Orexin Receptor Antagonism, a New Sleep-Enabling Paradigm: A Proof-of-Concept Clinical Trial

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The orexin system is a key regulator of sleep and wakefulness. In a multicenter, double-blind, randomized, placebo-controlled, two-way crossover study, 161 primary insomnia patients received either the dual orexin receptor antagonist almorexant, at 400, 200, 100, or 50 mg in consecutive stages, or placebo on treatment nights at 1-week intervals. The primary end point was sleep efficiency (SE) measured by polysomnography; secondary end points were objective latency to persistent sleep (LPS), wake after sleep onset (WASO), safety, and tolerability. Dose-dependent almorexant effects were observed on SE, LPS, and WASO. SE improved significantly after almorexant 400 mg vs. placebo (mean treatment effect 14.4%; $P < 0.001$). LPS (−18 min ($P = 0.02$)) and WASO (−54 min ($P < 0.001$)) decreased significantly at 400 mg vs. placebo. Adverse-event incidence was dose-related. Almorexant consistently and dose-dependently improved sleep variables. The orexin system may offer a new treatment approach for primary insomnia.

The orexin system has been implicated in the regulation of functions such as reward seeking,[1] feeding behavior,[2] locomotion and physical activity,[3–5] and arousal from sleep and the sleep–wake cycle.[6,7] Orexin-A and orexin-B (also known as hypocretin-1 and hypocretin-2, respectively) are neuropeptides that bind to the G protein–coupled receptors orexin-1 and orexin-2.[8–10] In rats as well as in humans, orexin levels in cerebrospinal fluid have been shown to fluctuate with the circadian cycle.[11–13] The levels are highest at the end of the wake-active period and lowest at the end of the sleep period.[11–13] Orexin deficiency has been linked to narcoleptic symptoms such as sudden sleep attacks and cataplexy, in animals[14–16] as well as in humans.[17,18]

Experiments in mice and rats have shown that orexin receptor antagonists have sleep-enabling effects.[3,19] The dual orexin receptor antagonist almorexant elicits somnolence without cataplexy in healthy rats, dogs, and humans when given during the active phase of the circadian cycle.[20] A phase I study investigating single-dose daytime administration of almorexant in healthy human subjects showed dose-dependent pharmacodynamic effects, with reductions in vigilance, alertness, visuomotor, and motor coordination observed for the 400- and 1,000-mg doses.[21] In the same study, pharmacoelectroencephalography profiles showed that almorexant decreases alpha Pz–Oz and increases beta Fz–Cz activities, as well as delta and theta power.[21] The increase in delta and theta power may potentially

indicate slow-wave sleep. Hence, inhibiting the orexin system with almorexant could represent a novel approach to the treatment of insomnia.

Insomnia is a persistent problem in ~10% of adults,[22–24] with primary insomnia estimated to be present in ~25% of patients with chronic insomnia.[25] Sleep-maintenance problems and nocturnal awakenings are more prevalent than sleep-onset difficulties.[26,27] Nonpharmacologic treatments are often preferred;[28] however, eventually, most patients either seek pharmacologic treatment or remain untreated.[29] Current standard pharmacologic treatments for insomnia include the benzodiazepine receptor agonists, (which potentiate the activity of γ-aminobutyric acid at the ionotropic γ-aminobutyric acid-A receptor), and the melatonin receptor agonist ramelteon. Benzodiazepine receptor agonists include benzodiazepines that decrease sleep latency and increase sleep time,[30–32] with some agents also improving sleep maintenance.[30,32] However, benzodiazepines have been associated with daytime drowsiness, tolerance, dependency, and withdrawal symptoms.[33–35] Newer benzodiazepine receptor agonists (nonbenzodiazepines) and ramelteon decrease sleep latency,[33,36,37] whereas some agents, such as eszopiclone and modified-release zolpidem, increase sleep time and improve sleep maintenance.[38,39] The side-effect profiles of nonbenzodiazepines and ramelteon appear to be better than those of benzodiazepines, with fewer next-day effects observed.[33,35–37,40] New insomnia therapies with different mechanisms of action are currently under investigation with the aim of further improving tolerability and sleep maintenance and specifically targeting sleep–wake architecture.[41,42]

We performed a two-part clinical study to evaluate the effect of almorexant on sleep in patients with primary insomnia. The primary objective was to determine the minimum dose of almorexant that would have a significant effect on sleep efficiency (SE). In the first part of the study, the effect of almorexant on SE was evaluated at a high dose of 400 mg; thereafter, we conducted the dose-ranging part of the study, which aimed to identify the minimum effective dose. The safety and tolerability of almorexant and its effect on objective and subjective sleep variables were also evaluated.

RESULTS

Between May 2006 and August 2007, 368 patients were screened and 161 were enrolled. **Supplementary Figure S1** online shows a summary of study enrollment and the patients treated at each dose level. The main reasons for screening failure were total sleep time (TST) >6 h and/or latency to persistent sleep (LPS) <20 min. Overall, eight patients were excluded from the per-protocol analysis: two of these did not complete the second treatment night because of adverse events (AEs) and six were excluded because of technical difficulties during polysomnography. The per-protocol analysis set therefore consisted of 153 patients. The mean age of all the patients treated was 45.2 years (60% in the age group 41–60 years); 66.5% were women; 98.8% were Caucasian, 0.6% were Hispanic, and 0.6% were Asian. The mean body mass index was 24.2 kg/m^2.

Efficacy

Objective polysomnographic assessments. The mean values for objective sleep variables on the screening/adaptation night and the almorexant and placebo treatment nights for almorexant 400, 200, 100, and 50 mg are reported in **Table 1**. SE significantly improved after a single 400-mg dose of almorexant, relative to placebo (mean treatment effect 14.4%; $P <$ 0.001), thereby demonstrating the efficacy of almorexant at this dose level (**Figure 1a**). A dose-related increase in SE was observed with almorexant 100, 200, and 400 mg relative to placebo (**Figure 1a**). The lowest effective dose was 100 mg (mean treatment effect 4.6%; $P = 0.02$). No significant treatment effect was observed for a dose of 50 mg (mean treatment effect 3.3%; $P = 0.23$), and enrollment was stopped in accordance with the study protocol. As a result, only six patients were recruited to the 25-mg dose group, and consequently the efficacy data for this dose group are not reported; however, the data for these patients were included in the safety analysis. The robustness of the main analysis was confirmed by similar findings for the all-treated analysis, which persisted even after adjustment for period and carryover effects (see **Supplementary Table S1** online). LPS was significantly reduced after the 400-mg dose of almorexant relative to placebo (mean treatment effect –18 min; $P = 0.02$; **Figure 1b**). Wake after sleep onset (WASO) declined significantly in response to the 400-mg dose relative to placebo (mean treatment effect –54 min; $P < 0.001$), and dose-related, clinically relevant, and nominally significant improvements were observed after almorexant doses of 200 ($P < 0.001$) and 100 mg ($P = 0.004$) (**Figure 1c**).

Exploratory polysomnography measurements showed dose-related increases in mean TST for almorexant 400, 200, and 100 mg as compared with placebo (**Figure 1d**). Almorexant 400 mg was associated with a mean of 411.8 min sleep vs. 342.1 min with placebo. Higher almorexant doses reduced latencies to S1 (400 mg), S2, S3, and S4 sleep stages (200 mg) (data not shown). Latency to rapid eye movement (REM) sleep decreased dose-dependently (**Figure 1e**). Almorexant 400 mg decreased the time spent in S1 and increased the time spent in S2, S3, S4, and REM sleep stages, as compared with placebo (**Table 2**). Similar but less pronounced effects were observed after the 200- and 100-mg doses. The percentage of TST spent in S1 decreased dose-dependently and the percentage of TST spent in REM increased dose-dependently after 400 and 200 mg as compared with placebo. The percentages of TST spent in S2, S3, and S4 were similar after placebo and after almorexant, irrespective of the almorexant dose.

Subjective assessments. The mean values for subjective assessments on the night of the screening/adaptation and on the nights of almorexant (400-, 200-, 100-, and 50-mg doses) and placebo treatments are given in **Table 3**. Self-reported patient assessments showed dose-related increases in subjective SE (**Figure 2a**) and decreases in subjective sleep latency after almorexant 400 and 200 mg relative to placebo (**Figure 2b**). No treatment differences were observed for mean subjective WASO at any almorexant dose relative to placebo (**Figure 2c**).

Table 1 Mean values of objective sleep variables for the screening/adaptation night and the treatment nights

Variable		Almorexant dose level										
	Screening/adaptation	Placebo (n = 39)	Almorexant 400 mg	Screening/adaptation	Placebo (n = 38)	Almorexant 200 mg	Screening/adaptation	Placebo (n = 38)	Almorexant 100 mg	Screening/adaptation	placebo (n = 32)	Almorexant 50 mg
SE												
% (SD)	63.4 (14.1)	71.0 (14.9)	85.4 (8.7)a	64.2 (15.2)	75.6 (15.2)	83.8 (11.8)a	60.7 (14.3)	78.1 (12.8)	82.7 (10.1)a	62.7 (10.1)	75.4 (16.7)	78.7 (9.1)
LPS												
min (SD)	74.3 (53.1)	54.2 (43.7)	36.2 (31.6)a	73.5 (55.1)	38.7 (35.5)	28.2 (24.1)	78.5 (41.1)	46.9 (49.5)	36.5 (29.3)	97.8 (59.8)b	49.6 (35.6)b	43.9 (33.4)b
WASO												
min (SD)	119.0 (46.0)	94.0 (56.5)	40.0 (29.0)a	124.1 (57.5)	86.9 (59.6)	52.7 (54.1)a	127.6 (50.0)	68.5 (46.7)	48.4 (40.3)a	115.2 (46.2)	69.6 (47.3)	62.9 (37.9)
TST												
min (SD)	306.8 (68.6)	342.1 (71.3)	411.8 (42.2)a	308.1 (71.8)	363.1 (73.6)	402.3 (56.9)a	291.7 (68.9)	375.8 (61.4)	397.2 (48.7)a	301.2 (48.5)	362.0 (80.1)	377.9 (43.5)
Latency to REM												
min (SD)	134.3 (60.7)c	121.9 (66.3)	76.6 (38.8)a	154.5 (74.6)d	127.6 (63.1)	96.9 (54.6)a	135.5 (59.9)e	105.7 (45.7)	93.6 (40.4)	151.7 (81.0)b	122.7 (50.4)b	108.2 (51.7)b

LPS, latency to persistent sleep; REM, rapid eye movement; SE, sleep efficiency; TST, total sleep time; WASO, wake after sleep onset.
aSignificant difference in treatment effect observed between the almorexant and placebo treatment nights (P < 0.05). P value determined by paired t-test. bn = 31 patients with evaluable data. cn = 38 patients with evaluable data. dn = 36 patients with evaluable data. en = 37 patients with evaluable data.

Subjective TST increased dose-dependently (**Figure 2d**). Subjective sleep quality improved after almorexant 400 and 200 mg relative to placebo (**Figure 2e**). Awakening quality and somatic complaint subscores were similar among treatments (data not shown).
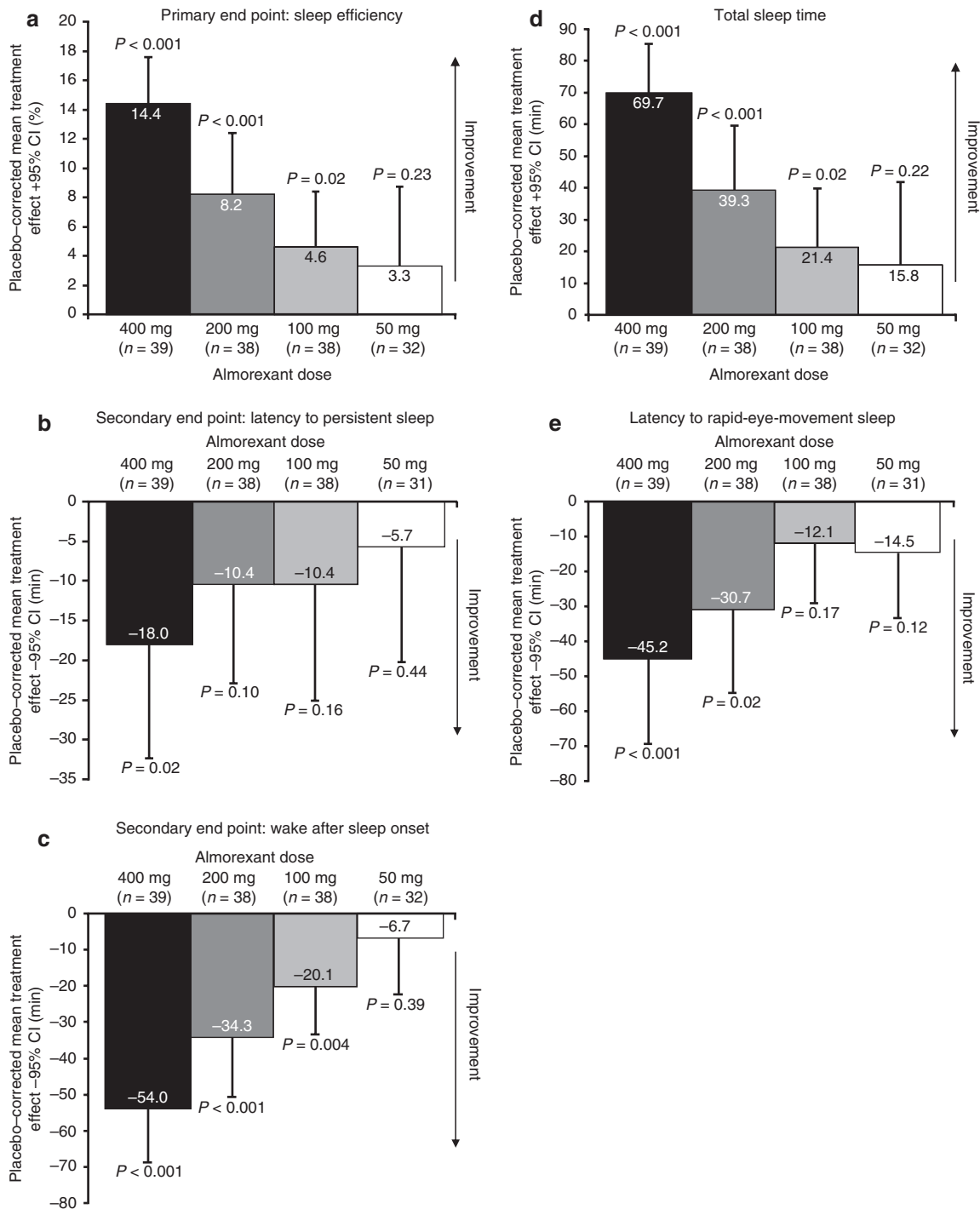
Next-day performance. Overall, no relevant negative residual effects of the previous night's treatment were observed after any of the almorexant doses relative to placebo with respect to subjective alertness levels (Bond and Lader visual analog scale; **Figure 3a**). With respect to mean reaction time (**Figure 3b**), there was a small increase after almorexant 400 mg only (mean treatment effect 34.7 ms (95% confidence interval 4.9–64.5 ms)). Almorexant had no effect on the scores in the fine-motor test (data not shown); in the digit span test, a lower forward score was observed for almorexant 200 mg as compared with placebo (mean treatment effect –0.41 (95% confidence interval –0.80 to –0.01)) (data not shown).

Safety
Overall, a larger proportion of patients reported at least one AE after almorexant administration than after placebo (21.9% vs. 13.8%). Dizziness, nausea, fatigue, headache, and dry mouth were the most common AEs reported after almorexant treatment (**Table 4**). The proportion of patients with one or more AE was highest after almorexant 400 mg (40.0%) but was markedly lower after doses of 200, 100, and 50 mg (12.8, 17.9, and 13.9%, respectively). There was one serious AE (vasovagal syncope after placebo treatment) and no deaths. Other than the serious event, all AEs were of mild or moderate severity. One patient discontinued the study because of a febrile upper respiratory tract infection after receiving almorexant 400 mg (unrelated to study treatment); another discontinued because of the aforementioned serious AE. No treatment-related changes in laboratory parameters, vital signs, or quantitative electrocardiogram variables were observed, nor did any electrocardiogram abnormalities develop. No narcolepsy or cataplexy events were reported, and no trend or dose relationship was detected by the narcoleptic effects questionnaire.

DISCUSSION
This is the first study to suggest that the orexin system may play a role in nocturnal sleep regulation in humans and that orexin receptor antagonism results in sleep-enabling effects in patients with primary insomnia. It extends and confirms previous preclinical and clinical observations.[20,21] In this two-part clinical study in patients with primary insomnia, a significant and clinically relevant increase in SE was observed after almorexant 400 mg, and the lowest effective dose was 100 mg. Almorexant 400 mg significantly improved sleep initiation as assessed by LPS, and at doses ≥100 mg almorexant dose-dependently and significantly decreased WASO, a measure of sleep maintenance. Almorexant decreased latency to REM sleep in a dose-dependent manner and decreased latencies to S1–S4 sleep stages at the higher doses, while increasing TST. The drug reduced the time spent in S1 and increased the time spent in REM sleep. At all doses ≥100 mg, it had similar effects on the percentages

Figure 1 Treatment effects of almorexant on objective sleep variables. Mean changes relative to placebo with 95% CIs. CI, confidence interval.

of TST spent in the S1 and REM sleep stages. The increase in time spent in REM sleep brought the percentage of TST spent in REM to approximately within the normal range, generally 20–25% in normal young adults[43] and decreasing with age to 18% in men and 19% in women (age range 61–70 years).[44] These results indicate that the administration of an almorexant dose as low as 100 mg caused an increase in the TST during the night, mainly by improving sleep maintenance. This dose level administered in the daytime did not result in detectable impairment

of vigilance, alertness, or visuomotor and motor coordination in healthy subjects.[21]

Consistent effects were generally observed between objective and subjective assessments of sleep variables, particularly with respect to SE, TST, and LPS, although differences were apparent at the lower almorexant doses. The drug had no effect on subjective WASO at any dose, although objective WASO decreased dose-dependently and patients reported improved sleep quality. This absence of an effect on subjective WASO warrants

Table 2 Mean values of time in sleep stages and percentage of each sleep stage of total sleep time for the treatment nights

	Almorexant dose level					
	Placebo	Almorexant 400 mg	Placebo	Almorexant 200 mg	Placebo	Almorexant 100 mg
Sleep stage	*n* = 39		*n* = 38		*n* = 38	
Stage 1						
Time, min (SD)	35.4 (18.0)	29.2 (16.1)[a]	34.8 (17.1)	28.4 (12.4)[a]	28.0 (12.2)	24.2 (11.2)[a]
Percentage of TST, % (SD)	10.6 (5.4)	7.1 (4.0)[a]	10.1 (6.0)	7.5 (4.6)[a]	7.8 (4.3)	6.3 (3.2)[a]
Stage 2						
Time, min (SD)	194.3 (53.8)	231.8 (45.8)[a]	210.4 (57.4)	227.4 (49.3)	209.9 (48.6)	227.9 (49.3)[a]
Percentage of TST, % (SD)	56.6 (9.0)	56.2 (9.2)	57.9 (11.0)	56.7 (10.6)	55.9 (10.3)	57.7 (11.6)
Stage 3						
Time, min (SD)	24.7 (14.3)	33.8 (22.1)[a]	28.3 (17.1)	30.4 (16.7)	38.4 (25.9)	40.4 (25.2)
Percentage of TST, % (SD)	7.3 (4.0)	8.2 (5.2)	8.1 (6.4)	7.3 (3.7)	9.9 (6.2)	10.0 (5.9)
Stage 4						
Time, min (SD)	31.2 (30.4)	37.4 (35.7)[a]	30.4 (31.2)	36.6 (32.4)[a]	26.9 (30.1)	28.8 (28.7)
Percentage of TST, % (SD)	9.3 (9.0)	9.3 (9.0)	8.0 (7.3)	8.9 (7.6)	7.1 (7.9)	7.0 (6.7)
REM						
Time, min (SD)	56.5 (23.5)	79.6 (27.7)[a]	59.1 (24.8)	79.5 (26.6)[a]	72.7 (27.0)	75.9 (24.9)
Percentage of TST, % (SD)	16.2 (5.1)	19.2 (5.9)[a]	15.9 (5.6)	19.7 (5.7)[a]	19.3 (6.4)	19.0 (5.7)

Data for almorexant 400, 200, and 100 mg doses only shown; no significant differences were observed between the almorexant 50 mg and placebo treatment nights.

REM, rapid eye movement; TST, total sleep time.

[a]Significant difference in treatment effect observed between the almorexant and placebo treatment nights ($P < 0.05$). *P*-value determined by paired *t*-test.

further investigation in future studies using other sleep-diary assessments. With respect to all the objective sleep variables, improvements were observed in the mean values from the night of screening/adaptation to that after placebo treatment. This may be due to an improvement in the patients' comfort with the equipment and sleep laboratory procedures.

Two residual effects on next-day performance, namely, increased mean reaction time and a lower forward score on the digit span test, were detected in association with the higher almorexant doses (400 and 200 mg, respectively). Given that the major goal for insomnia therapies is to improve sleep maintenance without affecting next-day performance, further investigation of any potential next-day effects after almorexant treatment at the therapeutic dose would be valuable. Of the current standard pharmacologic treatments used for sleep maintenance indications, benzodiazepines are associated with next-day effects; however, nonbenzodiazepine agents such as eszopiclone and controlled-release zolpidem cause minimal or no residual daytime effects.[35,39,40,45]

The safety profile of almorexant was similar to that of placebo for the lower doses, and an increase in the incidence of AEs was evident only for the 400-mg dose. There was no evidence of narcolepsy or cataplexy as seen from AE reporting and the narcoleptic effects questionnaire. The decrease in latencies to REM after almorexant administration deserves further attention and should be studied in larger trials at the therapeutic doses. Shortened REM latency could potentially point to narcolepsy-like changes in sleep architecture or potential direct effects of dual orexin receptor antagonists on sleep; alternatively, it could

indicate REM rebound in individuals susceptible to chronic partial REM deprivation.

The strengths of this study include the trial design, which involved the initial administration of a high dose of almorexant followed by decreasing doses in subsequent stages. This design allowed the efficacy of almorexant at a high dose to be established first before determining the lowest effective dose. Limitations of the study include the shortness of the treatment duration from which to assess the efficacy, safety, and tolerability of almorexant. The sleep-enabling and -maintaining effects and long-term safety and tolerability of dual orexin receptor antagonist treatment need to be assessed in large, long-term, randomized trials. This step is essential for new insomnia therapies because long-term use of current pharmacologic treatments can be associated with the development of tolerance and dependence, and with rebound insomnia on their withdrawal.[22,46,47] The clinical development of almorexant was discontinued in January 2011 for reasons not related to any of the observations in our study.[48]
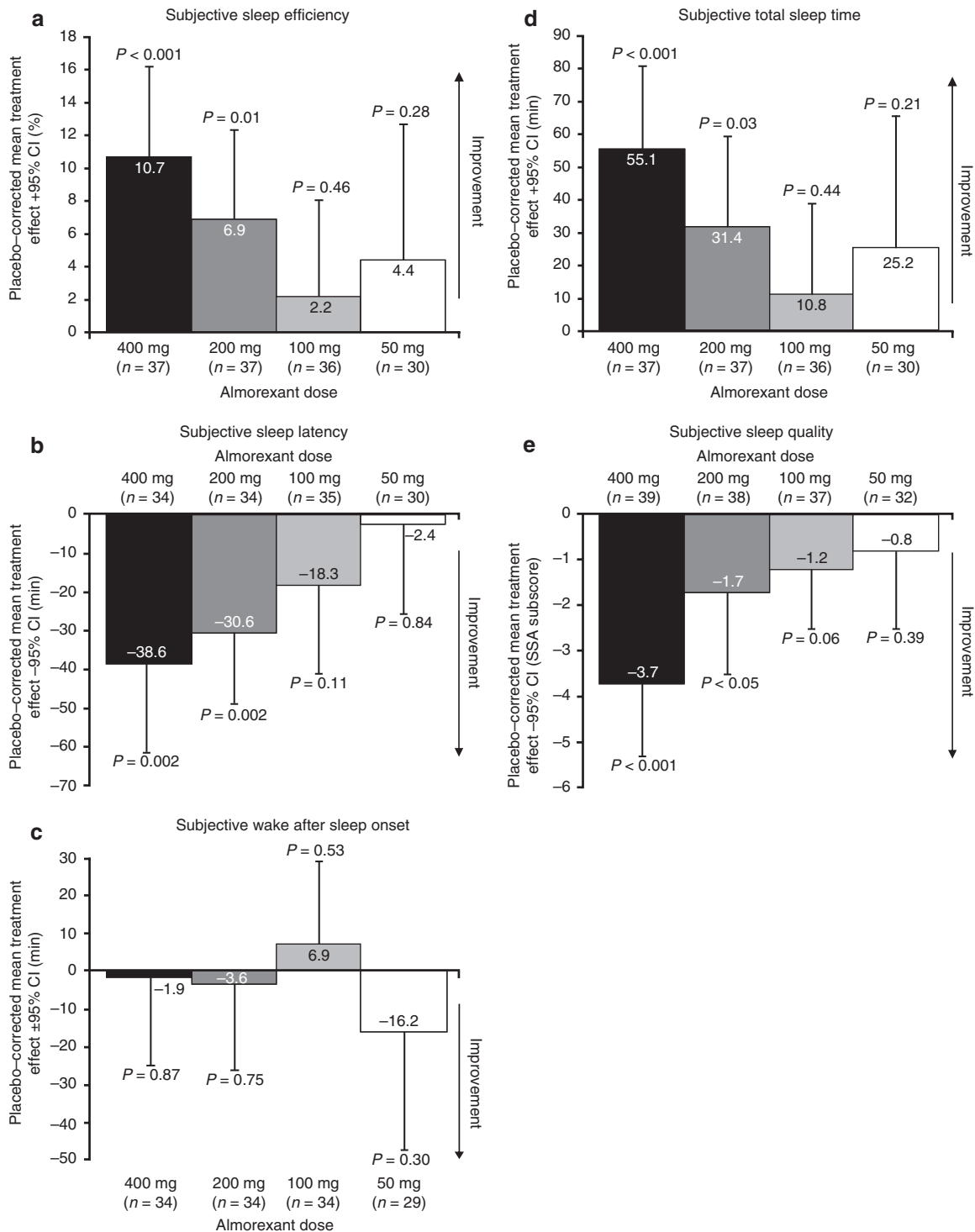
In conclusion, in this study, a single dose of the dual orexin receptor antagonist almorexant enabled initiation and maintenance of sleep in patients with primary insomnia. Almorexant treatment was associated with a dose-dependent improvement in SE, strongly suggesting that the endogenous orexin system plays an important role in the sleep–wake cycle. Consistent improvements were observed in sleep time, initiation, and maintenance, and in the patients' perception of sleep. Small effects on next-day performance were observed only after the higher almorexant doses. No safety concerns were revealed. Overall,

Table 3 Mean values of subjective sleep variables and next-day performance for the screening/adaptation night and the treatment nights

Variable	Almorexant dose level											
	Screening/ adaptation	Placebo	Almorexant 400 mg	Screening/ adaptation	Placebo	Almorexant 200 mg	Screening/ adaptation	Placebo	Almorexant 100 mg	Screening/ adaptation	Placebo	Almorexant 50 mg
		n = 39			n = 38			n = 38			n = 32	
SE												
Evaluable data, n	36	37	37	37	37	37	35	36	36	30	30	30
% (SD)	56.7 (17.8)	62.3 (16.1)	73.0 (14.8)[a]	52.3 (22.0)	61.7 (23.3)	68.5 (17.8)[a]	52.3 (20.1)	66.7 (18.4)	68.9 (19.1)	61.2 (24.3)	65.2 (25.6)	69.7 (18.4)
Sleep latency												
Evaluable data, n	34	34	34	34	34	34	33	35	35	29	30	30
min (SD)	93.8 (63.2)	81.5 (65.8)	42.8 (31.8)[a]	101.2 (84.7)	76.3 (69.5)	45.7 (34.7)[a]	100.2 (90.8)	78.5 (73.7)	60.2 (45.1)	86.5 (59.8)	63.0 (55.8)	60.7 (50.0)
WASO												
Evaluable data, n	34	34	34	34	34	34	33	34	34	28	29	29
min (SD)	115.9 (75.5)	93.0 (65.1)	91.1 (73.9)	118.7 (63.5)	98.3 (68.9)	94.7 (48.6)	127.2 (69.1)	85.3 (56.8)	92.2 (68.2)	80.5 (64.3)	95.0 (79.2)	78.8 (69.1)
TST												
Evaluable data, n	36	37	37	37	37	37	36	37	37	30	30	30
min (SD)	276.4 (89.0)	300.9 (77.9)	356.0 (74.0)[a]	253.5 (106.6)	300.3 (115.2)	331.6 (83.6)[a]	255.6 (96.4)	322.3 (88.1)	333.1 (95.1)	278.2 (105.5)	303.7 (112.6)	328.8 (84.7)
Sleep quality												
Evaluable data, n	39	39	39	38	38	38	37	37	37	32	32	32
SSA (SD)	19.2 (4.0)	18.6 (3.8)	14.8 (4.5)[a]	20.9 (3.4)	18.3 (4.9)	16.6 (4.8)[a]	19.1 (4.3)	16.3 (4.9)	15.1 (4.6)	18.1 (3.7)	17.0 (4.4)	16.3 (4.5)
Next-day performance alertness												
Evaluable data, n	38	39	39	38	38	38	38	38	38	32	32	32
mm (SD)	57.5 (17.7)	60.4 (16.0)	56.7 (15.5)	58.9 (18.8)	60.1 (20.0)	62.5 (18.5)	61.9 (22.0)	62.7 (20.5)	67.3 (19.7)	64.0 (17.4)	64.3 (19.4)	64.7 (16.0)
Reaction time												
Evaluable data, n	25	29	29	33	34	34	30	34	34	27	28	28
ms (SD)	845.0 (174.2)	844.8 (145.2)	879.5 (167.3)[a]	797.9 (115.3)	830.9 (152.3)	811.2 (141.9)	899.9 (237.8)	900.1 (189.0)	898.9 (208.1)	826.1 (176.9)	830.1 (105.0)	840.1 (122.8)

SE, sleep efficiency; SSA, self-rating sleep and awakening quality questionnaire; TST, total sleep time; WASO, wake after sleep onset. *P* value determined by paired *t*-test.

[a]Significant difference in treatment effect observed between the almorexant and placebo treatment nights (*P* < 0.05). *P* value determined by paired *t*-test.
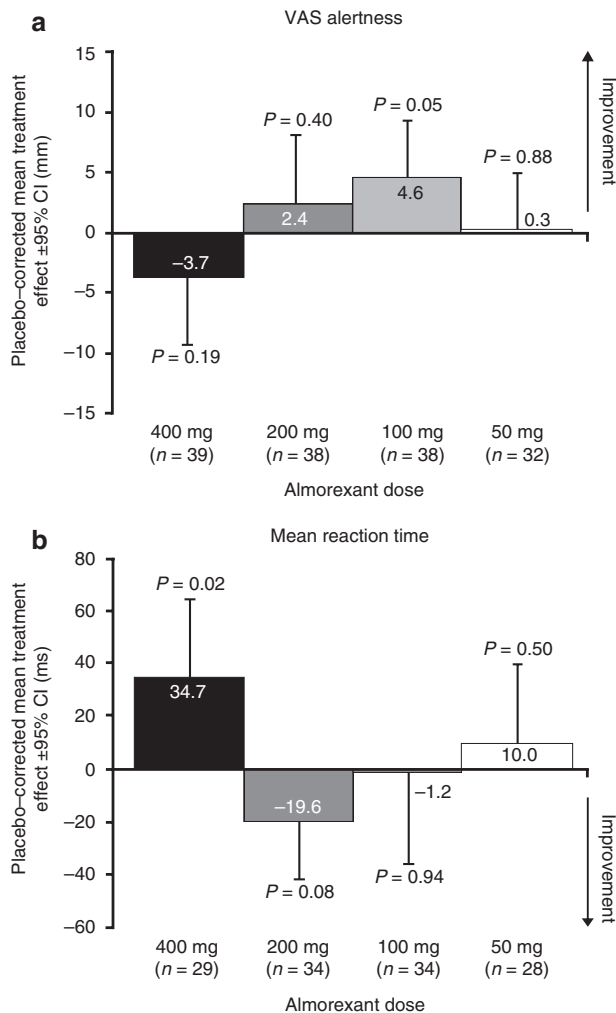
Figure 2 Treatment effects of almorexant on subjective sleep variables. Mean changes relative to placebo with 95% CIs. CI, confidence interval; SSA, self-rating sleep and awakening quality questionnaire.

dual orexin receptor antagonism may offer a potential new treatment approach for primary insomnia.

METHODS

Study design. This was a prospective, multicenter, multistage, double-blind, randomized, placebo-controlled, two-way crossover, single-dose study involving almorexant in patients with primary insomnia. The study was conducted at 20 centers across Europe and Israel (6 in Germany; 3 in Austria; 2 each in Finland, Sweden, and Switzerland; and 1 each in Denmark, Israel, the Netherlands, Spain, and the United Kingdom). The study design incorporated two parts: a first part to determine the efficacy of almorexant at a high dose followed by a second part with dose-descending stages to establish the minimum effective dose. In the first part of the study, the patients received a single dose of 400 mg oral almorexant and a placebo on different treatment nights 1 week apart, in a crossover design. The patients were allocated

Figure 3 Treatment effects of almorexant on next-day alertness and performance (reaction time test). Mean changes relative to placebo with 95% CIs. CI, confidence interval; VAS, visual analog scale.

in a 1:1 ratio to the two treatment sequences (placebo/almorexant and almorexant/placebo) on the first treatment night, using a computer-generated randomization list. The study treatments were indistinguishable with respect to packaging, appearance, and number of capsules administered. The duration of the washout period and the timing of study drug administration were based on pharmacodynamic and pharmacokinetic data from a previous ascending single-dose study of almorexant in healthy subjects. Almorexant was rapidly absorbed, with a median time to maximum concentration ranging from 0.7 to 2.3 h and rapid decreases to ~20% of peak plasma concentrations over the course of 8 h after time to maximum concentration.[21] The pharmacodynamics of the drug correlated well with the concentration levels and, in general, the onset of pharmacodynamic effects was observed within 1 h after the dose.[21]

If the primary end point treatment effect was found to be significant ($P < 0.05$), the study continued with the next lower dose (200, 100, 50, or 25 mg) in new patients sequentially until the treatment effect was no longer statistically significant. When the number of patients successfully screened reached the target sample size for a dose level, subsequent patients were assigned to the next dose level. Therefore some patients entered the next dose level before the outcome for the previous dose was known. After study enrollment was completed, all the patients were scheduled to complete all periods and assessments. If the primary end point was not significant at 400 mg, 1,000 mg was to be tested against

placebo in new patients. For each dose level, patients underwent a screening period (up to 4 weeks), first treatment night, 1-week washout period, second treatment night, and 28-day post-treatment follow-up. Patients were required to complete a daily sleep log for at least 1 week before the screening/adaptation night, 1 week before the first treatment night, and during the 1-week washout period before the second treatment night. The sleep log consisted of a "day questionnaire" and the self-rating sleep and awakening quality questionnaire (SSA).[49] The "day questionnaire" assessed the patient's daily habits regarding meals, caffeine-containing beverages, alcoholic drinks, smoking, nonstudy medication, and naps. In addition, information on the patient's well-being, physical and emotional stress, and expected stressful events was collected. The investigator reviewed the sleep log and assessed the patient's well-being and compliance before each polysomnography recording. Polysomnography was performed for 8 h overnight during the screening/adaptation night and each treatment night. The purpose of the screening/adaptation night was to objectively confirm the subjectively patient-reported pattern of sleep disturbances relating to TST and sleep onset latency. The screening/adaptation night also served to accustom the patient to the sleep laboratory, the polysomnography equipment, and the psychometric tests. All polysomnography results were analyzed in accordance with the rules of Rechtschaffen and Kales, using the validated Somnolyzer 24 × 7 scoring tool[50–52] and including a structured expert review. Sleep was scored in 30-s epochs. During treatment nights, a single dose of almorexant or placebo was administered 30 min before the start of the polysomnography. The patients were required to remain at the center the morning following the polysomnography until they were free of symptoms possibly related to sleep-enabling medication, as judged by the physician responsible.

Patients. Male and female patients 18–65 years of age were eligible for inclusion if they had primary insomnia (by the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition—Text Revision*) and a history (≥3 months) of subjectively reported usual TST of 3–6 h, usual sleep disturbance with a subjective sleep onset latency of >30 min, and daytime complaints associated with poor sleep. Other inclusion criteria were polysomnographic confirmation of TST of <6 h and LPS ≥20 min during the screening/adaptation night; a body mass index of 18–30 kg/m^2; no clinically relevant abnormalities as shown by a 12-lead electrocardiogram and by hematology/biochemistry test results; willingness to refrain from central nervous system-active drugs for five half-lives of the drug (at least 1 week); and a negative urine test for barbiturates, cannabinoids, amphetamines, and cocaine. Psychotropic drugs were not permitted, except hypnotics with a short half-life of ≤10 h taken ≥48 h before each treatment night; other stable medications were allowed. Cytochrome P450 isoenzyme 3A4 inhibitors were not permitted within 1 week before screening. Key exclusion criteria included major depressive disorder, severe psychosis, or significant anxiety disorder; a score >2 on the symptom assessment questionnaire for diagnosis of apnea,[53] a raw score ≥50 on the Zung Self-Rating Depression or Anxiety Scale;[54,55] and restless legs syndrome or insomnia associated with or caused by sleep apnea or periodic limb movement disorder, as assessed by polysomnography during the screening night (defined as apnea/hypopnea index >10/h or periodic limb movement arousal index >10/h, respectively). Caffeine consumption of >7 U/day was not permitted on a regular basis (one unit of caffeine was defined as one cup of coffee or two cups of tea). Pregnancy and lactation were also exclusion criteria. Women with childbearing potential were administered urine pregnancy tests at predefined time points during the study and were required to use a reliable method of contraception during the entire study duration and for at least 3 months after intake of the study drug.

Study end points. The primary end point was SE as determined by polysomnography, where SE (%) = (TST in minutes/total time in bed in minutes (fixed to 480 min)) × 100. Secondary end points, determined by polysomnography, were LPS (the time in minutes from the start of recording to the beginning of the first 20 nonwake epochs) and WASO (the time in minutes spent awake after sleep onset until the end of the recording, where sleep onset is the time of the first occurrence of three

Table 4 Adverse events (safety population) occurring at least once in the overall almorexant group or the placebo group (includes related and unrelated events)

Event	Almorexant					Placebo (*n* = 160)
	50 mg (*n* = 36)	100 mg (*n* = 39)	200 mg (*n* = 39)	400 mg (*n* = 40)	Overall[a] (*n* = 160)	
Patients with at least one event, *n* (%)	5 (13.9)	7 (17.9)	5 (12.8)	16 (40.0)	35 (21.9)	22 (13.8)
Total events, *n*	8	11	6	42	73	30
Adverse events, *n* (%)						
Dizziness	1 (2.8)	1 (2.6)	1 (2.6)	2 (5.0)	7 (4.4)	0
Nausea	1 (2.8)	1 (2.6)	1 (2.6)	2 (5.0)	7 (4.4)	0
Fatigue	1 (2.8)	0	0	5 (12.5)	6 (3.8)	4 (2.5)
Headache	2 (5.6)	2 (5.1)	0	2 (5.0)	6 (3.8)	4 (2.5)
Dry mouth	0	1 (2.6)	0	4 (10.0)	5 (3.1)	0
Somnolence	0	0	1 (2.6)	3 (7.5)	4 (2.5)	1 (0.6)
Sleep apnea syndrome	0	1 (2.6)	1 (2.6)	1 (2.5)	3 (1.9)	0
Abdominal pain	0	0	0	2 (5.0)	2 (1.3)	0
Abnormal dreams	0	0	0	2 (5.0)	2 (1.3)	0
Cardiac murmur	0	0	0	2 (5.0)	2 (1.3)	0
Diarrhea	1 (2.8)	0	0	1 (2.5)	2 (1.3)	0

Includes patients who were randomized to receive at least one dose of study medication and had at least one postbaseline assessment.

[a]Includes six patients who received almorexant 25 mg.

consecutive epochs in S1 or first occurrence of S2). Exploratory end points measured by polysomnography included TST (the amount of actual sleep time in minutes in the total sleep period), latency to sleep stages (the time in minutes from the start of the polysomnography recording to the first occurrence of the respective sleep stage) including REM sleep (the time in minutes from sleep onset to the first occurrence of REM), and time spent (in minutes) and percentage of TST for each of the sleep stages; and subjective measures of SE, sleep latency, WASO, TST, and sleep quality, assessed using the SSA. Next-day performance and alertness after treatment nights were assessed using the Bond and Lader visual analog scale, which assesses 16 subjective feelings;[56] fine-motor testing, reaction time testing;[57,58] and both forward and backward digit span testing.[59]

Safety assessments. AEs and serious AEs occurring within 36 h of administration of study treatment were recorded, irrespective of whether they were considered to be related to the study treatment. Any AE that continued for 24 h after the last drug intake was monitored for up to 28 days. Clinical laboratory tests, vital signs, 12-lead electrocardiogram, and a subjective narcoleptic effects questionnaire were assessed the morning after study drug administration. The narcoleptic effects questionnaire was specifically designed for this study; it evaluated symptoms of cataplexy and sleep paralysis seen in narcolepsy with a series of yes/no questions on muscle relaxation/weakness and dreams.

Statistical analysis. This dose-ranging study was powered to detect a placebo-corrected mean difference in SE of 6.5%. SE was assumed to be normally distributed, with a standard deviation of 9.8%; no period or carryover effects were expected. The desired power for each dose level (1,000, 400, 200, 100, 50, and 25 mg) was 98%, 98%, 96%, 94%, 94%, and 94%, requiring 39, 39, 34, 31, 31, and 31 patients, respectively. This approach was used to maximize the power for the first dose tested (400 mg) and to have ≥80% actual power at the 50 mg dose level. By this calculation, a minimum of 78 patients (400 and 1,000 mg dose levels) and a maximum of 166 patients (400, 200, 100, 50, and 25 mg dose levels) were required for the study. The null hypothesis of no difference between each dose and placebo was tested using a two-sided paired *t*-test on the per-protocol analysis set, and rejected when *P* < 0.05. If the null hypothesis was rejected, secondary end points were to be

sequentially tested (i.e., first LPS, then WASO) using a two-sided paired *t*-test. Robustness analyses of the primary and secondary end points included the Wilcoxon signed-rank test, and analysis of the all-treated set using all available data. For the primary end point, carryover and period effects were investigated using mixed modeling. If the carryover effect was significant (at the *α* = 0.10 level), statistical analysis of only the first period was carried out. Exploratory end points were analyzed in the same manner as the main analysis of the primary end point, but any statistical inferences had no confirmatory value. Statistical analyses were performed using SAS software version 8.2 (SAS Institute, Cary, NC). Safety end points were analyzed descriptively.

Study oversight. All materials were reviewed and approved by the appropriate independent ethics committees before the study began. The study was conducted in accordance with the Declaration of Helsinki, followed the International Conference on Harmonization Guidelines for Good Clinical Practice, and was registered at ClinicalTrials.gov (NCT00640848). Written informed consent was obtained from each patient before any study procedure and after adequate explanation of the aims, methods, objectives, and potential hazards of the study. It was made clear to each patient that he or she was completely free to refuse to enter the study or to withdraw from it at any time for any reason. Data were collected by the investigators and analyzed by the sponsor. The authors had access to the data, and they vouch for the accuracy and completeness of the data.

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/cpt

AUTHOR CONTRIBUTIONS

P.H. wrote the manuscript, designed research, and analyzed data. G.D. wrote the manuscript, designed research, analyzed data, and contributed new reagents/analytical tools. H. Beneš performed research. T.P. performed research. H.D.-H. wrote the manuscript, performed research, and contributed new reagents/analytical tools. M.J.B. wrote the manuscript and performed research. G.P. performed research and analyzed data. B.S. designed and performed research. O.P. performed research and analyzed data. D.K. wrote the manuscript and performed research. J.Z. wrote the manuscript and performed research. S.B. performed research. M.P. wrote the manuscript and performed research. C.L.B. performed research. B.H. wrote the manuscript and performed research. I.O.E. performed research. E.H.-T. performed research. H. Bengtsson performed research. Y.P. performed research . U.-M.H. performed research. E.C. designed and performed research, and analyzed data. G.H. designed and performed research, and analyzed data. J.D. wrote the manuscript, designed research, and analyzed data.

CONFLICT OF INTEREST

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