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Cannabinol (CBN; 30 and 300 mg) effects on sleep and next-day function in insomnia disorder ('CUPID' study): protocol for a randomised, double-blind, placebo-controlled, cross-over, three-arm, proof-of-concept trial

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ABSTRACT

Objective Insomnia is the most prevalent sleep disorder, with few effective pharmacotherapies. Anecdotal reports and recent preclinical research suggest that cannabinol (CBN), a constituent of *Cannabis sativa* derived from delta-9-tetrahydrocannabinol, could be an effective treatment. Despite this, the isolated effects of CBN on sleep have yet to be systematically studied in humans.

Methods The present protocol paper describes a randomised, double-blind, placebo-controlled, single-dose, three-arm, cross-over, proof-of-concept study which investigates the effects of CBN on sleep and next-day function in 20 participants with clinician-diagnosed insomnia disorder and an Insomnia Severity Index Score ≥15. Participants receive a single fixed oral liquid dose of 30 mg CBN, 300 mg CBN and matched placebo, in random order on three treatment nights; each separated by a 2-week wash-out period. Participants undergo overnight sleep assessment using in-laboratory polysomnography and next-day neurobehavioural function tests. The primary outcome is wake after sleep onset minutes. Secondary outcomes include changes to traditional sleep staging, sleep-onset latency and absolute spectral power during non-rapid eye movement (NREM) sleep. Tertiary outcomes include changes to sleep spindles during NREM sleep, arousal indices, absolute spectral power during REM sleep and subjective sleep quality. Safety-related and exploratory outcomes include changes to next-day simulated driving performance, subjective mood and drug effects, postural sway, alertness and reaction time, overnight memory consolidation, pre and post-sleep subjective and objective sleepiness; and plasma, urinary, and salivary cannabinoid concentrations. The study will provide novel preliminary data on CBN efficacy and safety in insomnia disorder, which will inform larger clinical trials.

Ethics and dissemination Human Research Ethics Committee approval has been granted by Bellberry (2021-08-907). Study findings will be disseminated in a peer-reviewed journal and at academic conferences.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study uses a randomised, double-blind, placebo-controlled, cross-over design to investigate two active doses of cannabinol (CBN) isolate on sleep and neurobehavioural function over ~17 hours using gold-standard objective measures, validated subjective measures and rigorous design methodology.

⇒ Study participants undergo extensive screening with a sleep physician to confirm insomnia disorder diagnosis, including an overnight diagnostic polysomnogram to rule out other sleep disorders that commonly co-occur with insomnia.

⇒ The study wash-out period (2 weeks) was chosen to minimise participant burden, as participants cannot undergo insomnia treatment before (3 months) or during (~2 months) the protocol; however, this may lead to carry-over effects between doses as a recent cross-over study demonstrated oral cannabinoids can persist in plasma for >4 weeks (1500 mg; single dose).

⇒ The study involves a single-dose, in-laboratory proof-of-concept design and results may, therefore, lack ecological validity (repeated CBN dosing may be necessary to effectively treat insomnia).

BACKGROUND

Insomnia is a sleep disorder characterised by subjective difficulty falling asleep, maintaining sleep and/or achieving restorative sleep, where symptoms persist for ≥3 months and are accompanied by daytime sequelae of dysfunction and/or distress.[1] Insomnia disorder is the most prevalent sleep disorder

which affects between 10% and 30% of adults[2] and persists in 37% of cases at a 5-year follow-up.[3] Insomnia disorder increases the risk of mental and physical illnesses such as depression[4] and dementia.[5] The economic burden of insomnia disorder is estimated at >$A13 billion per annum in Australia.[6]

Insomnia disorder treatments are typically behavioural or pharmacological in nature. Cognitive behavioural therapy for insomnia (CBTi) is the first-line treatment and its efficacy has been confirmed in recent meta-analyses.[7] Nevertheless, significant access barriers to CBTi exist including limited providers, high cost[8] and delayed perception of subjective benefits.[9] CBTi may also be less effective in individuals with shorter sleep duration (ie, <6 hours/night).[10] As such, sedative-hypnotics are commonly used in primary care.[7] While effective in the short-term, well-documented side effects include daytime sedation, psychomotor impairment, addiction/dependence and premature mortality.[11] There is a clear need for safe pharmacological insomnia disorder treatments.

Cannabis sativa is a complex plant containing numerous potentially therapeutic chemical compounds, including over 140 constituent 'cannabinoids'.[12] As historical legal prohibitions against cannabis are relaxed, cannabinoids are being increasingly used to aid sleep.[13] The most well researched cannabinoids are Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD); however, in recent times, cannabinol (CBN) has become recognised as a putative 'sleep-enhancing' cannabinoid.[14] CBN is produced via the non-enzymatic oxidation of THC and is therefore present at higher concentrations in aged (rather than 'young') cannabis plant material.[15] This is significant as aged cannabis plant material is anecdotally reported to induce sleep.[16] CBN products are also being sold as sleep aids in unregulated markets.[17] Importantly, a recent narrative review of CBN highlighted insufficient evidence to support sleep-related claims, despite a plausible mechanism of action.

THC and CBN exert some of their physiological effects via interactions with the endogenous cannabinoid system (ECS),[12] comprising signalling molecules termed 'endocannabinoids' (eg, anandamide, 2-arachidonoylglycerol (2-AG)), their receptors and enzymes.[18 19] The ECS is involved in regulating a myriad of biological processes including circadian rhythmicity.[20] Systematic administration of anandamide has been shown to promote sleep in rodents and humans[18 21 22] via activation of the cannabinoid receptor 1 (CB_1R).[21 23] As such, the CB_1R is considered a promising therapeutic target.[24] The pharmacological effects of THC occur via partial agonism at CB_1R[25] and can include acute intoxication, psychomotor impairment, sedation and changes in sleep architecture.[18] CBN also has agonist actions at CB_1R, although approximately 10 times less potent than THC,[25] which may potentially account for the low levels of intoxication reported in historic CBN studies.[26 27] Minimal intoxication could render CBN a more practical and safer alternative to THC as a sleep aid.

Two recent clinical studies administered small quantities of CBN in multi-cannabinoid formulations.[28 29] The first, a cross-over randomised controlled trial (RCT) with a 1-week washout, investigated 2-weeks of an oral liquid containing 20 mg THC, 2 mg CBN, and 1 mg CBD in 20 adults with insomnia disorder. The drug significantly improved insomnia disorder symptoms (Insomnia Severity Index (ISI) −5.1 points, $p=0.0001$, d=0.94) compared with placebo, with no significant changes to polysomnography (measured on night 14 of dosing).[28] In an interventional open-label study, current medicinal cannabis users with subjective sleep difficulty reported improved sleep (assessed via an unvalidated subjective survey and a validated non-contact at-home tracking device) after 3-weeks of nightly administration of an oral capsule containing 10 mg THC and 5 mg CBN.[29] Importantly, the effects of CBN cannot be disentangled from those of THC in these studies. We are not aware of any clinical studies to-date that have tested the isolated effects of CBN on objectively measured human sleep.

Importantly, a recently published conference abstract reported on a preclinical study in which CBN isolate (10, 30 and 100 mg/kg intraperitoneally compared with zolpidem 10 mg/kg as a positive control) increased the proportion of non-rapid eye movement (NREM) sleep and sleep bout duration 4-hours post administration in Long-Evan rats.[30] With the lowest dose, biphasic effects were observed—CBN (10 mg/kg) initially decreased REM sleep proportion; however, 4-hours after administration, a significant increase in the percentage of REM sleep occurred. These results suggest CBN could reduce wake after sleep onset (WASO), given such delayed effects on sleep.

Study design and aim

Here, we described the protocol for a randomised, double-blinded, placebo-controlled, three-arm, cross-over, single-site, proof-of-concept study design to investigate the acute effects of oral CBN on sleep and next-day function in 20 participants with clinician-diagnosed insomnia disorder. The primary study aim is to investigate the effects of CBN (30 and 300 mg) versus placebo on sleep in insomnia disorder. This study aims to evaluate the safety and efficacy of CBN as a pharmacological therapy and generate preliminary data to inform larger clinical trials. Many outcome measures used in insomnia disorder clinical trials are known to be susceptible to placebo effects. A placebo control is scientifically and ethically defensible choice as the effects of CBN on human sleep are unknown and could not be properly elucidated if an active treatment such as zolpidem was the comparator rather than placebo.

Study outcomes

The primary study outcome is WASO measured in minutes using in-laboratory overnight polysomnography, from the first epoch after lights out until the last epoch, scored as any stage of sleep by an experienced polysomnographic

Box 1 Tertiary, safety and exploratory study outcomes

Tertiary outcomes

1. Sleep spindles during non-rapid eye movement (NREM) sleep: Sleep spindle and slow oscillation events in NREM sleep from in-laboratory overnight polysomnography. A sleep spindle and slow oscillation detection algorithm will be applied to electroencephalography (EEG) signals from polysomnography after artefacts are detected and removed (comparisons between each cannabinol (CBN) dose versus placebo).
2. EEG Arousal Index: Number of cortical arousals captured via the electroencephalogram per hour of sleep scored by the polysomnographic technician on the polysomnogram in accordance with American Academy of Sleep Medicine Sleep Scoring criteria (comparisons between each CBN dose versus placebo).
3. Absolute EEG power during REM sleep: Spectral power of delta (1–4.5 Hz), theta (4.5–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta (15–25 Hz) and gamma (25–40 Hz) frequency ranges between treatment arms. Power spectral analysis will be applied to EEG signals from polysomnography after artefacts are detected and removed (comparisons between each CBN dose versus placebo).
4. Next-day post wake subjective sleep evaluation (Leeds Sleep Evaluation Questionnaire, LSEQ): Richard Campbell Sleep Questionnaire (RCSQ) score. LSEQ score. LSEQ scores range from 0 to 100, with higher scores indicating better subjective experience. Assessed within 1 hour after wake (comparison between each CBN dose vs placebo).
5. Next-day post wake subjective sleep evaluation (RCSQ): RCSQ score. RCSQ scores range from 0 to 100, with higher scores indicating better subjective experience. Assessed within 1 hour after wake (comparison between each CBN dose vs placebo).

Safety outcomes

1. SD of lateral position (SDLP) during next-day post-wake simulated drive: SDLP ('weaving') is measured across the 'standard', 'car following' and 'divided attention' subsections of a ~30 min simulated driving task. Assessed within 2 hours after wake (comparison between each CBN dose vs placebo).
2. Speed during next-day post-wake simulated drive: Average speed and SD of speed is measured across the 'standard' and 'divided attention' subsections of a ~30 min simulated driving task. Assessed within 2 hours after wake (comparison between each CBN dose vs placebo).
3. Distance headway during next-day post-wake simulated drive: Average distance headway (ie, distance between the driver's vehicle and vehicle immediately in front) and SD of distance headway is measured across the 'car following' subsection of a ~30 min simulated driving task. Assessed within 2 hours after wake at both treatment sessions (comparison between each CBN dose vs placebo).
4. Subjective mood evaluation: The Abbreviated Profile of Mood States consists of 40 items measuring domains of 'tension', 'depressed', 'anger', 'vigour', 'fatigue' and 'concentration'. Participants respond to each item using 5-point Likert scales ranging from 0 (not at all) to 4 (extremely). A total mood disturbance score is calculated by summing negative domains and subtracting positive domains. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose vs placebo).
5. Subjective drug effects: The Drug Effects Questionnaire assesses the extent to which participants feel a drug effect, feel high, like the effects, dislike the effects, want more of the substance, and feel sedated, on self-rating 100 mm Visual Analogue Scales. A total mood disturbance score is calculated by summing negative

Continued

Box 1 Continued

domains and subtracting positive domains. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose vs placebo).
6. Postural Sway: Centre-of-pressure during computerised static posturography. Administered pre and post drug administration, as well as next-day (comparison between each CBN dose vs placebo).
7. Behavioural alertness and reaction time: Psychomotor Vigilance Test is administered twice the next-day (comparison between each CBN dose vs placebo).
8. Overnight verbal declarative memory consolidation: Word pair recall scores measured using the computerised Word Pairs Task. Administered predrug administration and next-day (comparison between each CBN dose vs placebo).
9. Overnight procedural memory consolidation: Motor sequence learning measured using the computerised Finger Tapping Task. Administered predrug administration and next-day (comparison between each CBN dose vs placebo).
10. Resting wake EEG power after sleep: Resting wake EEG power during the Karolinska Drowsiness Test (KDT) on wake: delta (1–4.5 Hz), theta (4.5–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta (15–25 Hz) and gamma (25–40 Hz) frequency ranges. Power spectral analysis is applied to EEG signals from polysomnography, after artefacts are detected and removed. Comparison between each CBN dose versus placebo.
11. Subjective sleepiness after sleep: The Karolinska Sleepiness Scale (KSS) is a 10-item measure of subjective drowsiness. Participants respond to each item using a 9-point Likert scale ranging from 1 (extremely alert) to 9 (extremely sleepy). Higher scores are indicative of increased drowsiness. The KSS will be collected in accordance with the KDT protocol but will not be analysed due to insufficient statistical power.

Exploratory outcomes

1. Resting wake EEG power before sleep (Acute Effects of CBN). Resting wake EEG power during the KDT prior to sleep: delta (1–4.5 Hz), theta (4.5–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta (15–25 Hz) and gamma (25–40 Hz) frequency ranges. Power spectral analysis is applied to EEG signals from polysomnography, after artefacts are detected and removed. Comparison between each CBN dose versus placebo.
2. Subjective sleepiness before sleep (acute effects): The KSS is a 10-item measure of subjective drowsiness. Participants respond to each item using 9-point Likert scales ranging from 1 (extremely alert) to 9 (extremely sleepy). Higher scores are indicative of higher drowsiness. The KSS will be collected in accordance with the KDT protocol but will not be analysed due to insufficient statistical power.
3. Plasma cannabinoid concentrations: Presence of cannabinoids (CBN, delta-9-tetrahydrocannabinol (THC) and cannabidiol (CBD)) (eg, 11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD), and endocannabinoids and related molecules (eg, 2-Arachidonoylglyceroland anandamide) and their metabolites in plasma samples.
4. Urinary cannabinoid concentrations: Presence of cannabinoids (CBN, THC and CBD) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD) in urine samples.
5. Salivary cannabinoid concentrations: Presence of cannabinoids (THC, CBN) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC) in saliva samples.

technician in accordance with American Academy of Sleep Medicine (AASM) 2020 Sleep Scoring criteria (Version 2.6).[31] Secondary study outcomes include:

1. Traditional sleep staging: Proportion of the sleep opportunity scored at the five stages (wake, and N1, N2, N3 and REM sleep) between lights out and lights on, measured using overnight in-laboratory polysomnography, scored by a polysomnography technician in accordance with AASM Sleep Scoring criteria.
2. Sleep-onset latency (SOL): SOL measured in minutes using in-laboratory polysomnography, calculated from the time of lights out to the first sleep epoch as scored by a polysomnographic technician in accordance with AASM Sleep Scoring criteria.
3. Absolute electroencephalographic (EEG) power during NREM sleep: Spectral power of delta (1–4.5 Hz), theta (4.5–8 Hz), alpha (8–12 Hz), sigma (12–15 Hz), beta (15–25 Hz) and gamma (25–40 Hz) frequency ranges between treatment arms. Power spectral analysis will be applied to EEG signals from polysomnography after artefacts are detected and removed.

Tertiary, safety and exploratory study outcomes are described in box 1 (see ClinicalTrials.gov Identifier: NCT05344170).

Safety
The investigational product, CBN, is currently a Schedule 9 'Prohibited Substance' in Australia.[32] The safety profile of CBN can be inferred from human studies conducted between 1973 and 1987. These studies explored oral doses up to 1200 mg CBN with some administering repeated daily doses of CBN for up to 4 weeks.[26 33–35] There were no notable safety concerns, aberrations or toxicity concerns from these studies; with no adverse changes in parameters such as heart rate, blood pressure, body temperature, respiratory rate, perception, intoxication and postural stability.[26 33–35] None of these studies measured sleep, sedation or residual next-day effects of CBN (such as adverse effects on driving performance and cognitive function). These measures are obtained in the current study. CBN is currently being investigated as an analgesic agent (ClinicalTrials.gov Identifier: NCT03675971) and topical treatment for epidermolysis bullosa (ClinicalTrials.gov Identifier: NCT04908215). An RCT of oral CBN isolate (compared with combination CBN/CBD and placebo doses) on the subjective sleep of healthy adults was retrospectively registered in May 2022 (ClinicalTrials.gov Identifier: NCT05839964). We will monitor and review safety reports from these trials as they become available.

METHODS
Participants, interventions and outcomes
Trial setting
The trial sponsor and site is the Woolcock Institute of Medical Research, a specialist sleep and respiratory research institute and clinic (Sydney, Australia). The study funder is the Lambert Initiative for Cannabinoid Therapeutics, University of Sydney (Sydney, Australia), a philanthropically funded centre for cannabinoid science. Recruitment commenced in August 2022 and is anticipated to conclude in late 2023. The first participant was enrolled 24 August 2022 and the first participant was randomised on 13 October 2022. The study has ethical approval from Bellberry Human Research Ethics Committee (HREC; 2021-08-907; Version 2.1, 1 August 2022) and is registered on ClinicalTrials.gov (Identifier: NCT05344170; 25 April 2022). The clinical trial protocol is available on request. Significant changes to the study protocol will be documented on ClinicalTrials.gov. The study protocol was developed according to the Standard Protocol Items: Recommendations for Interventional Trials 2013 statement.[36] The Therapeutics Goods Administration has acknowledged the use of CBN in this study through the Clinical Trial Notification (CTN) scheme (CTN Number: CT-2022-CTN-00543-1).

Participant eligibility
Eligible participants are aged between 25 and 65 years with physician diagnosed insomnia disorder as per the International Classification of Sleep Disorders-3 (ICSD-3)[37] and Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)[38] criteria as determined by the study physician (see box 2). The age range was selected to minimise age-related changes to sleep architecture.[39] As cannabinoids have been shown to improve symptoms (eg, anxiety, pain) that commonly impair sleep (ie, causing 'secondary' insomnia disorder),[40] a 'primary' insomnia disorder population is being recruited for this study.

Patient and public involvement
Patients and/or the public were not involved in the design, conduct, reporting, nor dissemination plans of this research.

Intervention
Study doses
Participants receive a single fixed 2 mL oral liquid dose of: (1) 30 mg CBN ('ECS310' 1.5% containing 15 mg/mL CBN); (2) 300 mg CBN ('ECS310' 15% containing 150 mg/mL CBN) and (3) matched placebo. Treatments are administered across three separate overnight treatment sessions in randomised order 2 hours prior to habitual lights out. The lower dose was selected to match the CBN products available in the USA and supported by customer testimonies.[17] The higher dose was informed by a preclinical study of CBN isolate and sleep.[30]

Investigational product
The investigational drug ('ECS310') is a liquid formulation of CBN suspended in medium chain triglyceride (MCT) oil. The placebo contains MCT oil only. The maximum detectable THC content of the active investigational products is ≤150 µg. Neither the study drug nor matched placebo contain any other cannabinoids or terpenes/terpenoids. The study drug and matched

Box 2   Inclusion and exclusion criteria for study participants

Eligible individuals must fulfil the following criteria:

1. Between 25 and 65 years of age.
2. Insomnia Severity Index score ≥15 at eligibility screening.
3. Insomnia disorder (symptoms occurring at least three times per week and present for longer than 3 months) as determined by the study physician.
4. Ability to take oral medication.
5. Provision of signed and dated informed consent form.
6. Stated willingness to comply with all study procedures and availability for the duration of the study.

Individuals who meet any of the following criteria are ineligible to participate in the study:

1. Medical condition or medication that is the cause of the insomnia disorder as determined by the study physician.
2. Known hypersensitivity to cannabis or cannabinoid products (including if this becomes evident during the trial).
3. Reported use of cannabis or cannabinoid products within the past 3 months as confirmed by at least one negative urine drug screen (or at the study physician's discretion).
4. Sleep apnoea (defined as Apnoea Hypopnea Index >15 and Oxygen Desaturation Index >10) as confirmed by polysomnography at screening.
5. Sleep-related movement disorder as determined by the study physician.
6. Delayed or advanced sleep phase syndrome (based on actigraphy and sleep diary) as confirmed during screening.
7. Any medical condition that produces an abnormal electroencephalographic (ie, epilepsy, brain injury).
8. Clinically relevant cardiovascular abnormalities as determined by the study physician and a 12-lead ECG at screening.
9. Shift work or trans meridian travel (two time zones) within the last month.
10. History of major psychiatric disorder in the past 12 months at the study physician's discretion, except clinically managed mild depression and/or anxiety.
11. History of suicide attempt or current suicide ideation (score greater than 1 on Q9 of the Patient Health Questionnaire).[45]
12. Pregnancy or lactating. Female participants are required to complete a urine pregnancy test at screening and treatment sessions and all participants are instructed to use a reliable form of contraception throughout the study duration.
13. History of drug or alcohol dependency or abuse within approximately the past 2 years.
14. Use of central nervous system (CNS)-active drugs (cannabis, amphetamines, cocaine, antidepressants, opioids, benzodiazepines) in the past 3 months as confirmed by a positive urine drug test at screening or at the study physician's discretion.
15. Use of medications that may have a clinically significant impact on the metabolism and excretion of cannabinoids as determined by the study physician (eg, CYP450 enzyme inducers/inhibitors).
16. Excessive caffeine use that in the opinion of the study physician contributes to the participant's insomnia disorder, or the inability to abstain from caffeine use 24 hours prior to each overnight sleep study.
17. Inability to refrain from alcohol consumption 24 hours prior to each overnight sleep study.
18. Individuals with nicotine dependence (ie, daily smokers).

Continued

Box 2   Continued

19. Medical conditions that result in frequent need to get out of bed (eg, sleep walking, nocturia).
20. Psychological or behavioural treatment for insomnia disorder, including cognitive behavioural therapy for insomnia, within 3 months before screening (excluding sleep hygiene advice).
21. Occupational or judicially ordered drug screening.
22. Has held an unrestricted driving license <1 year.
23. Cannot speak English fluently.

placebo are expected to be identical in their visual appearance, taste and smell. The MCT oil contains paprika oleoresin colourant to ensure the double-blind is maintained and prevent oxidation. The sponsor purchased the investigational product from BOD Australia (Sydney, Australia), who sourced investigational products from Medropharm (Zürich, Switzerland). Stability studies supporting the shelf life applied to the drug product were conducted in accordance with the International Conference on Harmonisation (ICH) Guideline Q1A Stability Testing of new Drug Substances and Products and are ongoing. The CBN solution stability is 24 months at 15°C–25°C, 60% humidity and away from sunlight.

Recruitment and retention

We aim to recruit 20 participants over a 12-month period. The sample size was selected with consideration for practical factors such as time, cost and resource allocation (rather than using formal statistical techniques) as it is pilot in nature. Participants are recruited from social media, the Woolcock Institute Volunteer Database and locally displayed physical study advertisements. Participants are reimbursed a fixed amount equivalent to that used in previous clinical trials with similar participant involvement and loss of working hours.[41] To encourage retention, participants receive reimbursement on completion of the final Treatment Session. Study advertisements include an embedded link and QR code to the study website (described below).

Eligibility screening

Eligibility assessment is performed in three steps undertaken by the study team at the Woolcock Institute of Medical Research (see figure 1):

1. On-line and telephone prescreening: Interested individuals are directed to the study webpage. They provide electronic consent before completing an online prescreening questionnaire to assess initial eligibility (eg, age, location, ISI score, transmeridian travel, shift work). Potentially suitable individuals can enter their contact details and are provided the participant information statement so that the trial coordinator may conduct a brief telephone interview before scheduling screening appointments. Individuals deemed unsuitable are provided with support resources.
2. Screening visit 1: Willing and potentially suitable participants attend an on-site medical screening. The
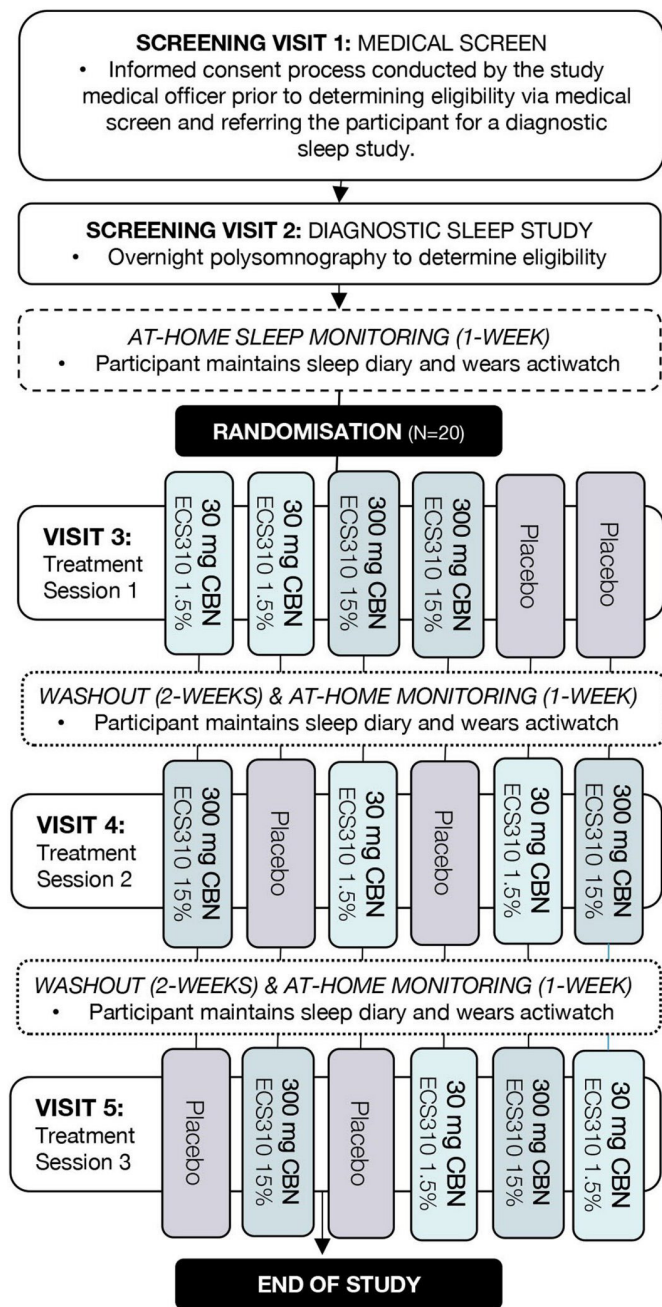
Figure 1 Study flow diagram. CBN, cannabinol.

study physician obtains written informed consent before conducting a screening interview to compile medical history, assess suitability against study eligibility criteria (box 2), and diagnose insomnia disorder (ICSD-3[37] and DSM-V[38]). The following validated self-report questionnaires are used to evaluate eligibility: ISI,[42] a measure of insomnia disorder symptom severity over the past 2 weeks; Epworth Sleepiness Scale,[43] a measure of sleep propensity in daily life; Hospital Anxiety and Depression Scale,[44] a measure of anxiety and depression; and, the Patient Health Questionnaire-9,[45] an instrument for screening and monitoring the severity of depression and suicidal ideation. An ECG and urinary screen (pregnancy and recent alcohol, cannabis,

cocaine, benzodiazepines, opiates or amphetamines, use) are also conducted. Participants must agree to the study protocol, including use of contraception and refraining from driving until the day after Treatment Session discharge.

3. Screening visit 2: Participants are required to complete a clinical overnight polysomnography to rule out the presence of other sleep disorders (obstructive sleep apnoea or sleep-related movement disorder, see box 2). Diagnostic polysomnography results within the previous 12 months may be used where weight has not changed substantially in that time (at discretion of the study physician). Diagnostic sleep studies are scored by an experienced sleep technician[46] and reviewed by the study physician.

Randomisation, allocation concealment and blinding

Participants are allocated to one of six possible treatment orders in a 1:1:1:1:1:1 ratio using a prepopulated randomisation schedule generated by the study epidemiologist (NM). The six orders constitute a balanced Latin square. Sequences were computer generated and are stored in a password-protected system inaccessible to blinded study personnel (centralised computerised randomisation). Allocation concealment is managed by the unblinded study epidemiologist and an independent staff member who do not have any contact with participants, nor involvement in day-to-day trial activities. A study physician may request to be unblinded in the event of a medical emergency. Participants who are enrolled in the study, but not yet randomised, are assigned a unique screening number. Randomisation occurs at the outset of the first treatment session assuming participant eligibility persists. Working under the study physician, the trial coordinator records the decision to randomise the patient. The study database then issues an irrevocable randomisation number for that participant. Investigational product is stored in opaque sequentially numbered containers labelled with the patient randomisation and treatment session number to maintain blinding of all patients, staff and outcome assessors. The drug manufacturer packaged and labelled the investigational product according to the randomisation list generated by the study epidemiologist.

Pretreatment session procedures

Participants are asked to maintain a regular sleep-wake schedule (ie, lights out between 22:00 and 23:00 hours each evening) for 1 week prior to each treatment session. Participants wear a wrist-worn actigraphy monitor (GENE-Active, Activinsights, UK) and complete a daily sleep diary (Karolinska Sleepiness Scale (KSS)[47] modified to capture additional information, such as naps, alcohol/caffeine consumption and GENEactiv removal times) throughout this period. If the sleep schedule is significantly irregular during the seven-night leadup (eg, >2 hours on >2 nights), the principal medical and non-medical investigators may reschedule the visit. Participants are required to abstain from consuming cannabis, cannabinoids, and other
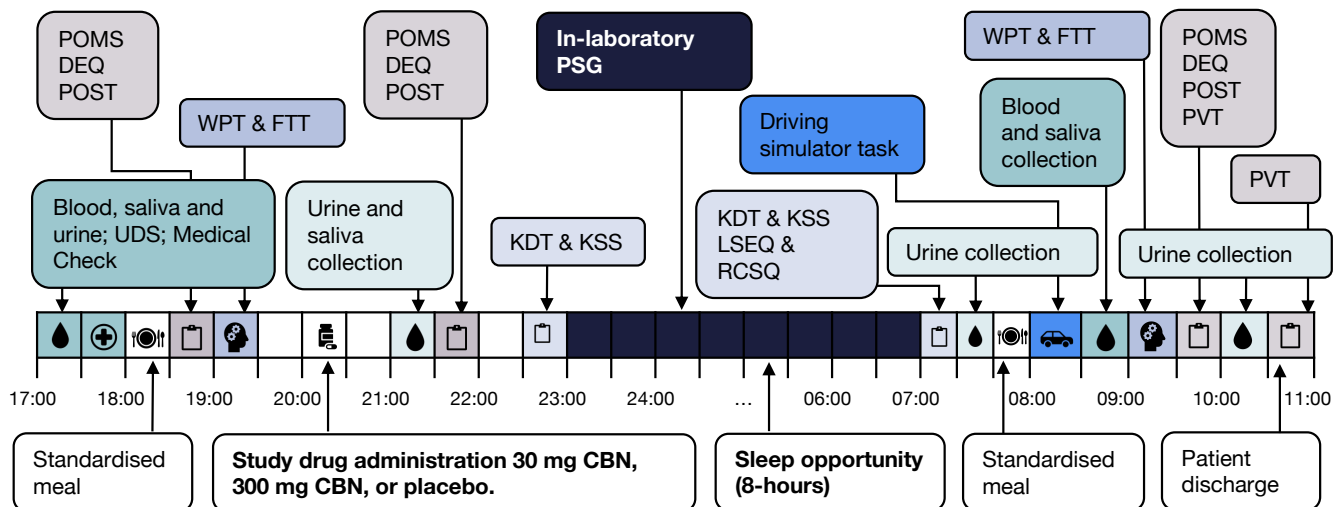
Figure 2 Schedule of events during each treatment session. CBN, cannabinol; DEQ, Drug Effects Questionnaire; EEG, electroencephalography; FTT, Finger Tapping Task; ISI, Insomnia Severity Index; KSD, Karolinska Sleep Diary; KSS, Karolinska Sleepiness Scale; LSEQ, Leeds Sleep Evaluation Questionnaire; POMS, Profile of Mood States; POST, Posturography; PSG, polysomnography; PVT, Psychomotor Vigilance Task; RCSQ, Richard Campbell Sleep Questionnaire; UDS, Urinary Drug Screen; WPT, Word Pairs Task.

Measure (ordered first to last)	Screening	Treatment session 1–3		
		At-home sleep monitoring	Night	Day
Informed consent	•			
Medical examination	•		•	
Baseline questionnaires (ISI, ESS, HADS and PHQ-9)	•			
ECG	•			
Urinary drug and alcohol screen	•		•	
Pregnancy test	•		•	
Overnight polysomnography (clinical EEG)	•		•	
Actigraphy		•		
Sleep diary (KSD)		•		
Oral fluid drug screen (Securetech DrugWipe)			•	•
Quantisal saliva collection			•	•
Blood plasma collection			•	•
Urine collection			•	•
Mood (POMS)			•	•
Drug effects (DEQ)			•	•
Balance (posturography)			•	•
Memory (FTT and WPT)			•	•
KDT with EEG			•	•
Sleep questionnaires (LSEQ, RCSQ and KSS)				•
Simulated driving performance				•
PVT				•

Table 1 A summary of assessments completed throughout the investigation

DEQ, Drug Effects Questionnaire; ECG, electrocardiogram; EEG, electroencephalography; ESS, Epworth Sleepiness Scale; FTT, Finger Tapping Task; HADS, Hospital Anxiety Depression Scale; ISI, Insomnia Severity Index; KDT, Karolinska Drowsiness Test; KSD, Karolinska Sleep Diary; KSS, Karolinska Sleepiness Scale; LSEQ, Leeds Sleep Evaluation Questionnaire; PHQ-9, Patient Health Questionnaire-9; POMS, Profile of Mood States; PSG, polysomnography; PVT, Psychomotor Vigilance Task; RCSQ, Richard Campbell Sleep Questionnaire; WPT, Word Pairs Task.

central nervous system (CNS)-active drugs throughout the entire study; and avoid alcohol and caffeine 24 hours prior to treatment sessions. urinary drug, alcohol (Drug-Check NxStep OnSite, USA) and pregnancy (SureStep, Germany) screening occurs at the outset of each treatment session.

Treatment session procedures

Participants arrive at the Woolcock Sleep Clinic in the early evening (~16:30-17:30 hours, depending on habitual lights out), where they remain until late morning (~10:00–11:00 hours) the following day, as per the treatment session schedule in figure 2. Standardised meals are provided. All treatment sessions are separated by a washout period of ≥2 weeks to minimise carry-over effects between doses.[48]

Study drug administration

After a brief medical examination, the study physician prescribes and dispenses the study drug, drawing a fixed 2 mL volume of liquid into a prelabelled amber plastic syringe. Participants self-administer the randomly allocated study drug 2-hours prior to lights out. The trial coordinator observes drug administration to ensure accuracy of dosing. The dose is documented in participant records and accountability logs. Timing of administration has been informed by delayed onset of effects observed with oral dosing of other cannabinoids[49] and delayed effects observed in a preclinical study of CBN.[30]

Data collection

Study measures are described below (see table 1).

Blood collection and plasma cannabinoid levels

Venous blood samples are taken at baseline (~17:00 hours) and approximately 13-hours post drug administration (~08:45 hours). Samples are drawn into EDTA vacutainers (Becton, Dickinson and Company, New Jersey, USA) and centrifuged (2500×g for 15 min at 4°C). Supernatant plasma is aliquoted into 1.5 mL Eppendorf Tubes and stored at −80°C in the Woolcock Institute of Medical Research until study completion when samples will be transferred to the Lambert Initiative Laboratory. Samples will undergo liquid chromatography–mass spectrometry (LC–MS/MS) analysis, using previously published methodology.[50–52] Plasma will be analysed for cannabinoids (eg, CBN, THC, CBD), their metabolites (eg, 11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD) and endocannabinoids and related molecules (eg, 2-AG and anandamide). THC and CBD and their metabolites are being tested to verify abstinence from common cannabinoids, notwithstanding the trace quantities of THC that the investigational product may contain (≤150 µg/2 mL).

Urine collection and urinary cannabinoid levels

Urine collection occurs at baseline (~17:00 hours), and approximately 90-min (~21:30 hours), 11-hours (~07:00 hours) and 14-hours (~10:00 hours) post drug administration. Urine (~6 mL) is aliquoted into 1.5 mL Eppendorf Tubes and stored at −80°C before being transferred to the Lambert Initiative Laboratory for quantification of cannabinoids (primarily THC, CBD and CBN) and their metabolites (11-OH-CBN, 11-COOH-CBN, 11-OH-THC, 11-COOH-THC, 7-OH-CBD, 7-COOH-CBD) and endocannabinoids at study completion. LC–MS/MS analyses will be conducted using our previously described methods.[50 51] It is important to conduct urinary analyses to further characterise the pharmacokinetic profile of CBN, which is currently not well described.

Salivary drug testing

Oral fluid testing occurs at baseline (~17:00 hours), and at approximately 75-min (~21:15 hours) and 13-hours (~08:55 hours) post drug administration using the Drug-Wipe 5S (DW5s; Securetec, Neubiberg, Germany) and Quantisal collection (Immunalysis, Pomona, California, USA) devices. The DW5s collects oral fluid (approximately 10–20 µL) from the tongue. The DW5s is commonly used in routine roadside drug tests; however, demonstrates variable sensitivity (22%–89%), specificity (50%–100%) and accuracy (53%–94%).[53] While the investigational product contains negligible quantities of THC, similarities in chemical structure between CBN and THC may conceivably lead to false-positive test results that would be a concern should CBN become widely used by patients.[54] As such, the Quantisal device is used to collect oral fluid from participants (~1 mL) which will be used in LC–MS/MS quantification of cannabinoid (CBN and THC) and metabolite concentrations. Samples are placed in a stabilising buffer and stored at 4°C before being transferred to the Lambert Initiative analytical chemistry laboratory for analysis. Analyses occur within 3 months of collection.

Cognitive and psychomotor performance

Overnight declarative and procedural memory consolidation is assessed at each treatment session using the Word Pairs Task (WPT) and Finger Tapping Task (FTT), respectively. For both tasks, memory encoding during a learning phase occurs prior to drug administration (~19:15–20:00 hours) with next-day retest (~09:00 hours). As THC can acutely impair cognitive function and memory, it is necessary to examine CBN effects in this regard. Contrary to oral dosing studies, in one study, CBN reportedly caused a mild intoxication when administered intravenously at high doses (1.2 mg/min).[27] To investigate possible intoxication-induced bodily sway, postural balance ('posturography') is being measured using the stabilometric balance platform (Advanced Medical Technologies, AccuSwayPLUS Balance platform, Massachusetts, USA), which measures centre of pressure in anterior-posterior (y-axis) and medial-lateral (x-axis) directions using four strain gauge load cells at the bottom of the device. Centre of pressure is assessed in eyes open and eyes closed conditions. Postural balance assessment takes <5 min to complete and is repeated three times (ie, baseline, 60-min post drug administration and next-day

at ~09:30 hours). The psychomotor vigilance test (PVT) is a commonly used reaction time based measure of attention and fatigue related changes in alertness[55] involving a hand-held box with a red-light emitting diode display of a three-digit millisecond counter (PVT-192, Ambulatory Monitoring, Adsley, New York, USA). During the 10 min task, participants are instructed to respond as quickly as possible to stimuli appearing at variable intervals (ranging 2–10 s). The PVT is repeated twice during next-day testing (~09:00 and ~10:00 hours).

Self-report questionnaires

Mood is evaluated using the Profile of Mood States (POMS; 40 items with 5-point Likert scales)[56] questionnaire, a commonly used rating scale of transient and distinct moods. The Drug Effects Questionnaire (DEQ; 7-items with unipolar 100 mm Visual Analogue Scales (VAS))[57] is used to evaluate subjective drug effect; including strength of drug effect, feeling stoned ('high'), liking/disliking the drug effect, and feeling sedated. The POMS and DEQ are completed at baseline, ~1-hour post-drug administration (~21:00 hours) and the next-day (~09:30 hours). The Leeds Sleep Evaluation Questionnaire (LSEQ; 10 items with 100 mm VAS)[58] is a validated measure of the previous night's sleep quality (sleep onset, maintenance and quality; including behaviour following waking) in comparison to usual. The Richard Campbell Sleep Questionnaire (RCSQ; 5 items with 100 mm VAS)[59] is used to measure perceived sleep depth, sleep latency (time to fall asleep), number of awakenings and sleep efficiency and quality. The LSEQ and RCSQ are administered on wake (~06:15 hours).

Subjective and objective sleepiness

KSS and Karolinska Drowsiness Test (KDT) are used to measure subjective and objective sleepiness, respectively.[60] The KSS is a single-item rating scale assessing current sleepiness, ranging from 1 (extremely alert) to 9 (extremely sleepy—fighting sleep). KSS scores are typically compared with the objective drowsiness ascertained using the KDT. During the KDT, resting awake EEG is measured while participants focus on a wall marker, with eyes open and closed across 2 min intervals. The KSS and KDT are administered immediately prior to sleep (~22:00 hours) and on wake (~06:00 hours).

Polysomnography

Sleep is assessed using polysomnography (Grael polysomnography, Compumedics, USA), a multiparametric tool used for monitoring and diagnosing sleep disorders. Polysomnography consists of 18 channels recording biophysical changes that occur during sleep, including brain activity (via EEG); muscle activity or skeletal muscle activation (electromyography); eye movement (electrooculogram); cardiac function (ECG); respiratory effort using chest and abdominal belts; and digital pulse oximetry. To improve participant comfort/retention, the pulse oximeter, nasal cannula, and thermistor are only used during

screening to detect sleep disordered breathing (visit 2). Polysomnography equipment is fitted by an experienced sleep technician prior to sleep and is removed the next morning once the KDT has been completed. Sleep and associated events are scored according to AASM 2020 criteria in 30 s epochs.[46] Each patient's three polysomnograms are scored by the same sleep technician blinded to treatment allocation. For quantitative EEG analyses, sleep and KDT polysomnography recordings will be processed using a previously validated automated artefact detection programme.[61 62] Power spectral and spindle analyses will be derived from artefact-free epochs in central channels using previously published methodology.[61 62]

Simulated driving performance

Next-day driving performance is measured at ~08:00 using a fixed-based driving simulator (Hyperdrive, Adelaide, Australia) equipped with standard vehicle controls (described elsewhere[41 48]) and using custom-built scenario developed using the SCANeR Studio Simulation Engine (version 2022.2 r25, AVSimulation, Paris, France). The 30 min driving scenario incorporates three independent epochs: (1) a 'car following' drive (~7 min duration) that closely resembles one that has previously demonstrated sensitivity to THC-induced impairment;[63] (2) a 'highway' drive (~17 min duration) and (3) a novel divided-attention drive (~6 min duration), designed to replicate a casual mobile-phone conversation that does not require mental rehearsal.[63] During the divided-attention task, pre-recorded five-word sentences are played every 10 s (through a hands-free speaker). Participants immediately indicate whether the sentence was sensical (eg, 'the truck delivered the package') or non-sensical (eg, 'the octopus burned the onions') in nature then; and at 7 s, they are asked to recall the last word in the sentence. The main outcome measure for all epochs of the driving task is SDLP, a widely used measure of lateral vehicular control that is sensitive to drug and alcohol effects. Previous reports show a dose-dependent increase in SDLP with THC (higher represents more erratic driving), particularly in occasional cannabis users.[64 65] Secondary outcomes for each driving epoch include: (epoch 1) average and SD of car-following headway (ie, distance relative to car in front) and speed coherence (ie, correlation between speed of lead and following car); (epochs 2 and 3) average speed and SD of speed. The driving scenarios were programmed by investigator CI.

Adverse events

Safety outcome measures are described above. Blood pressure is measured four times during each treatment session (~17:00, 18:45, 21:00 and 09:30 hours). Adverse event collection occurs via standardised interview at three timepoints (prior to discharge as well as 7-hours and 7-days post discharge). Passive adverse event collection occurs throughout the study protocol via open-ended interview and is recorded on paper form. A study physician assesses the severity and causality of each adverse event.

Investigator	Role
Dr Camilla Hoyos	Principal non-medical investigator
Professor Brendon Yee	Principal medical investigator
Professor Iain McGregor	Advisory committee member (pharmacologist and cannabinoid expert)
Professor Ronald Grunstein	Advisory committee member (sleep physician)
Professor Bandana Saini	Advisory committee (study pharmacist)
Associate Professor Nathaniel Marshall	Advisory committee (study statistician/epidemiologist)
Associate Professor Christopher Gordon	Advisory committee member (registered nurse and sleep disorder expert)
Dr Angela D'Rozario	Advisory committee member (sleep neurobiology expert/consultant)
Dr Danielle McCartney	Driving simulator and cannabinoid expert
Dr Chris Irwin	Advisory committee member (driving simulation expert)
Dr Anastasia Suraev	Advisory committee member (cannabinoid and sleep expert)
Ms Isobel Lavender	Clinical trial coordinator/PhD student

Post-treatment session care

Participants leave the research facility at approximately 10:00–11:00 hours and are contacted by the study co-ordinator that evening (~16:30 hours) and 7-days later, to assess well-being and record adverse events (including changes to sleep). On study completion, if deemed necessary in the study physician's opinion, a clinical follow-up is arranged for participants on an individual basis.

Data management

Any information obtained for the purpose of this research that could identify participants is treated as confidential and securely stored adhering to guidelines of the Sponsor, HREC, NHMRC National Statement on Ethical Conduct in Human Research (2007) and Note for Guidance on Good Clinical Practice (CPMP/ICH-135/95). All onboarding, consent and questionnaire data are captured through SPARDAC (Single Page Application-Research Data Capture) developed by Wappsystem and hosted on Amazon Web Services in Sydney, NSW. Participant data are identified by unique codes. The code linking participant data to identity (eg, name, date of birth, contact details) is stored securely with password protection, and is not accessible via the internet. Paper files are stored in locked storage cabinets on-site. Digital participant data are stored on a secure electronic data system, that is regularly backed up with disaster recovery features. All data will be stored securely for at least 15 years. Only study

investigators have access to participant data. Data monitoring occurs on a regular basis by the study investigators. Data integrity is being enforced through a variety of mechanisms including data rules, range checks and consistency checks against data already stored in the database. Written documentation of changes is available through electronic logs and audit trails. Study safety is internally monitored and evaluated regularly, with adverse events documented and reported according to sponsor and HREC requirements. The decision to terminate the study lies with the principal investigators and will be based on the recruitment target and safety data. The PIs and trial coordinator are monitoring data integrity throughout the trial.

Trial management structure

This is a pilot study and does not require an intricate management structure. The advisory committee provide advice or complete discrete tasks (table 2). The management of day-to-day trial activities is overseen by the principal investigators and the trial coordinator (IL, CMH and BY). This trial does not have an independent data safety monitoring committee because it is a single-site single-night small sample study with a medication that is thought to have a low side effect profile in a group of patients that are known not to be at high risk of life-threatening events.[66]

Roles and responsibilities

The study investigators have led the design and conduct of this trial, will conduct the analyses and make all publication decisions.

Statistical analyses

Statistical analyses will be completed using a commercial statistical package (eg, SPSS (Version 29.0.1.0) and SAS (Version 9.4)). Descriptive statistics will be described using means and SD or counts and percentages as appropriate. Adverse events and serious adverse events will be tabulated with the total number of incidents by each time point. No formal hypothesis testing will be conducted on adverse event and serious adverse event data. Due to insufficient statistical power, the KSS will only be described using a median and IQR. Continuous outcome data will be analysed using linear mixed models due to expected inter-participant variability with repeated measures and ability to handle missing data. Where variables are collected predrug administration (ie, treatment session 'baseline') for the outcome being analysed, those scores will also be included in the model as a covariate (eg, posturography, WPT, FTT, DEQ, POMS). Participants will be random factors. Fixed factors will be treatment (placebo, 30 mg CBN and 300 mg CBN) and the arm (first, second and third) of the study. Analysis will be by intention to treat. Difference in least squared means inside the treatment main effect will only be investigated for the hypotheses that the two active treatments are superior to placebo. We will not present a p value comparison for the two active doses. The critical p values for each of these hypotheses

is set at 0.05 (two tailed). Effect sizes will be calculated as partial eta squared (η_p^2), Cohen's d and Hedges' g, where appropriate. A statistical analysis plan will be finalised before last patient last visit and will be publicly available (eg, Clinical Trial Registry) and/or on request.[67] We are not undertaking any interim or stratified analyses.

Ethics and dissemination

Human Research Ethics Committee approval has been granted by Bellberry (2021-08-907). Study findings will be disseminated via scientific peer-reviewed publications, conferences and media, as applicable.

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