (7.1)	9 (5.8)	20 (12.7)	14 (8.9)	13 (8.2)
Anticholinergic	19 (12.3)	15 (9.8)	8 (5.2)	10 (6.5)	14 (9.1)	8 (5.1)	8 (5.1)	14 (8.8)
Caffeine daily use, No. (%)								
None	57 (37.0)	42 (27.5)	43 (28.1)	46 (29.7)	39 (25.3)	46 (29.3)	54 (34.4)	51 (32.1)
1 Cup or glass	52 (33.8)	68 (44.4)	65 (42.5)	67 (43.2)	67 (43.5)	61 (38.9)	52 (33.1)	64 (40.3)
>1 Cup or glass	45 (29.2)	43 (28.1)	45 (29.4)	42 (27.1)	48 (31.2)	50 (31.8)	51 (32.5)	44 (27.7)
Off time, mean (SE), h[e]	5.8 (0.2)	5.8 (0.2)	6.1 (0.2)	5.7 (0.2)	5.6 (0.2)	5.9 (0.2)	5.9 (0.2)	5.7 (0.2)
On time without troublesome dyskinesia, mean (SE), h[e]	9.5 (0.2)	9.8 (0.2)	9.7 (0.2)	10.2 (0.2)	9.9 (0.2)	9.6 (0.2)	9.7 (0.2)	9.3 (0.2)

Abbreviations: COMT, catechol-*O*-methyl transferase; PD, Parkinson disease.

[a] The table does not include a small number of patients (maximum of 2 per treatment group) who had a Hoehn-Yahr stage of 2.

[b] Based on randomized patients (see study flowcharts for sample sizes).

[c] Includes levodopa combination treatments such as levodopa plus carbidopa.

[d] Primarily entacapone.

[e] Based on the full analysis set (see study flowcharts for sample sizes).

primary analysis model. Additional analyses evaluated results according to geographic area and time when patients entered the trial.

Results

Patients

In trial 1, a total of 778 eligible patients were randomized to the addition of preladenant (2 mg, 5 mg, or 10 mg twice daily), placebo, or rasagiline mesylate (1 mg/d) in a 1:1:1:1:1 ratio. The full analysis set included 769 patients, and 106 discontinued treatment (Figure 1A). In trial 2, a total of 476 eligible patients were randomized to the addition of preladenant (2 mg or 5 mg twice daily) or placebo in a 1:1:1 ratio. The full analysis set included 473 patients, and 50 discontinued treatment (Figure 1B). In both studies, discontinuations were similar across treatment groups.

Patient characteristics are summarized in **Table 1**. Baseline demographics were broadly similar between the 2 trials in terms of patient age and PD history. Treatment groups were similar in baseline disease severity.

Efficacy

In trial 1, neither preladenant nor rasagiline was superior to placebo in reducing off time from baseline to week 12 (eFigure 1A in Supplement 3). The differences vs placebo were −0.10 hour (95% CI, −0.69 to 0.46 hour) for preladenant 2 mg twice daily, −0.20 hour (95% CI, −0.75 to 0.41 hour) for preladenant 5 mg

Table 2. Key Efficacy Results at Week 12 (Full Analysis Set)

Variable	Preladenant, 2 mg Twice Daily	Preladenant, 5 mg Twice Daily	Preladenant, 10 mg Twice Daily	Placebo	Rasagiline Mesylate, 1 mg/d
Trial 1					
Mean off time, h					
Change from baseline	−0.9	−0.9	−0.8	−0.8	−1.1
Difference vs placebo (95% CI)	−0.10 (−0.69 to 0.46)	−0.20 (−0.75 to 0.41)	−0.00 (−0.62 to 0.53)	NA	−0.30 (−0.90 to 0.26)
P value	.70	.56	.87	NA	.28
Responders with ≥30% decrease in off time at 12 wk, %					
Estimate	31.0	33.3	33.8	33.9	36.1
Difference vs placebo (95% CI)	−3.60 (−14.97 to 7.84)	−0.70 (−12.36 to 10.93)	−0.50 (−12.00 to 10.95)	NA	1.90 (−9.81 to 13.58)
P value[a]	.61	.92	.98	NA	.71
Mean on time without troublesome dyskinesia, h					
Change from baseline	0.8	0.9	0.5	0.4	0.7
Difference vs placebo (95% CI)	0.40 (−0.21 to 1.09)	0.50 (−0.18 to 1.12)	0.20 (−0.49 to 0.80)	NA	0.40 (−0.29 to 1.01)
P value	.18	.16	.64	NA	.28
Trial 2					
Mean off time, h					
Change from baseline	−1.0	−1.1	NA	−0.8	NA
Difference vs placebo (95% CI)	−0.20 (−0.72 to 0.35)	−0.30 (−0.86 to 0.21)	NA	NA	NA
P value	.49	.24	NA	NA	NA
Responders with ≥30% decrease in off time at 12 wk, %					
Estimate	37.1	36.9	NA	30.5	NA
Difference vs placebo (95% CI)	7.00 (−4.17 to 18.05)	6.50 (−4.63 to 17.61)	NA	NA	NA
P value[a]	.24	.26	NA	NA	NA
Mean on time without troublesome dyskinesia, h					
Change from baseline	0.6	0.7	NA	0.5	NA
Difference vs placebo (95% CI)	0.10 (−0.47 to 0.63)	0.10 (−0.44 to 0.67)	NA	NA	NA
P value	.78	.68	NA	NA	NA

Abbreviation: NA, not applicable.

[a] *P* value is for the estimated odds ratio based on a generalized linear mixed model with baseline mean off time (hours per day) as a covariate, treatment × time interaction as a fixed effect, and patient as a random effect.

twice daily, −0.00 hour (95% CI, −0.62 to 0.53 hour) for preladenant 10 mg twice daily, and −0.30 hour (95% CI, −0.90 to 0.26 hour) for rasagiline mesylate 1 mg/d (**Table 2**). The percentage of responders with at least 30% decrease in off time at week 12 was similar among the preladenant, placebo, and rasagiline groups and ranged from 31.0% to 36.1%. Both the odds ratios and the proportions of responders showed no significant differences between preladenant or rasagiline vs placebo. All preladenant groups and the rasagiline group had numerically larger increases in on time without troublesome dyskinesia than the placebo group; however, none of the preladenant or rasagiline vs placebo differences were significant, nor was there a dose response. In general, changes in UPDRS part 3 scores were similar among treatments, with no significant differences from placebo other than for rasagiline at week 12 (eFigure 2A in Supplement 3).

In trial 2, preladenant was not superior to placebo in reducing off time from baseline to week 12 (eFigure 1B in Supplement 3). The differences vs placebo were −0.20 hour (95% CI, −0.72 to 0.35 hour) for preladenant 2 mg twice daily and −0.30 hour (95% CI, −0.86 to 0.21 hour) for preladenant 5 mg twice daily (Table 2). Mean increases in on time without troublesome dyskinesia at week 12 were similar (0.6, 0.7, and 0.5 hour) among treatment groups, and there were approximately 37% responders in the preladenant groups compared with 30.5% for the placebo group. Baseline UPDRS part 3 scores were similar across treatment groups and ranged from 26.2 to 27.7 points. In general, changes from baseline were similar among treatments, with no significant differences from placebo other than for preladenant 5 mg twice daily at week 12 (eFigure 2B in Supplement 3).

Table 3. Post Hoc Analysis of Differences in Change in Off Time (Hours per Day) at Week 12 by Region in Trial 1 (Full Analysis Set)[a]

Model Estimate	Eastern Europe (n = 268)	European Union (n = 200)	India (n = 72)	Latin America (n = 62)	North America (n = 116)	Turkey (n = 35)
Baseline off time, h	5.75	5.96	5.91	5.55	5.89	4.61
Change From Baseline, h						
Preladenant, 2 mg twice daily	−0.64	−1.17	−0.66	−0.78	−1.43	1.32
Preladenant, 5 mg twice daily	−0.92	−1.63	−0.13	−0.49	−1.29	0.88
Preladenant, 10 mg twice daily	−0.61	−1.18	−0.60	0.44	−1.11	−0.94
Rasagiline mesylate, 1 mg/d	−1.10	−1.09	−0.59	−1.61	−1.48	0.53
Placebo	−0.53	−0.45	−1.00[b]	−2.15[b]	−0.76	−1.87[b]
Pairwise Comparisons, h						
Preladenant, 2 mg twice daily vs placebo	−0.11	−0.72	0.34	1.37	−0.66	3.19
Preladenant, 5 mg twice daily vs placebo	−0.39	−1.18	0.87	1.66	−0.52	2.75
Preladenant, 10 mg twice daily vs placebo	−0.08	−0.73	0.40	2.59	−0.34	0.94
Rasagiline mesylate, 1 mg/d vs placebo	−0.57[c]	−0.63[c]	0.41	0.54	−0.71[c]	2.40

[a] Eastern Europe includes Bulgaria, the Czech Republic, Poland, and Russia. The European Union includes Austria, Finland, France, Germany, Israel, Italy, the Netherlands, Portugal, Spain, Sweden, and the United Kingdom. Latin America includes Brazil and Peru. North America includes the United States and Canada.

[b] Strongest placebo response.

[c] Strongest rasagiline response.

Safety

Adverse events, pooled over preladenant dosages, are listed in eTable 1 in Supplement 3. In trial 1, preladenant was generally well tolerated, with adverse events reported by approximately 55% of patients in each treatment group. Overall rates by type of event were similar among treatments and were not different from placebo. Few patients discontinued treatment because of adverse events, including 7.2% (33 of 460) receiving preladenant, 11.7% (18 of 154) receiving rasagiline, and 11.0% (17 of 155) receiving placebo. One death was reported, a respiratory arrest in the placebo group, considered by the investigator to be unlikely related to the study drug. The most common adverse event that showed an increase for preladenant over placebo was constipation (5.7% [26 of 460] vs 0.6% [1 of 155]). The most common adverse event that showed an increase for rasagiline over placebo was dyskinesia, occurring in 4.8% (22 of 460) receiving preladenant, 13.6% (21 of 154) receiving rasagiline, and 5.2% (8 of 155) receiving placebo.

In trial 2, preladenant was generally well tolerated, although the incidence of adverse events was higher for preladenant (occurring in 60.5% [190 of 314]) than placebo (occurring in 45.9% [73 of 159]). Few patients (5.1% [16 of 314] of those receiving preladenant and 2.5% [4 of 159] of those receiving placebo) discontinued treatment because of adverse events. One death was reported, a suicide (self-inflicted gunshot wound to the chest) in the preladenant 2 mg twice daily group, which was considered possibly related to the study drug by the investigator. The most common adverse event that showed an increase for preladenant over placebo was constipation (8.0% [25 of 314] vs 2.5% [4 of 159]).

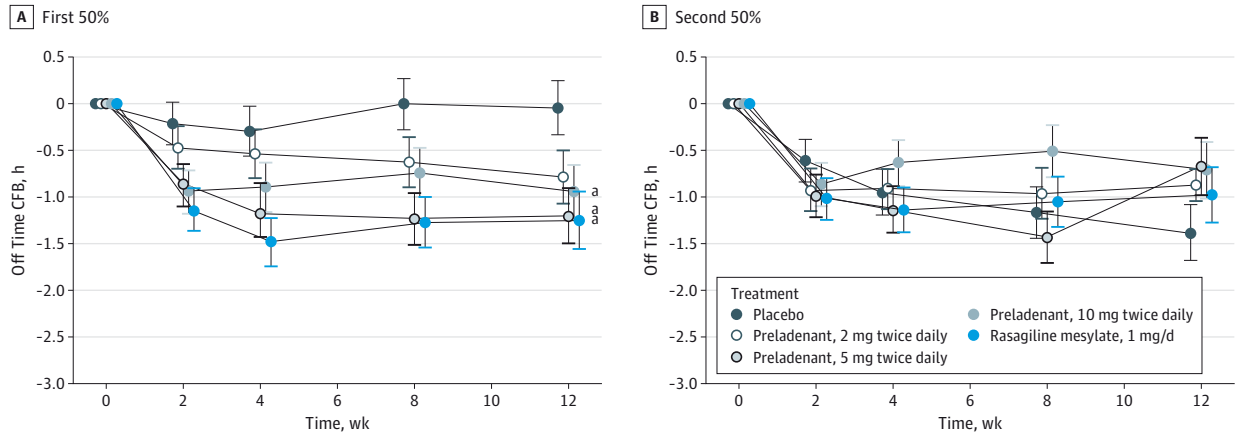
Post Hoc Analyses (Trial 1 Only)

Investigations determined that the randomization code and treatments were correctly administered. Treatment groups in trial 1 were generally comparable in terms of demographics, baseline disease characteristics, and concomitant medications (including caffeine use). Review of demographic data and baseline disease characteristics did not reveal any notable differences between the phase 2b trial[17] and the phase 3 trial or differences vs other similar published PD trials[24,25] (eTable 2 in Supplement 3). Plasma concentrations of preladenant in trial 1 were largely consistent with those in the phase 2b trial (eTable 3 in Supplement 3). The observed mean steady-state rasagiline level in trial 1 was 2.4 ng/mL at 2 hours after dosing, similar to previous trials.[26] Baseline caffeine use was not associated with off time change from baseline (P = .40 for <1 vs ≥1 cup per day and P = .54 for ≤1 vs >1 cup per day).

Analyses of potential regional differences (**Table 3**) found that Turkey, India, and Latin America had the largest mean reductions in off time in the placebo group (range, −1.00 hour to −2.15 hours), leading to numerically greater reductions in off time in the placebo group than in the preladenant or rasagiline groups. The mean placebo group reductions in off time were smaller in North America, the European Union, and Eastern Europe (range, −0.45 to −0.76 hour), leading to treatment responses that were directionally consistent with expectations that rasagiline and preladenant would show benefit vs placebo. However, improvements were still modest, with reductions from −0.39 hour to −1.18 hour in off time for preladenant 5 mg twice daily vs placebo and reductions from −0.57 hour to −0.71 hour in off time for rasagiline 1 mg/d vs placebo. There was no evidence of a dose response in the preladenant groups in any region.

Analyses by enrollment found that the first 50% of patients to be enrolled in trial 1 took approximately 18 months to enroll, whereas the second 50% took only approximately 9 months. Notably, patients in the placebo arm enrolled in the first half had a small response (reduction of −0.03 hour in off time) compared with patients enrolled in the second half (reduction of −1.40 hour in off time) (**Figure 2**). In addition, reductions in off time in the preladenant and rasagiline arms were slightly larger in the first half. Overall, patients randomized to preladenant (5 mg or 10 mg twice daily) or rasagiline enrolled in the first half demonstrated significant improvement vs placebo in reduction in off time (approximately 1 hour or more) (eTable 4 in Supplement 3).

Figure 2. Post Hoc Analysis of Change in Off Time by Enrollment Half in Trial 1



Shown is the estimated mean (SE) change from baseline in mean off time (hours per day) over time by enrollment half in trial 1 for the first 50% and the second 50%. CFB indicates change from baseline.

[a] $P < .05$ vs placebo.

Discussion

In these phase 3 trials, preladenant did not significantly reduce off time compared with placebo. However, because the active control (rasagiline) also failed to demonstrate a significant reduction in off time, it is not possible to determine from these results whether they represent a finding of inefficacy for preladenant or are related to issues of study design or conduct.

All 3 A_{2A} antagonists (istradefylline, preladenant, and tozadenant) have yielded positive results in reducing off time in phase 2 trials (eTable 5 in Supplement 3).[8-10,17] However, istradefylline had mixed phase 3 results,[11-14] and our 2 preladenant trials failed (while tozadenant has not yet completed a phase 3 trial). We also note that the designs of the phase 3 trials have been essentially the same as those of the phase 2 trials, which suggests that A_{2A} antagonists may have efficacy as adjuncts to levodopa but that problems with the execution of the phase 3 trials have hindered our ability to demonstrate this efficacy.

Demonstrating reduction in off time by diaries requires enrolling patients who actually have motor fluctuations, who can accurately recognize the various PD motor states (on, off, and dyskinesia), and who will accurately record those states over time in their diaries. Therefore, the investigator must select patients who actually have PD, confirm that the patient has true motor fluctuations, teach the patient to recognize the PD motor states, and verify that the patient understands them. It is then up to the patient to complete the diary in a timely and accurate manner. It seems likely that these requirements would be easiest to accomplish at a small number of expert sites with a successful clinical trial track record and a large population of well-known patients from which to draw, which appears to be the case for phase 2 trials but becomes more difficult in phase 3 trials, when more sites and more participants are required. In fact, the negative phase 3 studies enrolled the most patients (eTable 5 in Supplement 3).

In our post hoc analyses of trial 1, we identified a large placebo effect in Turkey, India, and Latin America, with numerically greater reductions in off time in these regions in the placebo group than in the preladenant or rasagiline groups. The exact reason for this finding is not known, but a large placebo response was also observed in a phase 3 monotherapy trial of preladenant in Latin America, India, Turkey, and Eastern Europe compared with North America and the European Union.[27] We are also aware of a phase 2 trial of fipamezole as an antidyskinetic agent in which benefit was demonstrated in the United States but not in India.[28] The differences could potentially be owing to clinical trial experience, cultural or language variations, genetic variation, or as yet unidentified reasons. Because no stratification or block randomization was used, our subgroup analyses may be subject to bias because they do not represent a fully randomized sample. A subtype analysis was not performed, and subtype response variance may also have affected results.

We found that there was a striking difference in results between the first 50% of patients enrolled and the second 50% of patients enrolled in trial 1, the only trial to evaluate this. In fact, if just the first 50% of patients were considered, there was a significant reduction in off time in the preladenant and rasagiline groups compared with the placebo group. Analyses did not suggest that this effect was because of site or regional influences. We hypothesize that the most likely explanation for this finding is that sites enrolled their most ideal patients first. After that, less ideal patients may have been enrolled to satisfy enrollment targets. That the second 50% was enrolled in half the time it took to enroll the first 50% raises concern that an enrollment push by the

Box. Lessons Learned

Some Regions May Have Large Placebo Effects

In our trial 1, we observed a large placebo effect in Turkey, India, and Latin America. In a phase 3 preladenant monotherapy trial,[27] a large placebo effect was observed in Latin America, India, Turkey, and Eastern Europe. The reasons for this are not clear. Careful choice of sites and regions may help reduce placebo effects. Treatment stratification by region should be considered for smaller trials to assure balanced assignment of arms. Specialized training to mitigate placebo response is recommended for all sites.

More Patients Is Not Always Better

In our trial 1, post hoc analysis found that if only the first 50% of enrolled patients were considered, both preladenant and rasagiline mesylate significantly reduced off time compared with placebo. Similar observations were made for paroxetine trials.[29] We hypothesize that sites enroll their most ideal patients first and then may "scrape the bottom of the barrel" to find additional patients to enroll. These patients may exhibit a larger placebo effect and may be less ideal in other ways, such as having less distinct motor fluctuations or more difficulty self-identifying their PD motor states. To mitigate this problem, one should avoid having to enroll more patients than are necessary (do not overpower the study and do not unnecessarily include dosage arms thought to be ineffective), consider more sites enrolling fewer patients if more competent sites are available, and avoid pressuring sites to enroll more patients or to enroll patients at a quicker pace.

Pressuring Sites to Increase Enrollment May Have Undesirable Consequences

Pressuring sites to enroll more patients or to enroll patients at a quicker pace may cause them to loosen their standards and enroll less ideal patients (as described above). In our trial 1, the second 50% of participants were enrolled in half the time it took to enroll the first 50%, suggesting that there may have been increased pressure to enroll during the second half of the study.

Avoid Unnecessary Exclusions

Unnecessary exclusions make enrollment more difficult, reduce the pool of eligible patients, and may increase the pressure on sites to enroll less ideal patients. This problem was not specifically identified in our studies but is commonly seen in clinical trials.

Active Control Arms Have Pros and Cons

Inclusion of an active control arm can be useful to confirm a trial's ability to detect efficacy using a medication known to be effective. In our trial 1, that the active control rasagiline did not exhibit efficacy compared with placebo suggested that there were problems with the design or conduct of the trial. This can be useful information to help interpret negative results regarding the active medication. On the other hand, because rasagiline was used as an active control, monoamine oxidase type B inhibitors were exclusionary, and this may have reduced the potential pool of eligible patients and placed an increased enrollment burden on the sites, leading to enrollment of less than ideal participants.

Understand Methodological Weaknesses and Institute Countermeasures Beforehand

In contemporary PD fluctuator trials, there are several responsibilities that are granted to the investigator and the patient such that trial data are dependent on the rigor with which the investigator and patient fulfill these responsibilities. First, the investigator is tasked with enrolling patients with a diagnosis of PD. This item could be strengthened by requiring the investigator or designate to complete a diagnosis criteria checklist that could be reviewed centrally. Second, eligible patients are required to have motor fluctuations, but there are often no clear criteria set. We suggest that UPDRS motor scores should be obtained in the clinic in the patient's usual on and off states, with a change criterion to be met to confirm the presence of motor fluctuations. Videotaped central ratings of on and off UPDRS motor scores would be even more rigorous but are more costly and time-consuming. Third, concordance diary testing to evaluate whether the patient understands the PD diary terminology and can self-identify his or her PD motor states is critically important. Unfortunately, this procedure is often conducted in an informal manner, with ongoing discussion between the rater and the patient. Strengthening this procedure by emphasizing that it is a test and that patient training should be completed before formal concordance testing is undertaken, with no discussion between the rater and patient regarding motor states during testing, should be emphasized. Fourth, receiving reliable data is dependent on the patient completely and accurately completing the diary in a timely fashion. A telephone call to the patient the day before the diary is completed to remind him or her to complete the diary and to review good practice completion instructions may be helpful. Sites should also exercise discretion and not enroll patients who are likely to be poorly compliant in diary completion. Electronic diaries may be helpful to remind patients to complete entries on time and limit entries to 1 per period.

sponsor or clinical research organization could potentially have degraded the quality of patients that sites were enrolling. These observations are consistent with results of an analysis of 4 phase 3 trials of paroxetine in major depression that found that a significant treatment effect was observed before approximately 100 patients had been enrolled per treatment arm.[29] However, continuing to enroll additional patients (up to approximately 150) did not maintain the achieved level of significance and in one case turned a potentially positive study into a negative study. Notably, pooled analysis indicated that patients in the fourth quarter of enrollment were more likely to be placebo responders than early-enrolling patients.

Inclusion of an active control arm was useful to identify trial 1 as a failed trial. Rasagiline has several positive qualities as an active control. Although mild in efficacy and typically well tolerated, it has consistently reduced off time in adjunctive trials. However, exclusion of monoamine oxidase type B inhibitors in our phase 3 trials may have placed an additional burden on enrollment and made it harder for investigators to enroll ideal patients.

To adequately evaluate adjunctive PD medications, investigators must enroll appropriate patients and ensure rigorous diary education. Improved documentation of on and off states may be helpful, including UPDRS motor scoring during the patient's routine on and off states, with a certain amplitude of change required. Although burdensome, central review of diagnosis and confirmation of fluctuations might be considered. Paper diaries are potentially subject to reduced compliance, recall bias, and diary fatigue, and elec-

tronic diaries may improve results, although they have their own set of limitations to consider.[30,31] There are also several objective motion sensors in development to assess PD throughout the day.[32] However, they are limited in many respects, including distinction of sleep or rest from the off condition and inability to distinguish troublesome from nontroublesome dyskinesia.

The most important lessons we learned from these trials are listed in the **Box**.

Conclusions

In our phase 3 trials, preladenant did not significantly reduce off time using essentially the same methods as in the phase 2b trial, in which efficacy was observed. However, we also did not observe a significant reduction in off time with the active control rasagiline, suggesting that our trials failed to adequately assess these medications.

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Study concept and design: Hauser, Stocchi, Rascol, Huyck, Ho, Sklar, Michelson, Hewitt.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Hauser.
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