

# Prolonged release melatonin for improving sleep in totally blind subjects: a pilot placebo-controlled multicenter trial

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**Introduction:** Melatonin, secreted by the pineal gland during the night phase, is a regulator of the biological clock and sleep tendency. Totally blind subjects frequently report severe, periodic sleep problems, with 50%–75% of cases displaying non-24-hour sleep–wake disorder (N24HSWD) due to inability to synchronize with the environmental day–night cycle. Melatonin immediate-release preparations are reportedly effective in N24HSWD. Here, we studied the efficacy and safety of prolonged-release melatonin (PRM), a registered drug for insomnia, for sleep disorders in totally blind subjects living in normal social environments. The primary endpoint was demonstration of clinically meaningful effects on sleep duration (upper confidence interval [CI] limit >20 minutes whether significant or not) to allow early decision-making on further drug development in this indication.

**Trial registration:** ClinicalTrials.gov registry – NCT00972075.

**Methods:** In a randomized, double-blind, placebo-controlled proof-of-principle study, 13 totally blind subjects had 2 weeks' placebo run-in, 6 weeks' randomized (1:1) PRM (Circadin®) or placebo nightly, and 2 weeks' placebo run-out. Outcome measures included daily voice recorded sleep diary, Clinical Global Impression of Change (CGIC), WHO-Five Well-being Index (WHO-5), and safety.

**Results:** Mean nightly sleep duration improved by 43 minutes in the PRM and 16 minutes in the placebo group (mean difference: 27 minutes, 95% CI: –14.4 to 69 minutes;  $P=0.18$ ; effect size: 0.82) meeting the primary endpoint. Mean sleep latency decreased by 29 minutes with PRM over placebo ( $P=0.13$ ; effect size: 0.92) and nap duration decreased in the PRM but not placebo group. The variability in sleep onset/offset and latency tended to decrease during PRM but not placebo treatment. The potentially beneficial effects of PRM persisted during the 2 weeks of discontinuation period, consistent with clock stabilizing effects. PRM was well-tolerated, adverse events were of mild or moderate severity and similar between PRM and placebo.

**Conclusion:** Nightly use of PRM may potentially improve patient-reported sleep difficulties in totally blind individuals trying to adhere to normal social lifestyle. A larger study powered to demonstrate a statistically significant effect is warranted.

**Keywords:** biological clock, non-24-hour sleep–wake disorder, sleep, melatonin

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## Introduction

The 24-hour rhythm in sleep and wakefulness is regulated by an intrinsic body clock – the suprachiasmatic nucleus (SCN) within the brain that controls the timing of sleep and wake and thereby prepares for the upcoming period of activity.<sup>1</sup> The individual period of the endogenous clock is usually longer than 24 hours (average 24.2 hours) and is normally entrained (synchronized) by the daily light–dark cycle to match the environmental 24-hour day.<sup>2</sup> The light cues that are necessary for entrainment

are perceived by the retina.<sup>3</sup> Approximately 55%–70% of totally blind patients are thought to have non-entrained rhythms and if they report periodic difficulties with sleep or daytime alertness, they are diagnosed as having non-24-hour ('free running') sleep–wake disorder (N24HSWD).<sup>4</sup> In N24HSWD, the free-running sleep–wake rhythm reflects the intrinsic oscillation of the circadian pacemaker and passes periodically out of phase with the social time for sleep and wake.<sup>5–9</sup> The rest of totally blind individuals entrain at a normal or abnormal phase in relation to the social day,<sup>4,10–12</sup> with a minority of these patients being entrained by light.<sup>13–15</sup>

Totally blind individuals with abnormal phase or non-entrained rhythms encounter great difficulties when trying to maintain a lifestyle within the societal norm. When forcing themselves into sleep at night, they will experience periods of difficulty initiating sleep at night and waking up in the morning and periods of premature sleep onset with early awakenings. In sleep laboratory assessments, these subjects had significantly lower total sleep time and sleep efficiency ( $P=0.0001$  for both) compared to age-matched, sighted, healthy subjects.<sup>9</sup>

Melatonin is released from the pineal gland in close association with the light–dark cycle. In sighted humans, melatonin secretion is halted during the light phase and is induced in the evening to reach a peak in the middle of the night.<sup>16</sup> Melatonin is involved in the regulation of sleep in two ways: it facilitates synchronization of the circadian clock with the ambient day–night cycle (chronobiotic) and attenuates the wake-promoting signal of the circadian system (soporific).<sup>17</sup> During nighttime, there is an increase in endogenous melatonin secretion, which results in higher propensity for sleep. Sleep onset will normally occur 2 hours after the onset of the endogenous melatonin, usually 14 hours after spontaneous wake-up in the morning.<sup>18,19</sup> In a totally blind person with N24HSWD, melatonin production will drift continuously through the night and daytime phases. The daytime production of melatonin is associated with daytime naps and difficulty sleeping at night in these patients.<sup>20,21</sup>

Since melatonin has beneficial effects on sleep in addition to its ability to entrain free-running rhythms to a 24-hour day, melatonin therapy is a rational approach for sleep disorders in the totally blind population.<sup>5,7</sup> The administration of melatonin (0.5–10 mg, once daily in the evening for 1 day up to several months) to totally blind individuals with N24HSWD facilitated phase advances and entrainment to the societal sleep/wake norms.<sup>20,22–25</sup> In addition, melatonin therapy was reportedly able to entrain endogenous melatonin and cortisol rhythms to the 24-hour cycle.<sup>20,22,24,25</sup>

A melatonin receptor agonist, tasimelteon, was recently approved by the US Food and Drug Administration (FDA) for the treatment of N24HSWD in the blind.<sup>26</sup> The clinical benefit to the patients in terms of ease to fall asleep and total sleep time has however not been rigorously addressed and no data from double-blind placebo-controlled randomized studies are currently available.

Melatonin is rapidly metabolized by the liver (half-life ~40 minutes). Because a short-term increase in melatonin was believed to best facilitate phase shifts in humans, studies in N24HSWD typically used immediate-release melatonin formulations.<sup>20,22,24,25,27</sup> Recent studies, however, suggest that modified-release preparations can be as effective for insomnia, as well as for phase resetting in sighted individuals,<sup>28–30</sup> but such formulations have not yet been tested in N24HSWD. A prolonged-release melatonin (PRM) formulation (Circadin® 2 mg) is approved for insomnia in many countries.<sup>31–33</sup> It was therefore pertinent to ask whether PRM would be effective for sleep disorders in totally blind patients.

The aim of this placebo-controlled randomized study was to evaluate the efficacy and safety of PRM in improving night sleep duration in totally blind subjects living in the community with periodic sleep difficulties while maintaining their habitual lifestyle. It was assumed that due to its potential effects on the biological clock, beneficial effects of PRM, should they exist, would reduce fluctuations in sleep onset/offset times and potentially persist for some time after drug discontinuation.

## Materials and methods

### Patients and procedures

This was a multi-center, double-blind, placebo-controlled study of a once-daily PRM (Circadin® 2 mg) dose in totally blind subjects with periodic sleep disorders. The study was performed in four centers in the US, under written approval from the appropriate local site's Independent Ethics Committee/Institutional Review Board (IEC, Helsinki Committee/IRB; ClinicalTrials.gov website registry NCT00972075).

Male or female subjects aged 20–80 years, who had no conscious perception of light (as confirmed by electroretinography) were recruited. Subjects had to meet the criteria for diagnosis of periodic sleep difficulty in the clinical setting: 1) difficulty initiating sleep or difficulty in awakening, with average total night sleep duration <6 hours per night; 2) progressive delays of sleep phase, with inability to maintain entrainment to 24-hour day; and 3) presence of this sleep pattern for at least 6 weeks. Subjects with medical disorders other than those related to blindness and medical

treatments that may have influenced melatonin production, sleep or alertness, as ascertained by medical history, were excluded. Use of melatonin during the preceding 2 weeks and use of benzodiazepines or other hypnotics during the study and the preceding 2 weeks or five half-lives, whichever was longer, and throughout the study, was prohibited. All participants signed the Informed Consent form approved by their institutional Ethics Committees.

Some authors from studies of melatonin in the blind have suggested that the time at which the first dosing of melatonin occurs within the individual circadian cycle is critical for entrainment,<sup>20</sup> whereas others have suggested that eventual entrainment of the human circadian pacemaker by melatonin is independent of the circadian phase of treatment initiation.<sup>25</sup> We chose to start dosing close to enrollment as would be the case in clinical practice. Patients were initially treated with placebo for 2 weeks and then were randomly assigned in a 1:1 ratio to receive PRM 2 mg or placebo for 6 weeks followed by a 2-week washout period. They were instructed to take the study drugs between 9 pm–10 pm and to keep bedtime hours every day between 11 pm–7 am. During the entire study period, daily diary recordings were performed in the morning via an Interactive Voice Response System (IVRS). The IVRS called the subjects every day at a pre-specified time after they got up in the morning and the subjects answered questions related to the previous night's sleep and daytime napping. The Clinical Global Impression of Change (CGIC; severity of illness score and global improvement score) was evaluated by the investigator during each study visit. WHO-Five Well-being Index (WHO-5) was assessed by the subject and completed by the investigator at each study visit.

Safety and tolerability were assessed with physical examinations, vital signs, clinical laboratory tests (hematology, chemistry, and urinalysis), electrocardiogram (ECG), and adverse events (AEs) assessment. An AE was defined as any noxious, pathological, or unintended change in anatomical, physiological, or metabolic functions as indicated by physical signs, symptoms and/or laboratory changes, or spontaneous reports, whether associated with the study drug, and whether or not considered drug-related. Pulse, blood pressure, and body temperature were collected at each study visit. In addition, the investigator or qualified designee performed a complete physical examination.

## Determination of sample size

Based on previous studies<sup>20</sup> that reported mean + standard deviation (SD) total sleep time of  $5.99 \pm 0.88$  hours in placebo

treated and  $6.64 \pm 1.11$  in melatonin treated totally blind subjects groups, 21 subjects per group (42 total) would be required to have at least an 80% chance of detecting a significant difference between the groups at the 0.1 significance level. N24HSWD in totally blind subjects is an orphan disease. Therefore, the number of patients available for trials is very limited. From previous reports in the scientific literature, significant changes in sleep duration between baseline and treatment conditions were observed in groups of 1–10 patients. It was thus decided that 14 patients (seven subjects per group) would be recruited to a pilot study to evaluate the mean and variance in treatment effects. At the planned sample size, we did not expect clinical significance of the difference between the PRM and placebo groups. To allow early decision-making on further development of PRM for this indication, we decided to use, as proposed in a number of studies, the upper limit of 95% confidence intervals (CIs) to help decide if a particular statistical result (whether significant or not) may be of relevance in practice.<sup>34,35</sup> Total sleep time values (polysomnography) reported in this patient population were  $290 + 80$  minutes<sup>9</sup> and  $314 + 32$  minutes.<sup>23</sup> In patient-reported assessments, a mean total night sleep duration in this population was  $5.99 + 0.88$  hours ( $359.4 + 53$  minutes).<sup>20</sup> For a baseline value of total sleep time of  $<390$  minutes, we considered an increase in sleep duration of 20 minutes to be clinically relevant.

## Statistical analyses

All analyses were performed using SAS<sup>®</sup> (v8.2 or later; SAS Institute Inc., Cary, NC, USA). The safety population included all subjects who were randomized and received at least one dose of study medication. The full analysis set (FAS) included all subjects in the safety population who satisfied all major entry criteria, and had efficacy data for the primary parameter recorded for at least one post run-in period assessment. Mean and CI values of the diary recorded sleep and morning alertness variables were summarized on a weekly basis in the last 2 weeks of the double-blind period and the 2 weeks of the washout period. The mean rate of change per week in the recorded parameters was calculated for the two randomization groups.

## Efficacy analyses

Efficacy assessments included total night sleep duration, sleep latency, sleep onset/offset times, sleep maintenance, sleep quality, feeling upon awakening, morning alertness, and number and duration of naps assessed by a diary via daily recorded telephone calls to a study IVRS. Efficacy assessments also included CGIC and the WHO-5.

The primary efficacy variable was total night sleep duration assessed by daily-recorded telephone calls to an IVRS. A descriptive summary was provided with respect to actual values for baseline (weeks 1–2) and the last 2 weeks of the treatment period (weeks 7–8), change from baseline (weeks 1–2), to the last 2 weeks of the treatment period (weeks 7–8) as well as mean and 95% CI values of the difference of change in total night sleep duration during the last 2 weeks of the 6-week randomized treatment period and the 2 baseline weeks in the two groups. The differences of change from baseline in total night sleep duration to the last 2 weeks of the treatment period was compared between the PRM and placebo groups using one-way analysis of variance (ANOVA). Because of the planned sample size, statistical significance of the difference between the drug and placebo groups (parallel groups) was not expected in this pilot study. In line with Greenhalgh<sup>34</sup> and Fethney,<sup>35</sup> the primary endpoint was demonstration of clinically meaningful effects on sleep duration as evidenced by a treatment effect upper CI limit >20 minutes (whether significant or not).

For the secondary efficacy measures, descriptive summary statistics (n, mean, SD, median, minimum, and maximum) were provided and compared using 95% CI from one-way ANOVA for daily diary records of sleep onset/offset times, sleep latency, sleep maintenance (how many times did you wake up in the night?), sleep quality, number of naps, total duration of naps, feeling upon awakening, and morning alertness for the baseline period (weeks 1–2), the last 2 weeks of the treatment period (weeks 7–8), the washout period (weeks 9 and 10), and changes from baseline, weekly basis in the last week of the treatment period (week 8), the 2 weeks of the washout period (weeks 9 and 10), and change from week 8 to weeks 9 and 10. WHO-5 Well-being Index score and CGIC score for severity of illness and global improvement were compared at baseline (Visit 2), end of randomized treatment, and washout period.

For post-hoc analyses, we calculated the mean standard deviations in the various sleep parameters during the last 2 weeks in each treatment period and compared the variability between baseline and treatment periods in the PRM and placebo groups.

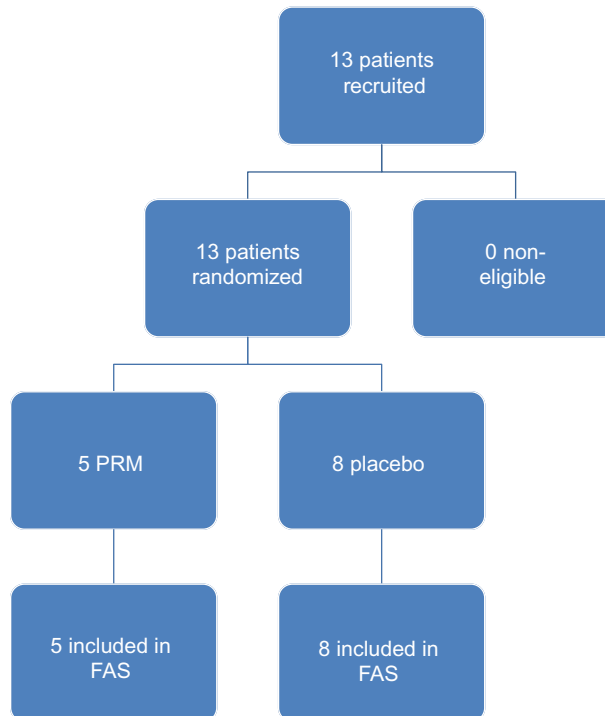
## Safety analysis

Descriptive statistics were provided for AEs, change in physical examination parameters, and vital signs. The number of subjects taking concomitant medications during the 6-week treatment period was summarized by WHO drug classification code for each treatment group. AEs were coded using

the MedDRA Dictionary (v10.0). The change in laboratory parameters from screening (Visit 1) to the end of the 6-week treatment period (Visit 3) was summarized using shift tables to show the number of subjects having values below, within, and above the normal range for each assessment by treatment group. The change in physical examination parameters from baseline (Visit 2) to the end of the 6-week treatment and washout period (Visit 3) was summarized as the number of subjects who had a normal or abnormal examination at each assessment for each treatment group separately. Vital signs were summarized at baseline (Visit 2), the end of the 6-week treatment period (Visit 3), and at the end of the washout period (Visit 4) in actual values and change from baseline in the melatonin and placebo groups using descriptive statistics.

## Results

A total of 25 patients were screened, of whom 13 were confirmed to be totally blind according to electroretinography and were randomized, with five subjects in the PRM group and eight subjects in the placebo group. All 13 subjects completed the study. Subject disposition is presented in Figure 1. More of the subjects were female (61.5%), the majority of



**Figure 1** Overall study patient disposition.

**Notes:** The study comprised of 2 weeks placebo run-in period followed by randomization 1:1 to receive PRM (Circadin® 2 mg) or placebo for 6 weeks followed by a 2-week washout period.

**Abbreviations:** FAS, full analysis set; PRM, prolonged-release melatonin.

the subjects (76.9%) were Caucasian, and the rest were black (one in the PRM and two in the placebo groups), ranging in age from 37–67 years. Of the 13 enrolled subjects, all 13 (100%) were included in the safety population and the FAS. There were no systematic differences in demographic variables between the PRM and placebo groups (Table 1).

## Total sleep duration

The primary efficacy variable was total night sleep time assessed by a diary via daily-recorded telephone calls to an IVRS. The mean change from baseline to end of treatment in total night sleep duration for the PRM 2 mg group was  $0.72 \pm 0.699$  (95% CI:  $-0.14$  to  $1.59$ ) hours (43 minutes) and for the placebo group it was  $0.27 \pm 0.449$  (95% CI:  $-0.11$  to  $0.64$ ) hours (16.2 minutes) with a mean difference between groups of 27 minutes ( $P=0.18$ ; 95% CI:  $-0.24$  to  $1.15$  hours; effect size: 0.82; Figure 2A). The upper limit of the 95% CI of the difference between PRM and placebo treatment effects on total sleep duration was 1.15 hours (69 minutes), namely larger than the predefined difference of 20 minutes, thus meeting the primary endpoint. No systematic difference was observed between the groups at baseline in the FAS population (Figure 3A). In the PRM group, despite the small sample, sleep duration under treatment was significantly longer at the 0.1 level of significance than at baseline ( $P=0.08$ ). No such improvement was found in the placebo-treated group ( $P=0.232$ ). Individual changes from baseline to end of treatment in total nighttime sleep duration for the PRM and placebo groups are also depicted (Figure 2B). Four of the five individuals in the PRM group showed >30 minutes

of improvement in sleep duration and none had clinically meaningful deterioration, whereas in the placebo group, only two of the eight subjects improved by >30 minutes, and one deteriorated (Figure 2B). In the washout period, the total night sleep duration was still longer in the PRM than the placebo-treated group, suggesting persistence of the effects beyond the active treatment period (Figure 2A).

## Sleep latency

Sleep latency was defined as the answer to the question ‘how long did it take you to fall asleep last night?’ as assessed by telephone calls to an IVRS. The mean change from baseline to end of treatment for sleep latency (measured in hours), was  $-0.48 \pm 0.765$  ( $-28.8$  minutes; 95% CI:  $-1.43$  to  $0.46$ ) for the PRM group and  $-0.001 \pm 0.314$  ( $-0.06$  minutes; 95% CI:  $-0.26$  to  $0.26$ ) for the placebo group (Figure 3A). There was a mean difference of 29 minutes between the PRM- and placebo-treated groups that did not reach statistical significance at this sample size ( $P=0.13$ ; Figure 3A). During the washout period, the mean difference from baseline to discontinuation in sleep latency for the PRM group was  $-0.45 \pm 0.766$  (95% CI:  $-1.40$  to  $0.50$ ) hours ( $-27$  minutes) and for the placebo group, it was  $0.062 \pm 0.6523$  (95% CI:  $-0.48$  to  $0.61$ ) hours ( $+3.7$  minutes; Figure 3A).

## Sleep onset/offset time

Sleep onset was the time subjects started trying to fall asleep. The results demonstrated a slight change in sleep onset time for subjects in the PRM group (8 minutes earlier) and no change for the placebo group. Essentially, there was no difference between the groups.

Sleep offset was the time subjects woke up. The results demonstrated a delay in sleep offset time for PRM subjects (37 minutes later) and advance for placebo (8 minutes earlier). Because total sleep time is a main complaint in the patients, the delay in sleep offset of 37 minutes represented an improvement, which was consistent with the improvement in total sleep time. There was a mean difference of 45 minutes in total sleep time between the PRM- and placebo-treated groups that showed a trend for a significant improvement ( $P=0.11$ ; data not shown).

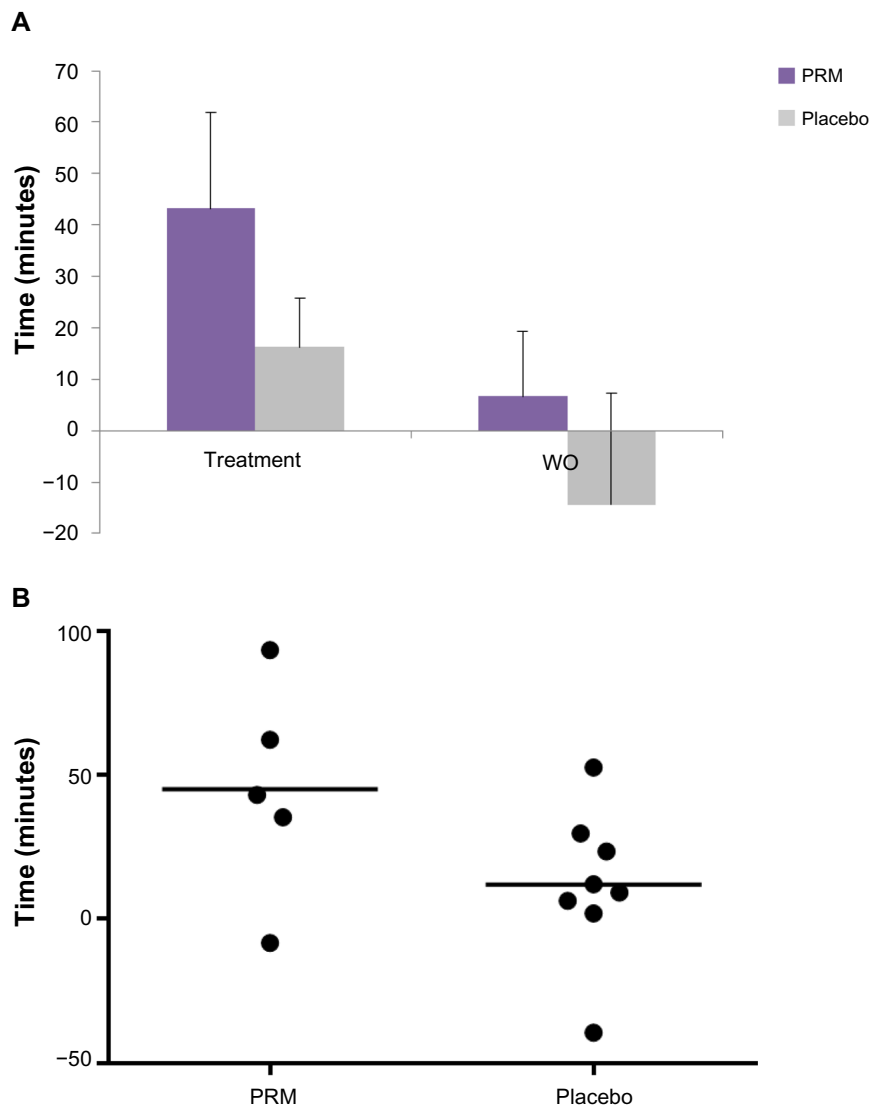
## Number and duration of daytime naps

At baseline, the mean number of daytime naps was not high for either group. Nevertheless, mean duration of naps in the PRM group demonstrated an improvement (reduction) at the end of treatment of 13 minutes compared to 2 minutes increase in mean nap duration in the placebo group. This effect was

**Table 1** Demographics and caffeine/alcohol history for safety population

Parameter	PRM N=5	Placebo N=8	Overall N=13
Mean age (years) $\pm$ SD (N)	52.2 $\pm$ 10.38	55.6 $\pm$ 7.58	54.3 $\pm$ 8.51
Age range (years)	37.0–66.0	44.0–67.0	37.0–67.0
Sex: males, N (%)	2 (40.0)	3 (37.5)	5 (38.5)
Height (inches) $\pm$ SD	64.5 $\pm$ 4.45	64.6 $\pm$ 4.15	64.5 $\pm$ 4.08
Weight (lbs) $\pm$ SD	197.6 $\pm$ 43.89	177.9 $\pm$ 35.45	185.5 $\pm$ 38.4
Race, N (%)			
Caucasian	4 (80)	6 (75)	10 (76.9)
Black	1 (20)	2 (25)	3 (23.1)
Caffeine history			
Never used, N (%)	0 (0)	0 (0)	0 (0)
Currently uses, N (%)	5 (100)	8 (100)	13 (100)
Alcohol history			
Never consumed, N (%)	0 (0)	1 (12.5)	1 (7.7)
Currently consumes, N (%)	4 (80)	5 (62.5)	9 (69.2)
Formerly consumed, N (%)	1 (20)	2 (25)	3 (23.1)

**Abbreviations:** PRM, prolonged-release melatonin; SD, standard deviation.



**Figure 2** Endpoint of sleep variables: sleep duration.

**Notes:** (A) Change from baseline for the total night sleep duration ( $\pm$  SEM). Treatment period took place from weeks 3–8; WO period from weeks 9–10. (B) Individual changes from baseline to end of treatment in total nighttime sleep duration for the PRM and placebo groups.

**Abbreviations:** PRM, prolonged-release melatonin; SEM, standard error of the mean; WO, washout.

maintained during discontinuation for the PRM group, with a mean decrease from baseline to washout of 13 minutes, while in the placebo group, the mean duration of naps increased by 19 minutes from baseline values, demonstrating continued improvement in total duration of naps for the PRM subjects but not for the placebo subjects (Figure 3B).

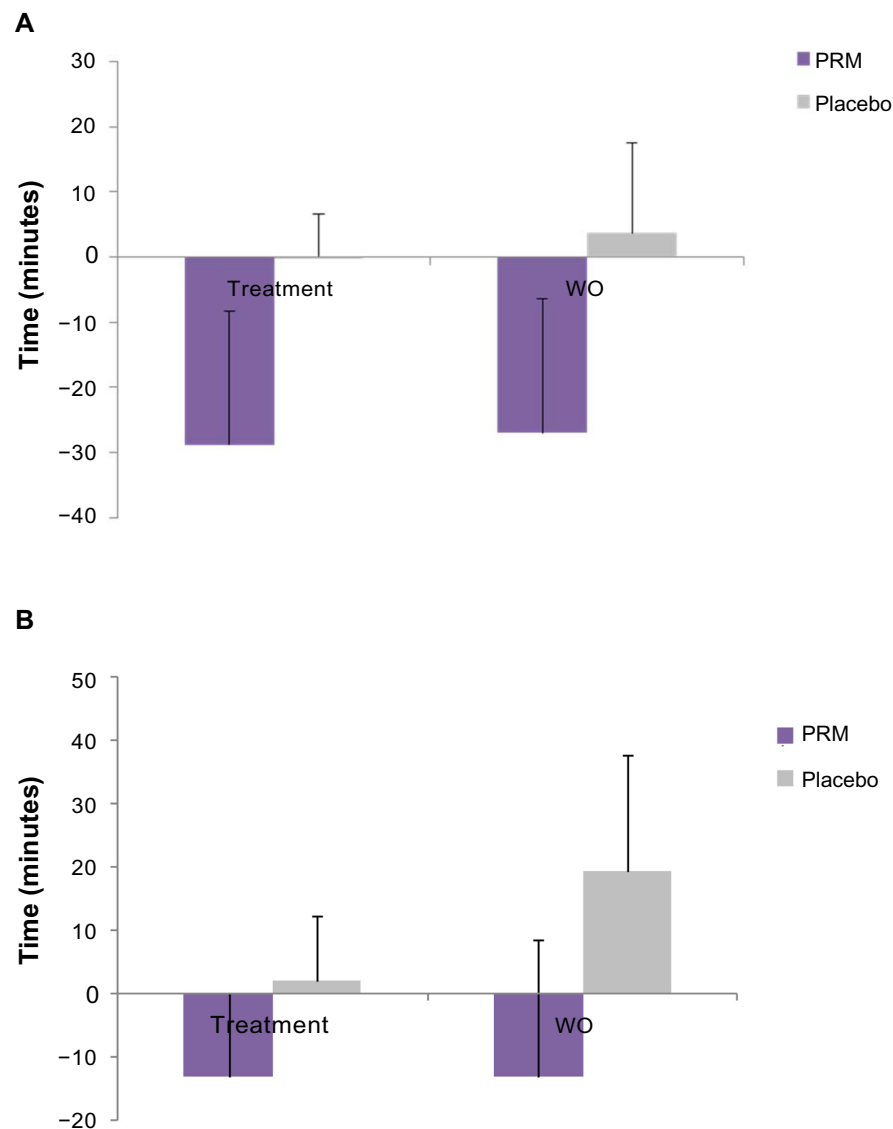
CGIC and WHO-5 did not show differences between the two groups.

## Onset and offset of treatment effects

Mean sleep duration and sleep latency by study week are presented in Figure 4. As can be seen in Figure 4A, sleep duration increased at each time point in the PRM treated group reaching a peak at week 6, whereas the placebo

treated group did not show the same trend. The mean sleep duration in the PRM-treated group showed a gradual decline toward baseline levels, but by the end of the discontinuation period (weeks 9 and 10) it was still better than that in the placebo group, suggesting persistence of the effect beyond the active treatment period (Figure 4A).

Mean week-by-week sleep latency values in the placebo group changed in a periodic manner, consistent with the cyclic nature of the sleep disorder. With PRM, the subject's sleep latency demonstrated a gradual improvement (decrease), reaching a plateau level at week 5 (Figure 4B). The results after discontinuation showed gradual increase in sleep latency toward baseline values but continued to show shorter mean sleep latency values for the PRM-treated



**Figure 3** Endpoint of sleep variables: sleep latency and daytime naps.

**Notes:** (A) Change from baseline for night sleep latency ( $\pm$  SEM) (B) Change from baseline for the total duration of naps ( $\pm$  SEM). Treatment period is calculated as weeks 3–8; WO period is calculated as weeks 9–10.

**Abbreviations:** PRM, prolonged-release melatonin; SEM, standard error of the mean; WO, washout.

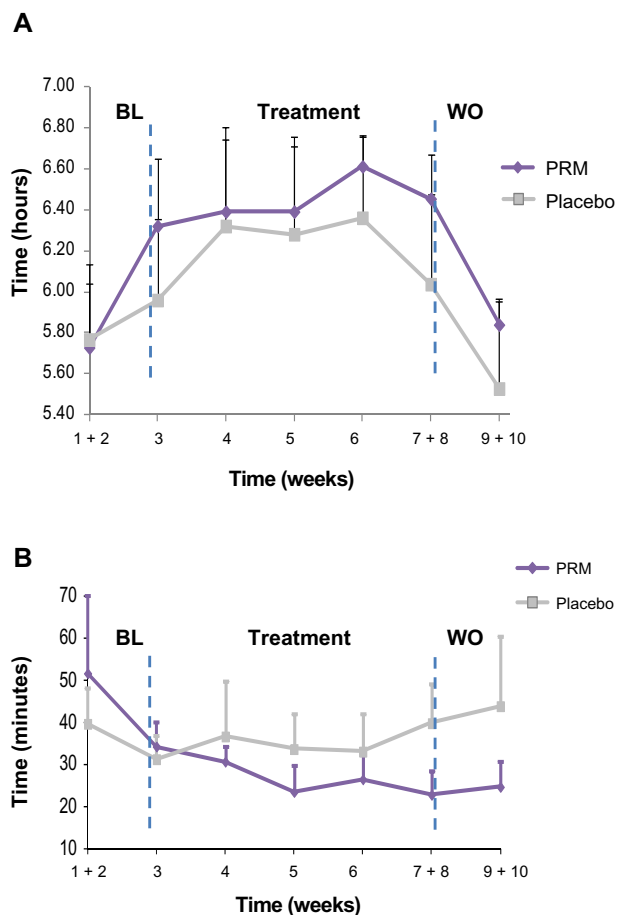
group but not for the placebo group, compared to baseline, demonstrating persistence of the effect beyond treatment (Figure 4B).

### Variability in sleep parameters

To demonstrate stabilization in sleep phase, the variability among individual patients in sleep onset/offset and latency should decrease during treatment. The variability represented by the mean standard deviation (SD) in sleep parameters (eg, sleep onset/offset and sleep latency), during the last 2 weeks of the double-blind treatment period was compared to the variability during the 2-week placebo baseline period on a patient-by-patient basis (paired *t*-test; Figure 5).

The mean SD of the sleep onset time values tended to decline with PRM treatment ( $P=0.078$ ,  $N=5$ ) but not with placebo treatment ( $P=0.12$ ,  $N=8$ ). As can be seen in Figure 5A, during treatment, variability in sleep onset decreased in four (80%) of the five patients in the PRM group and in four (50%) of the eight patients in the placebo group. The SD of the mean sleep offset time values declined significantly with PRM treatment ( $P=0.05$ ,  $N=5$ ), but not with placebo ( $P=0.19$ ,  $N=8$ ). As can be seen in Figure 5B, during treatment, the mean SD in sleep offset time decreased in four (80%) of five patients treated with PRM, and in four (50%) of those treated with placebo.

There was a similar trend in sleep latency: the mean SD of the sleep latency time values tended to decline with PRM treatment



**Figure 4** Onset and offset of treatment effects on sleep.

**Notes:** Analysis week-by-week (mean  $\pm$  SEM) in (A) total night sleep duration and (B) sleep latency. BL period is from weeks 1–2; treatment period is from weeks 3–8; WO period is from weeks 9–10.

**Abbreviations:** PRM, prolonged-release melatonin; BL, baseline; SEM, standard error of the mean; WO, washout.

( $P=0.099$ ,  $N=5$ ) but not with placebo treatment ( $P=0.44$ ,  $N=8$ ). As can be seen in Figure 5C, variability decreased in five out of five (100%) patients in the PRM-treated group but only four of eight (50%) in the placebo-treated group.

## Safety

Of the 13 safety subjects, one (20%) PRM and two (25%) placebo subjects experienced one or more treatment-emergent AEs during the study. All of the AEs experienced were considered mild in severity and none led to study withdrawal. There were no serious AEs reported during the study, and there were no deaths. No clinically significant changes in physical examinations or vital signs were observed during the study. There were no distinct trends or clinically meaningful differences between treatment groups for any hematology and blood biochemistry and urinalysis parameters. No individual changes in

hematology and blood biochemistry parameters were considered clinically significant.

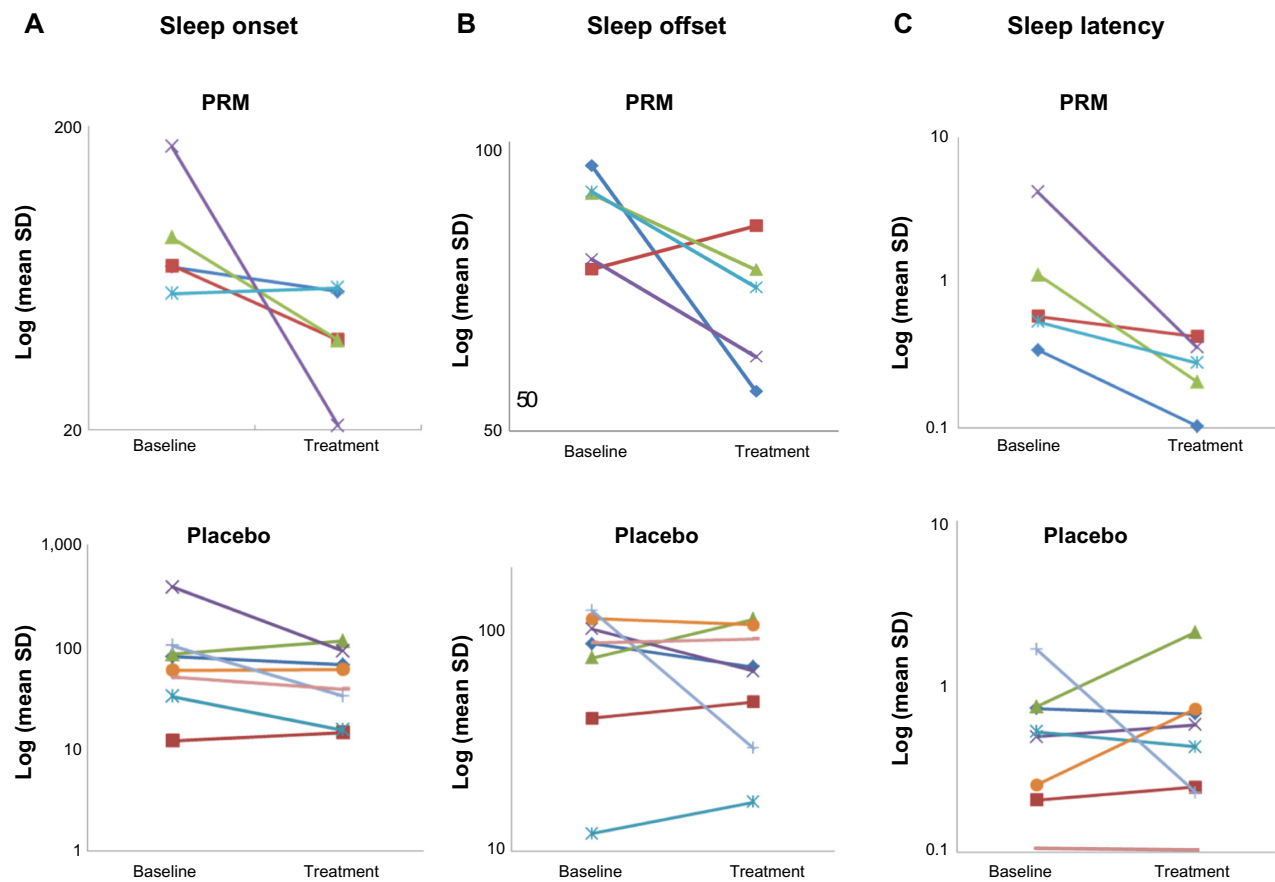
The only drug-related clinically significant laboratory value reported during the study was in the placebo group. This subject had 3+ fold leukocytes present, which was considered to be clinically significant and reported as an AE. At a follow-up visit, the subject had trace leukocytes still present, but the finding was not considered to be clinically significant.

## Discussion

The study provides preliminary evidence of beneficial effects of PRM on sleep duration and latency in totally blind subjects reporting disturbed sleep. The mean difference between the change from baseline in sleep duration in the PRM and placebo groups of 27 minutes is of relevance in practice and may justify a larger trial to confirm a significant treatment benefit. Another way of interpreting the study results is calculation of the effect size. Effect size is a name given to a family of indices that measure the magnitude of a treatment effect. Unlike significance tests, these indices are independent of sample size. Thus, this is a good measure for interpreting this trial with a limited sample size.<sup>36</sup> Accordingly, effect sizes are defined as ‘small,  $d=0.2$ ’, ‘medium,  $d=0.5$ ’, and ‘large,  $d=0.8$ ’. For total sleep time, the mean change of 27 minutes over placebo represented an effect size (‘Cohen’s  $d$ ’) of 0.82. This is a point estimate of effect size, and this estimate has a CI that we cannot calculate. Similarly, for sleep latency, the mean difference of 29 minutes between the groups represented an estimated effect size of 0.92. The estimated large effect sizes of PRM of 0.82 and 0.92 thus suggest a potentially strong treatment effect of PRM over that of placebo on total night sleep duration and sleep latency, respectively.

Three studies reported on the effects of regular melatonin formulations on sleep in totally blind subjects. Acute administration of regular-release melatonin 5 mg or placebo to 12 totally blind subjects for 1 hour before bedtime resulted in significantly longer mean total sleep time (by 89.8 minutes) and delayed sleep offset, recorded by polysomnography after melatonin, than placebo intake in the subjects, whereas sleep latency did not differ between the treatments.<sup>23</sup> In another study, repeated administration of regular-release melatonin 0.5 mg or placebo single-blind, to ten totally blind subjects daily at 9 pm (treatment duration varied between 26–81 days depending on the individuals’ circadian period) resulted in a significant increase in mean nighttime sleep duration (by 39 minutes), reduction in daytime nap number





**Figure 5** Mean SD of the individual changes in sleep onset/offset and latency variability during baseline and the last 2 weeks of PRM and placebo treatment.

**Notes:** Data from all individual patients in the study in the PRM (top panels) and placebo (bottom panels) treatment are depicted using logarithmic scales. The different colored lines in the figure represent different patients.

**Abbreviations:** PRM, prolonged-release melatonin; SD, standard deviation.

(by 0.33 naps per day on average), and mean nap duration (by 18.6 minutes) as recorded by daily sleep and nap diaries during the melatonin period compared to the placebo period. Mean sleep offset was delayed by 15 minutes, and there was no significant effect on sleep latency or onset.<sup>20</sup> In a third study, 10 mg melatonin or placebo was administered daily to seven totally blind free-running subjects 1 hour before their preferred bedtime for 3–9 weeks before being switched over to the other treatment; there was less time awake after sleep onset (77.5 minutes) and higher sleep efficiency (16.7%) as assessed by polysomnography by the end of the melatonin and placebo treatment periods, whereas total sleep time and sleep latency did not differ.<sup>24</sup> The results of the current study with PRM 2 mg for sleep in the totally blind subjects are thus consistent in nature and magnitude to those observed with 0.5 and 5 mg regular-release melatonin.

A limitation in our study is that we did not measure circadian variation in neuroendocrine markers, and we are thus unable to determine whether the improvement in sleep represents adjustment of the biological clock to the night

phase. Nevertheless, there are some hints that there is a stabilization of the clock phase with PRM treatment. First, the changes in mean total sleep duration and sleep latency in the placebo group are consistent with the periodic nature of the sleep disorder in the study population. With PRM, the mean subjects' sleep variables improved gradually with treatment time rather than improving immediately as would occur in the case of hypnotic drugs. Secondly, the variability in sleep onset/offset and sleep latency during the last 2 weeks of treatment declined in 80%–100% of patients treated with PRM but only 50% patients treated with placebo. Last but not least, the treatment effect did not dissipate immediately following discontinuation of PRM, but rather, declined gradually in the 2 weeks withdrawal period toward baseline values. Furthermore, there was a reduction in daytime napping, which is also consistent with better sleep at night and possibly also phase adjustment to the nightly melatonin signal.

These effects of PRM as demonstrated in the present study are compatible with melatonin's effects at the biological clock to control circadian phase stability.<sup>37–39</sup> It should be noted that

the treatment period of 6 weeks might have been too short to attain synchronization in some patients.<sup>25</sup> Also, in our study, the ingested melatonin at treatment initiation did not coincide with the circadian phase, and some patients may have started the treatment when their endogenous phases were too far off the environmental night phase to allow effective response to melatonin.<sup>20</sup>

Melatonin administration can elicit phase advances between 13 and 5 hours before sleep mid-point (essentially overlapping afternoon–evening hours) and phase delays between 5–7 hours after sleep mid-point (essentially overlapping the early morning hours).<sup>30,40–43</sup> While the phase-advancing effects of appropriately timed melatonin have been confirmed across many studies, the phase-delaying efficiency of morning melatonin ingestion is smaller and not as certain.<sup>44</sup>

This is the first demonstration of efficacy of a PRM in totally blind persons. It adds to published data from studies in sighted subjects indicating that slow-release melatonin preparations are at least as effective as immediate-release preparations in phase shifting of the clock in healthy individuals kept in near darkness environment<sup>28,29</sup> and facilitating early circadian sleep in operational air transport aircrews.<sup>45</sup> In another study, modified- and slow-release melatonin preparations were found to be at least as effective as immediate-release preparations in advancing the clock phase under laboratory conditions mimicking eastward and westward travel.<sup>30</sup> Also, timed  $\beta$ 1-adrenergic antagonists and PRM treatment was reported to reset the clock and restore sleep in a circadian disorder, Smith–Magenis syndrome.<sup>46</sup> Apparently, if the time of administration ‘hits’ the phase-advance period, PRM and immediate-release melatonin will both be effective even if PRM (which releases melatonin over 8 hours) is ‘spilling over’ this period, because melatonin does not phase shift the clock during the 8 hours night phase anyway. However, if the time of administration ‘hits’ the phase delay period, PRM but not immediate-release melatonin (which provides melatonin for ~2 hours) will be able to counteract the phase delay effects, by ‘spilling over’ into the phase advance period of the clock on the very same day. Such studies extend the utility of PRM to the treatment of circadian rhythm sleep disorders.

## Conclusion

Totally blind subjects with periodic sleep-wake disorder may potentially benefit from PRM given orally once daily between 9 pm–10 pm, in improving the patient-reported duration of sleep and sleep latency and attaining greater stability of sleep onset/offset times. Treatment effects appear to persist beyond the treatment phase into the withdrawal period, without

rebound, consistent with stabilization of the circadian clock. Treatment with 2 mg daily of PRM was safe and well-tolerated in this group of patients. A larger study powered to demonstrate a statistically significant effect is warranted.

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## Author contributions

NZ and TN were responsible for the integrity of the work as a whole, from inception to the published article. NZ and TN made substantial contribution to the conception and design of the study and were involved in drafting the protocol, interpretation of data, and preparation of the manuscript. TR was a consultant and contributed to the interpretation of data and critical revision of the manuscript. All authors approve the final version.

## Disclosure

The study was funded by Neurim Pharmaceuticals, Tel Aviv, Israel. TR has acted as a paid consultant to Neurim Pharmaceuticals. NZ and TN have disclosed that they are employees of Neurim Pharmaceuticals.

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