Evaluation of the Effects of Neurexan® on Short-Term Insomnia, Daytime Performance and Stress Response by Polysomnography (PSG), Electroencephalogram (EEG), Stress Biomarkers and Patient-Reported Outcomes (PROs)

An Exploratory, Placebo-Controlled Trial in Short-Term Insomnia Patients

Protocol:	Approved Version 4.0, dated 27-Feb-2025
EudraCT Number:	2022-003565-38
EU CT Number:	2024-514391-41-00
NCT Number:	NCT06278077

Clinical Trial Protocol

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An Exploratory, Placebo-Controlled Trial in Short-Term Insomnia Patients

Development Phase:	II
EudraCT Number:	2022-003565-38
EU CT Number:	2024-514391-41-00
Sponsor's Protocol Code:	C2104
Investigational Product:	Neurexan®
Indication:	Short-term insomnia
Coordinating Investigator:	Prof Dr. Martin Walter, MD Chair of Department of Psychiatry and Psychotherapy Jena University Hospital Philosophenweg 3 07743 Jena Germany
Sponsor:	Biologische Heilmittel Heel GmbH Dr.-Reckeweg-Straße 2-4 76532 Baden-Baden Germany

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Conduct: In accordance with the ethical principles that originate from the Declaration of Helsinki and that are consistent with the Good Clinical Practice guideline of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) and regulatory requirements as applicable.

RESPONSIBILITIES AND SIGNATURES

Protocol Approval Signature Page

I have carefully read this protocol and agree to conduct the clinical trial according to the protocol specifications and in compliance with the ICH guideline for Good Clinical Practice (GCP) and the Declaration of Helsinki and after having obtained approval from the Ethics Committee and consent in writing from the patients. Signing this form constitutes a written agreement between the Investigator, Biologische Heilmittel Heel GmbH, and their representatives.

Coordinating Investigator

[redacted]

3.3.2025

Prof Dr Martin Walter, MD — Date
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Sponsor's Representatives

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Head of Clinical Development/ Medical Responsible Person
Biologische Heilmittel Heel GmbH

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03/03/2025
Date

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04 Mar 2025
Date

Senior Clinical Project Manager
Biologische Heilmittel Heel GmbH

Contract Research Organization

Biostatistician

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28 Feb - 2025
Date

Clinical Trial Statistician
AMS Advanced Medical Services GmbH

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28 Feb. 2025
Date

Project Manager
AMS Advanced Medical Services GmbH

Protocol Signature Page

I have carefully read this protocol and agree to conduct the trial according to the protocol specifications and in compliance with the ICH GCP Guideline and the Declaration of Helsinki and after having obtained approval from the Ethics Committee and consent in writing from the patients as stipulated in the protocol. Signing this form constitutes a written agreement between the Investigator, Biologische Heilmittel Heel GmbH, and their representatives.

Coordinating Investigator:

[redacted]

3.3.2021

Prof Dr Martin Walter, MD
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Date

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REVISION HISTORY

Number	Date	Section of clinical trial protocol	Amendment or update	Reason
0	V. 1.0 24 November 2022			
1	V. 2.0 15 February 2023	Signature page of Sponsor's Representatives	[redacted] Head of Medicine & Research [redacted] Senior Medical Manager	Organizational changes
		In Schedule of Trial Procedures/ and accordingly in whole document	Daytime/Performance Questionnaires, Level of Anxiety and Depression Paper PROs: BAI, BDI-II, WEIMuS, SF-36v2, ***AMS***-ePRO®: ESS	BDI-II questionnaire will be used on paper (paper PRO) instead of ***AMS***-ePRO®. FSS questionnaire was replaced by WEIMuS questionnaire measuring the same construct. WEIMuS will be used on paper (paper PRO)
		Whole document	Perceived Stress Scale-10 (PSS-10)	PSS questionnaire further specified that it is the 10-item scale
		Whole document	Short Form-36 version 2 (SF-36v2)	SF-36 questionnaire further specified that version 2.0 is used
		Exclusion criteria (synopsis and section 6.2)	No. 5. History of psychiatric disorders within the last 6 months prior to Screening Visit, according to the Structured Clinical Interview for DSM-5® Disorders – Clinician Version (SCID-5-CV)	Excl. criterion no. 5 further specified; SCID-5-CV used to screen for psychiatric disorders in the last 6 months
		Whole document		Minor corrections of typos and commas
		Section 6.4	Insufficient treatment compliance as specified in the protocol (see section 11.3.2)	Withdrawal criterion no. 3 further specified
		Section 9.1.2	Saliva samples are collected in 2 ml SaliCap sampling tubes (TECAN/ IBL International, Hamburg, Germany, ref. no. RE69985) using short drinking straws	Further information added
		Section 9.1.9	Venous blood for transcriptome analysis will be collected in PAXgene® Blood RNA Tubes (Qiagen/ PreAnalytiX, purchased from Becton Dickinson, Heidelberg, Germany, ref. no. 762165) using a BD Vacutainer Safety-Lok blood collection set (Becton Dickinson, ref. no. 367282) before and after each	Further information added

			adaptation PSG night (4 samples of 2.5 ml/ sample)	
		Section 9.1.15.4	Würzburg Fatigue Inventory (WEIMuS) The WEIMuS is a 17-item scale designed to assess fatigue as a symptom of a variety of different chronic conditions and disorders. The scale addresses fatigue's effects on daily functioning, querying its relationship to motivation, physical activity, work, family, and social life, and asking respondents to rate the ease with which they are fatigued and the degree to which the symptom poses a problem for them. The WEIMuS will be completed on paper at Baseline 1 and at Visit 1	Fatigue questionnaire FSS was replaced by WEIMuS measuring the same construct
2	V. 3.0. 19 July 2023	Signature page of Contract Research Organization	[redacted] [redacted] Clinical Trial Statistician	Organizational change
		Synopsis Section 4.1	The primary endpoint of this trial is to assess Neurexan® related changes in sleep efficiency (SE) at Visit 2 compared to Baseline 2 as revealed by polysomno-graphy.	Further clarification which Visits are used to assess the primary endpoint
		Synopsis Section 4.2	Added sentence for objective sleep parameters: To be assessed as change at Visit 2 compared to Baseline 2 For subjective sleep pattern parameters o Insomnia Severity Index (ISI): changes in ISI at Visit 1 compared to Baseline 1	Further clarification which Visits are used to assess the secondary endpoints
		Synopsis Section 4.2	• Stress questionnaires Changes in subjective stress levels compared to Baseline as assessed by • Subclinical Stress Symptom Questionnaire-25 (SSQ-25) • Primary Appraisal Secondary Appraisal (PASA) • Perceived Stress Scale-10 (PSS-10) • Continuous (over the whole trial period) daily stress assessment using AMS-ePRO® functionality.	Shift of stress questionnaires for logic reasons and addition of sentence for clarification

		Inclusion criteria (synopsis and section 6.1)	No. 2 Short-term insomnia with moderate symptoms according to ISI of at least 8 and below 22 being present for at least one week, but no longer than 3 months prior to Screening Visit ~~and Baseline 1~~	Inclusion criterion 2 was amended for clarification (deleted “and Baseline 1”)
		Inclusion criteria (synopsis and section 6.1), Section 10.7	No. 8 Females (Women, in section 10.7) of childbearing potential must agree to maintain highly effective or acceptable birth control throughout the trial (CTFG 2020). Highly effective (failure rate of less than 1% per year) ~~Such methods include:~~ • ~~Oral or intravaginal or transdermal c~~**Combined** (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal • **Progestogen-only** hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable • **Intrauterine** device • ~~oral or injectable or implantable hormonal contraception associated with inhibition of ovulation, or~~ **I**~~i~~**ntrauterine** hormone-releasing system ~~(IUS)~~ • Bilateral **tubal** occlusion • **Vasectomized** partner (provided partner is sole sexual partner and if vasectomized partner has received medical assessment of the surgical success) • Sexual **abstinence** (only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments)	Inclusion criterion 8 was amended to also allow females with medically acceptable contraception according to CTFG 2020. In order to allow enrollment of female patients with acceptable non-hormonal contraception Section 10.7 on pregnancy adapted accordingly

			Acceptable birth control methods which may not be considered as highly effective (failure rate of more than 1% per year) • **Progestogen-only** oral hormonal contraception, where inhibition of ovulation is not the primary mode of action • Male or female **condom** with or without spermicide • **Cap, diaphragm** or **sponge** with spermicide • **Combination** of male condom with either cap, diaphragm or sponge with spermicide (**double barrier** methods)	
		Exclusion criteria (synopsis and section 6.2)	No. 3 Based on the first polysomnographic screening night at Baseline 1, insomnia due to sleep apnea or periodic limb movement disorder (PLMD): OSA (Apnea Hypopnea Index of >5 events/ hour), PLMD (Periodic Limb Movement Index (PLMI) >15 events/ hour	Exclusion criterion 3: Added "the first" and "at Baseline 1" for clarification Adjusted number of Periodic Limb Movement Index (PLMI) to >15 events/ hour in order to avoid screening failures due to normal PLMI exclusion criterion
		Exclusion criteria (synopsis and section 6.2)	No. 8 Cognitive impairment (~~age-adapted~~ cut-off of 24 points in the Montreal Cognitive Assessment [MoCA], Thomann, Berres et al. 2020) at Screening Visit	Deleted "age-adapted", addition "of 24 points" and a reference for higher specificity
		Exclusion criteria (synopsis and section 6.2) Section 7.6.1	No. 13 Excessive consumption of xanthine-containing beverages (more than 7 cups daily of coffee or tea or other beverages containing xanthines)	Added "daily" to avoid misunderstanding
		Exclusion criteria (synopsis and section 6.2)	No 18. Hypertension defined as systolic blood pressure ~~>145~~ ≥140 mmHg (Burnier, 2018)	Threshold for hypertension was amended and reference added to align with current European hypertension guideline
		Schedule/ Flowchart of trial procedures	Inclusion and Exclusion criteria (except exclusion criterion 3)	Further clarification for in- and exclusion criteria that eligibility will be assessed at Screening Visit, except

		(following synopsis section 2.2, section 8.1)	~~Randomization~~ Exclusion Criterion 3	exclusion criterion 3 which will be assessed at first polysomnographic screening night at Baseline 1. Harmonized flowchart in order to address changes made in exclusion criterion 3, and which is no longer defined as randomization criterion
		Synopsis and section 11.3.2	**Criteria for Evaluation:** The ~~enrolled~~ screening population is defined as all patients who signed informed consent.	Wording amended for clarification “The screening population is defined as all patients who signed informed consent.”
		Synopsis and section 11.4.2	Secondary Efficacy Variables *Biochemical parameters* Serum cortisol levels ~~(ug/dl)~~ and other stress-related parameters in response to the stress induction by Cold Pressor Test (CPT).	Units are not given anywhere else. Deleted to harmonize
		Synopsis and section 11.4.2	Secondary Efficacy Variables *Exploratory Efficacy Variables* • Cognitive performance in three attentional ~~distraction~~ tasks • PSG ~~Adaptation~~ sleep parameters ~~data of~~ derived ~~polysomnography~~ from the two adaptation nights at Baseline 1 and Visit 1	Wording changed for correction and clarification reasons
		Synopsis and section 11.4.2	**Statistical Methods:** Correlations ~~will~~ may be analyzed using Spearman's rank correlation coefficient or another appropriate method.	Wording changed to allow other methods than Spearman’s.
		Section 4.1	The primary objective is to demonstrate an improvement of sleep efficiency (SE) measured by polysomnography on daily treatment with Neurexan® in short-term insomnia. The primary endpoint of this trial is to assess Neurexan® related changes in ~~sleep efficiency~~ SE ~~(SE)~~ at Visit 2 compared to Baseline 2 as revealed by polysomnography (PSG).	Further clarification which Visits are used to assess the primary endpoint

		Section 4.2	The secondary objectives are to assess further PSG-based objective as well as subjective sleep parameters, daytime performance and stress processing in patients with short-term insomnia treated daily with Neurexan® compared to placebo.	Further specified in objective, that trial medication is to be taken daily
		Section 6	An enrollment of 80 patients suffering from short-term insomnia is planned for the trial. If a patient fulfils all of the inclusion criteria and none of the exclusion criteria, his/her eligibility will be assessed during the Screening Visit and he/she will be randomly assigned to Neurexan® or placebo treatment with 1:1 allocation and stratification by sex ~~as soon as the randomization criterion is fulfilled~~.	Amended in order to address changes made in exclusion/ randomization criterion 3
		Section 6.1	Inclusion criterion 3. Reports habitual bedtime, defined as the time the participant attempts to sleep, between 21:00 ~~24:00~~ and ~~and habitual wake time between 05:00 and 09:00~~ 01:00	Inclusion criterion 3 was accurately stated in the synopsis and has now been harmonized
		Section 6.2	Added sentence regarding blood tests taken at Screening: The eligibility of patients based on safety laboratory parameters (except CRP) are at the discretion of the investigator.	Addition to further clarify that it is at the investigator's discretion to decide upon eligibility of patients based on safety laboratory parameters (except CRP, because a clear threshold is defined in exclusion criterion no 17)
		Section 6.3	~~Randomization Criterion Only patients who do not meet the exclusion criterion number 3 following Baseline 2 PSG night at Day 0 are eligible for randomization.~~	Randomization criterion is no longer needed. Exclusion criterion 3 will be assessed at first polysomnographic screening night at Baseline 1.
		Section 6.4	Intake of prohibited concomitant medication (see section 7.6.1) that might influence the trial procedures or the results	Included cross-reference to section 7.6.1 to increase readability
		Section 7.6.1	**Prohibited Medications** Abuse of alcohol and/or use of amphetamines, benzodiaze-	Clarification that not only the abuse but also "use of" amphetamines, benzodiaze-pines, cocaine, marijuana,

			pines, cocaine, marijuana, methaqualone, methadone, opioids, propoxyphene, barbiturates, phencyclidine	methaqualone, methadone, opioids, propoxyphene, barbiturates and phencyclidine are considered as prohibited
		Section 8.1	**Screening Visit** Restructuring of assessments; Informed consent followed by diagnosis of short-term insomnia directly after and completion of ISI • Instruction in the use of the actigraphy watch, i.e., Actiwatch Spectrum Plus, and its handing over.	Restructuring for clarification and addition of instruction in the use of Actiwatch
		Section 8.1	From Screening Visit onwards daily until the End of Trial (Follow-up Visit/ Day 28+3) patients must wear an actigraphy watch ~~portable actimetric device~~ recording their ~~rest~~ sleeping and activity cycles, complete a sleep diary and answer digital questionnaires regarding stress level (AMS-ePRO®).	Wording harmonized throughout the document
		Section 8.1	**Baseline 1** (…) patients will have their first night at the trial site for PSG adaptation and screening for exclusion criterion 3. In the evening before the ~~second~~ Baseline 1 night urine and breath alcohol tests will be conducted on drugs and alcohol, respectively PSG for screening of exclusion criterion 3 and for adaptation to the unfamiliar sleep lab environment ~~to measure SOL, WASO, TST, NWAK and ARI during sleep~~ in the night between Day -2 and Day -1 (section 9.1.1) Check exclusion criterion 3 at Day -1	Addition of “and screening for exclusion criterion 3” to reflect changes of evaluation timepoint of exclusion criterion 3 Correction: Baseline 1 night Clarification that PSG at Baseline 1 is for screening of exclusion criterion 3 and for the adaptation of the patients to unfamiliar sleep lab environment and not to measure any endpoint parameters Addition since exclusion criterion 3 to be assessed at Baseline 1 now

		Section 8.1	**Baseline 1** Collection of approximately ~~15.1~~12.5 ml blood for measuring cortisol, DHEA-S, adrenaline, noradrenaline, inflammation markers (IL-6 and TNF-α) prior to CPT, 20 minutes and 50 minutes after CPT at Day -1 (section 9.1.8)	Amount of blood to be collected reduced from approximately “15.1 ml” to “12.5 ml”
		Section 8.1	**Baseline 2** ~~After fulfilment of the randomization criterion~~ ~~p~~Patients will be randomly assigned to treatment group Neurexan® or placebo with a 1:1 allocation and stratification by sex. Baseline 2 PSG night ~~recording~~ to measure primary, i.e., SE and secondary endpoints, i.e., SOL, WASO, TST, NWAK, and ArI during sleep in the night between Day -1 and Day 0 (section 9.1.1) ~~Check randomization criterion at Day 0 (section 6.3)~~	Changed because there is no longer a randomization criterion Clarification of time point and endpoints to be measured. Deleted, no longer needed
		Section 8.1	**Visit 1** PSG night, again~~to~~ for adaptation to the unfamiliar sleep lab environment ~~measure SOL, WASO, TST, NWAK, and ArI during sleep in the night~~ between Day 12 and Day 13 (section 9.1.1)	Clarification that PSG between Day 12 and Day 13 night is for the adaptation of the patients to unfamiliar sleep lab environment and not to measure any endpoint parameters
		Section 8.1	**Visit 1** Collection of approximately ~~15.1~~12.5 ml blood for measuring cortisol, DHEA-S, adrenaline, noradrenaline, inflammation markers (IL-6 and TNF-α) ~~and alpha-amylase~~ prior to CPT, 20 minutes and 50 minutes after CPT at Day 13 (section 9.1.8)	Changed “approximately 15.1 ml” to “approximately 12.5 ml”, deleted “and alpha-amylase”

		Section 9.1.1	**Polysomnography (PSG)** (…) Sleep efficiency (SE) is calculated as the ratio of total sleep time (TST) to time in bed (TIB) (i.e., both sleeping and attempting to fall asleep or fall back asleep).	Sentence added to define sleep efficiency
		Section 9.1.2	Salivary samples are stored frozen at -80°C (+/- 10°C fluctuation) […].	Further specified storage temperature
		Section 9.1.5	**Actigraphy** (…), which will continuously monitor movement information over the whole trial duration (Screening Visit/ Day -28 to -8 until Follow-up Visit/ Day 28) to assess ~~time asleep~~ SE, SOL, WASO, TST and NWAK ~~sleep stages (time spent in REM, light sleep, and deep sleep)~~ as well as to track daily activity to investigate habitual sleep–wake patterns	Parameters that will be measured by actigraphy are further specified
		Section 9.1.7	~~Immediately b~~Before conduction of the CPT (~~at~~ Baseline) ~~value~~, 20 minutes and 50 minutes after CPT blood (~~15.1~~ 12.5 ml/ blood sample) will be taken to assess cortisol and DHEA-S, adrenalin and noradrenalin and inflammatory response (IL-6 and TNF-α).	Wording changed for clarification, amount of blood changed “15.1 ml” to “12.5 ml”, “adrenalin and noradrenalin” added
		Section 9.1.8	Each blood sample consists of ~~15.1~~ 12.5 ml blood that will be split up in ~~4~~ 3 monovettes (all from Sarstedt, Nümbrecht, Germany) as following:	Changed “15.1 ml” to “12.5 ml”, and “4 monovettes” to “3 monovettes”
		Section 9.1.8	4.9 ml ~~serum~~ blood will be collected in an S-Monovette® Serum Gel (ref no 04.1935.001) for the analysis of cortisol, DHEA-S and TNF-α,	Replaced “serum” with “blood”, added “DHEA-S”
		Section 9.1.8	~~4.9 ml serum will be collected in a S-Monovette® Serum Gel (ref no 04.1935.001) for the analysis of DHEA-S,~~	Deleted second bullet point (DHEA-S will be analyzed together with cortisol and TNF-α from one S-Monovette®)

		Section 9.1.8	~~2.6~~ 4.9 ml ~~plasma~~ blood will be collected in a S-Monovette® K3 EDTA (ref no 04.193~~1~~01.001) for the analysis of adrenalin and noradrenalin, and	Replaced “2.6 ml plasma” with “4.9 ml blood” and adapted ref no
		Section 9.1.8	2.7 ml ~~plasma~~ blood will be collected in a S-Monovette® Lithium-heparin gel (ref no. 04.1928.001) for the analysis of IL-6.	Replaced “plasma” with “blood”
		Section 9.1.9	(…) interim storage at -80°C (+/- 10°C fluctuation) in the biobank (…).	Further specified storage temperature
		Section 9.1.11	With neuropsychological tests the Investigator assesses the patients’ alertness, vigilance and sustained attention ~~concentration, distraction as well as psychomotor speed~~.	Correction for consistency
		Section 9.2.4	**Safety Laboratory** Safety laboratory blood samples will be collected once at Screening Visit (and up to 2 working days later) (...).	Clarification that screening safety lab can be collected within 2 working days following the Screening Visit
		Section 11.3	**Definition of Baseline** For PSG derived parameters the baseline is defined as the night from Day -1 to Day 0 (Baseline 2).	Sentence added for further clarification of Baseline values for PSG parameters
		Section 11.3.1	**Hypothesis** The null hypothesis will be rejected and the superiority of a treatment (Neurexan® or placebo) will be claimed if the p-value will be equal or less than 0.05.	Added “equal or” for clarification
		Section 11.4.1	**Primary Efficacy** The primary efficacy variable for changes in sleep pattern will be sleep efficiency (SE) measured by polysomnography at Baseline 2 and Visit 2, calculated as the ratio of total sleep time (TST) to time in bed (TIB), as difference at Visit 2 to Baseline 2.	Clarification which Visits are used to assess primary efficacy endpoint
		Section 11.4.2	**Secondary Efficacy**	Specification of exploratory instead of descriptive

			For all secondary variables, exploratory ~~descriptive~~ statistics comparing the 2 treatment groups will be performed. Group comparisons of continuous numeric parameters will be tested using an ANCOVA including treatment, the stratification factor sex, and the baseline value of continuous numeric parameter~~s at Baseline~~ as covariate; (..). *Polysomnography at Baseline 2 and Visit 2*	Re-wording for better understanding Addition to clarify when these variables will be assessed
		Whole document		Minor corrections of typos and commas
3	V. 4.0. 27 February 2025	2.1 Synopsis	**Trial Duration:** First Patient First Visit ~~planned~~: ~~March~~ June 2023 Last Patient Last Visit planned: January ~~November~~ 2024~~6~~	Updating actual FPFV and planned LPLV
		2.1 Synopsis	**Stress Processing** Further secondary objectives are to assess correlations between: ~~• Changes in sleep parameters, stress parameters and daytime performance with whole blood transcriptomics.~~	It was decided to omit the analysis of whole blood transcriptomics for this trial due to the following limitations: • RNA Stability and Degradation RNA: in whole blood is prone to degradation, especially if samples are not processed immediately or properly. This instability can compromise data quality and reproducibility. • The WBT blood samples taken so far are incomplete (based on the currently 46 randomized patients, WBT blood samples are incomplete for 10 patients, >20%).
		2.1 Synopsis	**Subgroup Analyses** ~~Sex (female/ male) differences based on whole blood transcriptomics~~	See the justification above for decision to omit WBT for this trial.

		2.2 Schedule of Trial Procedures and 8.1 Visit Schedule Table 3	~~Venous Blood Samples for Whole Blood Transcriptomics[2]~~ | | ~~X~~ | | ~~X~~ | | | ~~[2]Venous blood sampling (2.5 ml in PAXgene® Blood RNA Tubes) for transcriptome analysis before sleep and after awakening and additionally before and 50 minutes after CPT stress induction.~~	See the justification above for decision to omit WBT for this trial.
		4.2 Secondary Objectives and Endpoints	**4.2 Secondary Objectives and Endpoints** ***Daytime Performance by*** • Resting state EEG: Regional changes in spectral characteristics of EEG using relative power in delta-theta-alpha-beta ~~gamma~~ frequency ranges at Visit 1 compared to Baseline 1 **Further secondary objectives** are to assess correlations between: (…) • ~~Changes in sleep parameters, stress parameters and daytime performance with whole blood transcriptomics~~	Correction See the justification above for decision to omit WBT for this trial.
		5.1 Trial Type and Design Features	It is planned to randomize ~~enroll~~ a total of 80 patients with short-term insomnia.	Substituted “enroll” by “randomize” in order to be more accurate
		5.3 Trial Duration	The ~~estimated~~ First Patient First Visit (FPFV) ~~is~~ was in June ~~March~~ 2023. A total of 80 patients will be randomized ~~recruited~~ within approximately 32 ~~21~~ months. The Last Patient Last Visit (LPLV) is planned to be in January 2026.	Updating actual FPFV and planned LPLV

		5.5.3 Central Laboratories	(…) (Institut für Klinische Chemie und Laboratoriums-diagnostik IKCL, University Hospital Jena) will analyze safety laboratory blood samples (see section 9.2.4) as well as blood biomarker samples (see section 9.1.8) and will provide frozen interim storage of saliva ~~and whole blood transcriptomic~~ samples in their biobank. Saliva biomarker samples (9.1.2) will be analyzed at Dresden LabService GmbH. ~~and whole blood transcriptomic samples (section 9.1.9) will be analyzed at German Center for Neurodegenerative Diseases DZNE, Göttingen.~~	See the justification above for decision to omit WBT for this trial.
		6. Patient ~~Enrollment~~ Screening, Randomizat-ion, Discontin-uation and Withdrawal	~~An enrollment~~ Randomization of 80 patients suffering from short-term insomnia is planned for the trial.	Substitution “enrollment” by either “screening” or “randomization” in order to be more accurate
		6.3 Patient Premature Withdrawal from Trial/ Withdrawal Criteria	If possible, a remotely prematurely withdrawn patient should be invited for a last onsite visit to return remaining IMP and the Actiwatch. The procedures of the Follow-Up Visit should be followed.	Addition for further clarification
		7.2 Dosage and Administration	The trial treatment consists of 2 tablets taken sublingually 3 times daily (a total of 6 tablets/ day) for a period of 14 consecutive days (+ up to 2 days allowance, see flow chart at section 2.2 and Table 3).	Insertions for time deviation allowance
		7.3 Blinding	The unblinding has to be documented by the Investigator. Details of the unblinding procedure will be explained in the Safety Reporting ~~Management~~ Plan for this trial.	Correction
		7.4 Manufacturing , Packaging and Labelling	(…) Labelling implements the use of the computer-generated randomization plan in a treatment ratio of 1:1 and stratification by sex**.** IMP containers for males and for females are provided, distinguishable by randomization numbers and IMP label color.	Insertion of further IMP details.

		8.1 Visit Schedule Baseline 1	(…) ~~Before sleep~~, ~~p~~Patients complete their electronic sleep diary (…).~~Venous blood for transcriptome analysis will be collected.~~ (…) ~~Venous blood for transcriptome analysis will be collected after awakening as well as before and after CPT.~~ The following procedures will be performed: (…) • Completion of the following questionnaires ~~at Day -2~~: ○ a) on paper: BAI, BDI-II, WEIMuS, PSQI, PSS-10, SF-36v2, SSQ-25 ○ b) on ***AMS***-ePRO®: ESS, ISI, PASA (section 9.1.14) • ~~Collection of 2.5 ml venous blood for transcriptome analysis before sleep at Day -2 and at awakening (Day -1) (section 9.1.8)~~ (…) • ~~Collection of 2.5 ml venous blood for transcriptome analysis before and 50 minutes after CPT at Day -1 (section 9.1.8)~~	Extended time frame to complete the patient diary at Baseline 1. See the justification above for decision to omit WBT for this trial. Extended time frame to complete the patient questionnaires at Baseline 1, either at Day -1 or Day -2. See the justification above for decision to omit WBT for this trial
		8.1 Visit Schedule Visit1	(…)~~Before sleep, venous blood for transcriptome analysis will be collected.~~ (…) ~~Venous blood for transcriptome analysis will be collected after awakening and before and after CPT.~~ The following procedures will be performed: (…) • ~~Collection of 2.5 ml venous blood for transcriptome analysis, before sleep at Day 12 and at awakening at Day 13 (section 9.1.8)~~ • ~~Collection of 2.5 ml venous blood for transcriptome analysis before and 50 minutes after CPT at Day 13 (section 9)~~	See the justification above for decision to omit WBT for this trial

			• Completion of the following questionnaires ~~at Day 12~~: ESS, ISI, PASA (***AMS***-ePRO®); BDI-II and WEIMuS (both paper-based; section 9.1.14)	Extended time frame to complete the patient questionnaires at Visit 1.
		8.1 Visit Schedule Visit 2	The following procedures will be performed: - (…) Last IMP intake in the night of Day 13 prior to sleep	Addition to specify when exactly the last IMP dose is to be taken.
		9.1.3 Electroencephalography (EEG)	In addition to EEG recordings during PSG nights, patients will undergo continuous EEG measurements at Baseline 1 (Day -1) and Visit 1 (Day 13). These include a resting-state EEG before the CPT, an EEG recording during the CPT, and a resting-state EEG after the CPT. ~~Besides EEG during PSG nights patients will have 4 additional EEG measurements during this trial.~~ ~~Two EEGs (rsEEG to measure the EEG vigilance fluctuations, EEG asymmetry index and spectral characteristics of EEG during resting state at Baseline 1 and at Visit 1 and two EEGs during stress induction by CPT at Baseline 1 and Visit 1.~~ (…) Regional changes in spectral characteristics of EEG using relative power indices in delta-theta-alpha-beta~~-gamma~~ frequency ranges are assessed.	Insertions to specify EEG data. Correction
		9.1.7 Cold Pressor Test (CPT)	~~Before and after CPT a 2.5 ml venous blood sample will be collected for transcriptome analysis.~~	See the justification above for decision to omit WBT for this trial
		~~9.1.9 Venous Blood for Whole Blood Transcriptomics~~	~~With whole blood transcriptomics a more detailed picture of the physiological condition of the patients can be obtained. Venous blood for transcriptome analysis will be collected in PAXgene® Blood RNA Tubes (Qiagen/ PreAnalytiX, purchased from~~	See the justification above for decision to omit WBT for this trial

			~~Becton Dickinson, Heidelberg, Germany, ref. no. 762165) using a BD Vacutainer Safety-Lok blood collection set (Becton Dickinson, ref. no. 367282) before and after each adaptation PSG night (4 samples of 2.5 ml/ sample). Additionally, 2 venous blood samples for transcriptome analysis (2.5 ml/ sample) will be collected in PAXgene® Blood RNA Tubes using BD Vacutainer before and 50 minutes after CPT at Day -1 and Day 13 (4 samples of 2.5 ml/ sample). Blood tubes will be gently inverted 8 to 10 times, stored upright for 2 hours at room temperature (18-25°C) and then transferred to -20°C for minimum 24 hours and maximum 72 hours followed by interim storage at -80°C (+/- 10°C fluctuation) in the biobank of the local laboratory of the investigational site (Institut für Klinische Chemie und Laboratoriumsdiagnostik, IKCL, Jena University Hospital). Analysis will be performed by the German Center for Neurodegenerative Diseases (DZNE), Göttingen.~~	
		9.1.1~~5~~4 Patient Question-naires	Patient-Reported Outcomes(…), at Baseline 1 (either in the evening prior to Baseline 1 PSG night or next morning following Baseline 1 PSG night) and either at Visit 1 (either in the evening prior to Visit 1 PSG night or next morning following Visit 1 PSG night), or at Follow-up Visit.	Insertions to specify completion timepoints
		9.1.1~~5~~4.1 Pittsburgh Sleep Quality Index (PSQI)	The PSQI is well suited for measuring change in insomnia and will be completed on paper at Baseline 1 and at Follow-up Visit ~~(*AMS*-ePRO®).~~	Correction
		9.1.1~~5~~4.6 Subclinical Stress Symp-tom Question-naire-25 (SSQ-25)	This questionnaire consists of ~~1~~25 questions (…) .	Correction
		9.1.1~~5~~4.8 Perceived	The English version of the PSS-10 comprises 10 items that are answered on a five-	Correction of used scale.

		Stress Scale-10 (PSS-10)	point rating scale ranging from 0~~1~~ = 'never' to 4~~5~~ = 'very often' and will be completed on paper at Baseline 1 and at Follow-up Visit. A German translation of the English PSS-10 version is used.	
		10.3 Recording of AEs	Information dated after the end of the trial for the patient concerned will not be part of the data collection in the eCRF.	Insertion to specify the follow-up of AEs after the End of Trial documentation
		11.3 Statistical Planning	**Subgroup Analyses** The following subgroups will be defined: • (…)~~Sex (female/ male) differences based on whole blood transcriptomics~~ **Descriptive Analyses** For continuous variables the number of observations (n), arithmetic mean, standard deviation (SD), minimum, median, 25%-, 75%-quantile, maximum, 95% confidence interval (CI) (2-sided), and the number of missing data will be presented.	See the justification above for decision to omit WBT for this trial. Insertion of 25%- and 75%-quantile
		11.4.2 Secondary Efficacy	Secondary efficacy variables are the following: (…) ~~*Whole blood transcriptomics*~~ Correlations between • (…)~~Changes in sleep parameters, stress parameters, and daytime performance with whole blood transcriptomics~~	See the justification above for decision to omit WBT for this trial
		12.2 Competent Authorities	Irrespective of the outcome of the clinical trial, the trial results are to be reported within one year from the end of the clinical trial to regulatory authorities and published in international databases. A summary of the results understandable to laypersons will be made available in the EU database irrespective of the outcome of the trial.	Addition to align with the CTR requirements

		12.3 Ethical Conduct of the Trial	The trial ~~will be~~ was originally submitted under the EU Clinical Trials Directive (EC) No. 2001/ 20/EC and transitioned in 2024 to the EU Clinical Trials Regulation (EU CTR No. 536/2014).	Added information on trial transition to CTR
		12.8 Serious Breaches	A serious breach is any deviation of the approved protocol version or the Clinical Trials Regulation that is likely to affect the safety, rights of trial participants and/or data reliability and robustness to a significant degree in a clinical trial. Serious breaches require immediate attention of the Investigator. Any suspected serious breaches must be reported to the Sponsor within 24 hours after becoming aware of it using the following contact details: Emails: C2104@heel.com, drugsafety@heel.com Phone +49 160 88 29 373	Addition of section regarding 'serious breaches' to align with the CTR requirements
		13.4.1 Investigator Site File (ISF)	(…) The Investigator records in the Patient Identification Log the following details for all persons giving their consent to participate in the trial: name (first and surname), date of birth~~, sex, postal address~~ and the assigned unique 7-digit trial patient number. The ~~date of enrollment~~ (~~Screening Visit~~) date ~~into the trial~~ must also be documented.	Correction Substituted "date of enrollment" by "Screening Visit date" in order to be more accurate
		Whole document	Minor corrections, typos and commas	Minor corrections, typos and commas

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1. List of Abbreviations

Abbreviation	Term
ADR	Adverse Drug Reaction
AE	Adverse Event
ALAT (ALT)	Alanine Transaminase (formerly GPT)
AMS	Advanced Medical Services (contract research organization)
ANCOVA	Analysis of Covariance (a collection of statistical models used to analyze the differences among means)
ANS	Autonomic Nervous System
AR	Adverse Reaction
ArI	Arousal Index
ASAT	Aspartate Aminotransferase (also GOT)
BAI	Beck's Anxiety Inventory (paper PRO)
BDI-II	Beck's Depression Inventory revision II (paper PRO)
BDRM	Blind Data Review Meeting
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte
BMI	Body Mass Index
CAR	Cortisol Awakening Response
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CI	Confidence Interval
CLIA	ChemiLuminescence Immune Assay
CONSORT	CONsolidated Standards Of Reporting Trials guideline
CPT	Cold Pressor Test
CRO	Contract Research Organization
CRP	C-Reactive Protein
CTFG	(European Union) Clinical Trials Facilitation and Coordination Group
CTR	Clinical Trials Regulation (EU CTR No. 536/2014)
DGSM	Deutsche Gesellschaft für Schlafforschung und Schlafmedizin
DHEA-S	Dehydroepiandrosterone Sulfate
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th edition
DSUR	Development Safety Update Report
eCRF	Electronic Case Report Form
EDA	Electrodermal Activity
EDC	Electronic Data Capture
EEG	Electroencephalogram
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	Ecological Momentary Assessment (used for daily stress assessment via ***AMS***-ePRO®)
ePRO	Electronic Patient-Reported Outcome
ESR	Erythrocyte Sedimentation Rate
ESS	Epworth Sleepiness Scale (***AMS***-ePRO®)
EoT	End of Trial
FDA	Food and Drug Administration
fMRI	Functional Magnetic Resonance Imaging
FPFV	First Patient First Visit
Gamma GT (GGT)	Gamma-Glutamyl Transferase
GAMP	Good Automated Manufacturing Practice, a risk-based approach to compliant GxP computerized systems
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
HPA axis	Hypothalamic-Pituitary-Adrenal axis
HR	Heart Rate
HRV	Heart Rate Variability
ICF	Informed Consent Form
ICH GCP	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use – Guideline on Good Clinical Practice
ICMJE	International Committee of Medical Journal Editors

Abbreviation	Term
ID	Identification
IEC	Independent Ethics Committee
IKCL	Institut für Klinische Chemie und Laboratoriumsdiagnostik, University Hospital Jena (local routine laboratory performing safety laboratory and blood biomarker analysis)
IMP	Investigational Medicinal Product
ISF	Investigator Site File
ISI	Insomnia Severity Index (***AMS***-ePRO®)
ITT	Intention-To-Treat (analysis population)
LF/HF	Low Frequency / High Frequency Power
LOCF	Last Observation Carried Forward
LPLV	Last Patient Last Visit
MedDRA	Medical Dictionary for Regulatory Activities
mmHg	Millimeters of mercury (unit of blood pressure)
MoCA	Montreal Cognitive Assessment
MSLT	Multiple Sleep Latency Test
NWAK	Number of Awakenings (by PSG and sleep diary)
OSA	Obstructive Sleep Apnea
PASA	Primary Appraisal Secondary Appraisal (***AMS***-ePRO®)
PK	Pharmacokinetics
PLMD	Periodic Limb Movement Disorder
PLMI	Periodic Limb Movement Index
PP	Per-Protocol
PPP	Per-Protocol Population
PRO	Patient-Reported Outcome (e.g., patient questionnaire and sleep diary)
PSG	Polysomnography
PSQI	Pittsburgh Sleep Quality Index (paper PRO)
PSS-10	Perceived Stress Scale-10 (paper PRO)
QoL	Quality of Life
QPPV	Qualified Person for Pharmacovigilance
RCT	Randomized Controlled Trial
REM	Rapid Eye Movement sleep
RLS	Restless Legs Syndrome
RMSSD	Root Mean Square of Successive Differences
rsEEG	Resting state electroencephalogram
S1, S2, S3	Sleeping states
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SADR	Serious Adverse Drug Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus-2
SBQ	STOPBang questionnaire
SCID-5-CV	Structured Clinical Interview for DSM-5® Disorders – Clinician Version
SD	Standard Deviation
SDV	Source Data Verification
SE	Sleep Efficiency
SF-36v2	Short Form-36 Health Survey version 2.0 (QoL questionnaire, paper PRO)
SI	Stress Index (Baevsky's SI)
SOL	Sleep Onset Latency (by PSG and sleep diary)
SOP	Standard Operating Procedure
SSL	Secure Sockets Layer
SSQ-25	Subclinical Stress symptom Questionnaire-25 (paper PRO)
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAP	Tests of Attentional Performance
TEAE	Treatment-Emergent Adverse Event
TIB	Time In Bed
TSST	Trier Social Stress Test
TST	Total Sleep Time (by PSG and sleep diary)

Abbreviation	Term
UAR	Unexpected Adverse Reaction
VIGALL	Vigilance Algorithm Leipzig software to objectively classify vigilance in resting EEG recordings, latest version 2.1
WASO	Wake After Sleep Onset (by PSG and sleep diary)
WHO	World Health Organization
WEIMuS	Würzburg Fatigue Inventory (paper PRO)

2. Overview

2.1 Protocol Synopsis

Sponsor: Biologische Heilmittel Heel GmbH	**Name of Medical Product:** Neurexan® tablets	**Active Ingredient(s):** Homeopathic preparation, containing herbal and mineral ingredients as described in the Clinical Trial Protocol
EudraCT number: 2022-003565-38		**Indication:** Short-Term Insomnia

Title:

Evaluation of the Effects of Neurexan® on Short-Term Insomnia, Daytime Performance and Stress Response by Polysomnography (PSG), Electroencephalogram (EEG), Stress Biomarkers and Patient-Reported Outcomes (PROs)
An Exploratory, Placebo-Controlled Trial in Short-Term Insomnia Patients

Investigational Sites:

Coordinating/ Principal Investigator: Prof Dr. Martin Walter
Chair of Department of Psychiatry and Psychotherapy
Jena University Hospital
Philosophenweg 3
07743 Jena, Germany
Phone: [redacted]

Trial Duration:		**Trial Phase:**
First Patient First Visit:	June 2023	**II**
Last Patient Last Visit planned:	January 2026	

Trial Objectives and Endpoints

The objective of this clinical trial is to demonstrate the effects of daily treatment with Neurexan® on improvements of objective and subjective measures of sleep quality, daytime performance and stress responsiveness in patients with short-term insomnia according to Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5).

The primary objective is to demonstrate an improvement of sleep efficiency (SE) measured by polysomnography (PSG) on daily treatment with Neurexan® in short-term insomnia.

Primary endpoint of this trial is:

Sleep Parameter

To assess Neurexan® related changes in sleep efficiency (SE) at Visit 2 compared to Baseline 2 as revealed by polysomnography.

The secondary endpoints are to assess:

Sleep Pattern

- <u>Further objective sleep parameters revealed by polysomnography (PSG)</u>
  To be assessed as change at Visit 2 compared to Baseline 2
  - Sleep Onset Latency (SOL): changes in SOL compared to Baseline 2
  - Wake After Sleep Onset (WASO): changes in WASO compared to Baseline 2
  - Total Sleep Time (TST): changes in TST compared to Baseline 2
  - Number of awakenings (NWAK): changes in NWAK compared to Baseline 2
  - Number of arousals during sleep (Arousal Index, ArI): changes in Arousal Index compared to Baseline 2
  - Latencies to sleeping stages S1, S2, S3, and Rapid Eye Movement (REM): changes in these latencies compared to Baseline 2.

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- Objective in-home assessment of sleep pattern revealed by actigraphy (24/7)
    - A portable device, i.e., an actigraphy watch will be used to continuously monitor movement information over the whole clinical trial duration for actimetric sleep–wake detection to investigate habitual sleep–wake patterns and daily activity.

- Subjective sleep pattern assessed using sleep questionnaires and sleep diary (adapted from Deutsche Gesellschaft für Schlafforschung und Schlafmedizin, DGSM)
    - Insomnia Severity Index (ISI): changes in ISI at Visit 1 compared to Baseline 1
    - Pittsburgh Sleep Quality Index (PSQI): changes in PSQI at Follow-Up Visit compared to Baseline 1
    - Sleep diary parameters: changes in subjective SE, SOL, WASO, TST, and NWAK compared to baseline.

Daytime Performance by

- Resting state EEG:
    - Changes in time course of EEG vigilance fluctuations during wakefulness assessed by Vigilance Algorithm Leipzig (VIGALL) version 2.1 (validated with the Multiple Sleep Latency Test (MSLT) by (Olbrich, Pawlowski et al. 2015) at Visit 1 compared to Baseline 1
    - Changes in EEG asymmetry index at Visit 1 compared to Baseline 1
    - Regional changes in spectral characteristics of EEG using relative power indices in delta-theta-alpha-beta frequency ranges at Visit 1 compared to Baseline 1
- Resting state heart rate (HR), heart rate variability (HRV) and electrodermal activity (EDA) at Visit 1 compared to Baseline 1
- Questionnaires: Epworth Sleepiness Scale (ESS), Würzburg Fatigue Inventory (WEIMuS) and Beck's Depression Inventory-II (BDI-II) at Visit 1 compared to Baseline 1, and Quality of Life (QoL) by QoL questionnaire Short Form-36 (SF-36v2) and Beck's Anxiety Inventory (BAI) at Follow-Up Visit compared to Baseline 1
- Neuropsychological tests assessing general cognitive functioning, alertness, vigilance, and sustained attention: three subtests from electronic Tests of Attentional Performance (TAP) test battery at Visit 1 compared to Baseline 1

Stress Processing

Given the reported Hypothalamic-pituitary-adrenal (HPA) axis dysregulation in the form of an altered cortisol secretion pattern in insomnia patients, a secondary outcome is to demonstrate changes in decreased resting state stress parameters in saliva as well as stress reactivity to physiological challenge using the Cold Pressor Test (CPT) in terms of changes in serum levels of cortisol in short-term insomnia patients treated with Neurexan®.

- Resting state stress response by
    - Changes in stress biomarkers in saliva compared to baseline
        - Cortisol
        - Alpha-amylase
        - Dehydroepiandrosterone sulfate (DHEA-S)
        - Time course of stress biomarkers release synchronized to the awakening time (5 time points after awakening: 0 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes for the assessment of Cortisol Awakening Response (CAR))
        - Diurnal profile of stress biomarkers by collecting saliva samples at 3-hourly intervals over 12 hours (4 time points) synchronized to the awakening time.

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- Stress response induced by CPT
  - Changes in subjective pain rating and pain tolerance at Visit 1 compared to Baseline 1.

  Blood sampling:
  - Changes in the cortisol time course: Visit 1 before CPT, 20 minutes, and 50 minutes after CPT compared to Baseline 1
  - Changes in dehydroepiandrosterone sulfate (DHEA-S) time course: Visit 1 before CPT, 20 minutes, and 50 minutes after CPT compared to Baseline 1
  - Changes in the time course of adrenalin, noradrenalin and the inflammatory response (IL-6 and TNF-α): Visit 1 before CPT, 20 minutes, and 50 minutes after CPT compared to Baseline 1.

  Autonomic stress response:
  - Changes in heart rate and associated HRV measures, electrodermal activity (EDA): Visit 1 compared to Baseline 1.

  EEG stress response:
  - Changes in time course of EEG vigilance fluctuations during wakefulness assessed by VIGALL version 2.1 (validated with the Multiple Sleep Latency Test (MSLT) by (Olbrich, Pawlowski et al. 2015) at Visit 1 compared to Baseline 1
  - Changes in EEG asymmetry index at Visit 1 compared to Baseline 1
  - Regional changes in spectral characteristics of EEG using relative power in delta-theta-alpha-beta frequency ranges at Visit 1 compared to Baseline 1.

- Stress questionnaires

Changes in subjective stress levels compared to Baseline 1 as assessed by
- Subclinical Stress Symptom Questionnaire-25 (SSQ-25) at Follow-Up Visit
- Primary Appraisal Secondary Appraisal (PASA) at Visit 1
- Perceived Stress Scale-10 (PSS-10) at Follow-Up Visit
- Continuous (over the whole trial period) daily stress assessment using AMS-ePRO® functionality and EMAs.

Further secondary objectives are to assess correlations between:
- Changes in sleep parameters, stress parameters, and daytime performance
- Changes in sleep parameters, stress parameters, and inflammatory parameters.

Methodology, Trial Design

Randomized, double-blind, placebo-controlled exploratory monocentric clinical trial

Number of Patients (Planned):

Estimation of sample size is based on the demonstration of improved sleep efficiency (SE) in short-term insomnia patients treated with Neurexan® as assessed by polysomnography. The standard deviation (SD) of SE is estimated at 10%. With α= 5%, two-sided and an expected mean difference of 6.7% between patients treated with Neurexan® and Placebo, a power of 80% is achieved with 72 patients. Expecting a dropout rate of 10%, the total number of patients needed is 80.

Patients will be randomly assigned to treatment group Neurexan® or Placebo with a 1:1 allocation and stratification by sex.

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Diagnosis and Inclusion Criteria:

The patients have to meet all of the following inclusion criteria at Screening Visit:

1. Insomnia definition according to DSM-5 criteria; episode duration less than 3 months
2. Short-term insomnia with moderate symptoms according to ISI of at least 8 and below 22 being present for at least one week, but no longer than 3 months prior to Screening Visit
3. Reports habitual bedtime, defined as the time the participant attempts to sleep, between 21:00 and 01:00
4. Reports regular time spent in bed, either sleeping or trying to sleep, between 6 and 9 hours
5. ≥18 years of age, not older than 65 years
6. Legally competent male or female patient
7. Signed Informed Consent
8. Females of childbearing potential must agree to maintain highly effective or acceptable birth control throughout the trial (CTFG 2020).

   Highly effective (failure rate of less than 1% per year)
   - **Combined** (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal
   - **Progestogen-only** hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable
   - **Intrauterine** device
   - **Intrauterine** hormone-releasing system
   - Bilateral **tubal** occlusion
   - **Vasectomized** partner (provided partner is sole sexual partner and if vasectomized partner has received medical assessment of the surgical success)
   - Sexual **abstinence** (only if defined as refraining from heterosexual intercourse during the entire period of risk associated with investigational treatment)

   Acceptable birth control methods which may not be considered as highly effective (failure rate of more than 1% per year)
   - **Progestogen-only** oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
   - Male or female **condom** with or without spermicide
   - **Cap, diaphragm** or **sponge** with spermicide
   - **Combination** of male condom with either cap, diaphragm or sponge with spermicide (**double barrier** methods)
9. Body Mass Index (BMI) between 18.5 and 29.9 kg/m^2 at Screening Visit
10. Use of digital device e.g., smartphone, tablet or laptop
11. German speaking and reading.

Exclusion Criteria:

Potential trial patients will be excluded if at least one of the following exclusion criteria is present:

1. Patients with insomnia symptoms present longer than 90 days prior to Screening Visit
2. Based on the diagnostic interview, reported history (within 2 years) of other sleep disorders (e.g., chronic insomnia, circadian rhythm sleep disorders, restless legs syndrome (RLS), obstructive sleep apnea (OSA)), i.e., STOPBang questionnaire (SBQ) score ≥5, International Restless Legs Scale score ≥16)

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3. Based on the first polysomnographic screening night at Baseline 1, insomnia due to sleep apnea or periodic limb movement disorder (PLMD): OSA (Apnea Hypopnea Index of >5 events/ hour), PLMD (Periodic Limb Movement Index (PLMI) >15 events/ hour)
4. Rotating shift work with overnight shifts
5. History of psychiatric disorders within the last 6 months prior to Screening Visit according to the Structured Clinical Interview for DSM-5® Disorders – Clinician Version (SCID-5-CV)
6. History of sensitivity to any component of Neurexan®
7. Unwilling or unable to comply with all the requirements of the clinical trial protocol
8. Cognitive impairment (cut-off of 24 points in the Montreal Cognitive Assessment [MoCA]; Thomann, Berres et al. 2020) at Screening Visit
9. Any history of or current abuse of alcohol and/or amphetamines, benzodiazepines, cocaine, marijuana, methaqualone, methadone, opioids, propoxyphene, barbiturates, phencyclidine; or expected to take during trial participation (urine drug screening at Screening Visit and adaptation nights)
10. Current use of medication affecting sleep, i.e., antidepressants, antipsychotics, diuretics, blood pressure drugs, anti-dementia drugs (e.g., piracetam), herbal and homeopathic medicine, hormone preparations (e.g., thyroxine) with the exception of hormonal contraceptives
11. Use of Neurexan® within the last two weeks from Screening Visit
12. Non-pharmacological insomnia therapies (e.g., cognitive behavioral therapy within the last 6 months of Screening Visit, sleep restriction therapy, complementary and alternative therapies as meditation, Traditional Chinese Medicine, aromatherapy)
13. Excessive consumption of xanthine-containing beverages (more than 7 cups daily of coffee or tea or other beverages containing xanthines)
14. Use of nicotine during the last 6 months prior to Screening Visit
15. Participation in any interventional clinical study within the past 30 days prior to Screening Visit
16. Any relationship of dependence with the Sponsor or with the Investigator
17. Active infection/ disease (C-reactive protein [CRP] >5 mg/l)
18. Hypertension defined as systolic blood pressure ≥140 mmHg (Burnier, 2018)
19. History of neurological, rheumatic, chronic pain, immune, cardiovascular, pulmonary, liver/ kidney, or metabolic disorder within the last 6 months prior to Screening Visit
20. Nocturia
21. Pregnancy (as proven by positive urine pregnancy test at Screening Visit) or breastfeeding
22. Patients with moderate to severe skin allergies and/or eczema
23. Raynaud's disease
24. Donation of blood or platelets 3 months prior to or in-between in-hospital visits.

Patient screening will include blood tests for complete blood count (CBC) and differentials, as well as thyroid hormone and blood glucose; urinary toxicology screens will also be conducted (exclusion criterion number 9). The medical history and physical examination will be conducted by a trial physician.

Test Product, Dose, Duration of Treatment and Mode of Administration:

Neurexan®: *Passiflora incarnata* dilution (Dil.) D2 (0.6 mg/ tablet), *Avena sativa* Dil. D2 (0.6 mg/ tablet), *Coffea arabica* Dil. D12 (0.6 mg/ tablet), *Zincum isovalerianicum* Dil. D4 (0.6 mg/ tablet), lactose monohydrate, magnesium stearate.
2 tablets sublingually 3 times daily for 14 days.

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Placebo Therapy, Dose, and Mode of Administration:

Placebo: Lactose monohydrate, magnesium stearate.
2 tablets sublingually 3 times daily for 14 days.

Rescue Medication, Dose and Mode of Administration:

None

Supportive Therapy:

None

Criteria for Evaluation:

Analysis Populations:

The screening population is defined as all patients who signed informed consent.

The safety population is defined as all patients who were randomized and received at least one dose of investigational medication. All safety analyses will be based on the treatment actually taken by the patient ('as treated').

The Intention-To-Treat (ITT) population is defined as patients who were randomized and received at least one dose of investigational medication and had at least one post-dose assessment.

The Per-Protocol Population (PPP) is defined as all patients who received investigational treatment and fulfil the following criteria:

- The absence of any important major protocol deviations
- No technical difficulties during PSG
- Patients who were correctly stratified and received the correct treatment
- Sufficient treatment compliance of 80%-120%.

Primary Efficacy Variable

- The primary efficacy variable for changes in sleep pattern will be sleep efficiency (SE) measured by polysomnography

Secondary Efficacy Variables

Polysomnography

- Sleep Onset Latency (SOL)
- Wake After Sleep Onset (WASO)
- Total Sleep Time (TST)
- Number of Awakenings (NWAK)
- Latencies to sleeping states 1, 2, 3 (S1, S2, S3), Rapid Eye Movement (REM) sleep
- Arousal Index (ArI).

Biochemical parameters

- Resting state cortisol and other stress-related parameters in saliva
- Serum cortisol levels and other stress-related parameters in response to the stress induction by Cold Pressor Test (CPT).
- Stress test induced inflammation parameters in blood serum.

Actigraphy

- Continuous in-home assessment of sleep
- Daytime activity.

Patient Questionnaires

- Subclinical Stress Symptom Questionnaire-25 (SSQ-25)
- Primary Appraisal Secondary Appraisal (PASA)
- Perceived Stress Scale-10 (PSS-10)

Sponsor: Biologische Heilmittel Heel GmbH	**Name of Medical Product:** Neurexan® tablets	**Active Ingredient(s):** Homeopathic preparation, containing herbal and mineral ingredients as described in the Clinical Trial Protocol
EudraCT number: 2022-003565-38		**Indication:** Short-Term Insomnia

- Epworth Sleepiness Scale (ESS)
- Würzburg Fatigue Inventory (WEIMuS)
- Short Form-36 (SF-36v2)
- Insomnia Severity Index (ISI)
- Pittsburgh Sleep Quality Index (PSQI)
- Beck's Depression Inventory-II (BDI-II)
- Beck's Anxiety Inventory (BAI).

Sleep diary data (adapted from DGSM): subjective SE, SOL, WASO, TST, NWAK

*Continuous digitally recorded stress assessments (**AMS**-ePRO®)*

Neurexan® response

Exploratory Efficacy Variables

- EEG based vigilance fluctuations during resting state
- EEG based vigilance fluctuations during CPT
- EEG based asymmetry index during resting state
- EEG based asymmetry index during CPT
- Regional changes in spectral characteristics of EEG during resting state
- Regional changes in spectral characteristics of EEG during CPT
- Cognitive performance in three attentional tasks
- PSG sleep parameters derived from the two adaptation nights at Baseline 1 and Visit 1

Safety Variables

- Adverse Events (AEs)
- Treatment-Emergent Adverse Events (TEAEs)
- Other observations related to safety (physical examinations, vital signs, laboratory assessments).

Subgroup Analyses

- Age
- Sex (female/ male)
- Neurexan® responder vs. non-responder
- Duration of short-term insomnia (≤ 1 month, > 1 month)
- Cortisol response influenced by hormonal contraceptives

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Statistical Methods:

The primary endpoint of this trial is sleep efficiency measured by polysomnography in patients treated with Neurexan®. Analysis of Covariance (ANCOVA) will be used to test the hypothesis H0: $\mu_{Neurexan} - \mu_{Placebo} = 0$ vs. H1: $\mu_{Neurexan} - \mu_{Placebo} \neq 0$. The model will include treatment and the stratification factor sex and will account for sleep efficiency at Baseline 2 as covariate. The test will be performed using a Type I error level of 5%, two-sided.

Analyses of secondary objectives on efficacy will include ANCOVA and Fisher's exact test, as appropriate. These analyses will be exploratory in nature without adjustment for multiplicity.

Correlations may be analyzed using Spearman's rank correlation coefficient or another appropriate method.

Efficacy analyses will be performed based on the ITT population. The PPP will be used additionally to explore the robustness of the findings. Further on, robustness analyses will include the Wilcoxon rank-sum test. Difference from baseline will be used, as appropriate.

Clinical safety will be addressed by assessing AEs, TEAEs, physical examinations, vital signs and as needed laboratory assessments in a descriptive manner. Safety analyses will be performed with the safety population.

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Statistics:	*AMS* Advanced Medical Services GmbH Am Exerzierplatz 2 68167 Mannheim/ Germany

2.2 Schedule of Trial Procedures

	Run-In (9 to 29 days)			Treatment (14 days)		Follow-Up (14 days)
Procedure	**Screening Visit** Day -28 to -8	**Baseline 1** Day -2 to -1	**Baseline 2** Day -1 to 0	**Visit 1** Day 12 to 13 +2 days^	**Visit 2** Day 13 to 14 +2 days^	**Follow-Up Visit** Day 28 + 3 days^^
Diagnosis according to DSM-5 (short-term insomnia)	X					
Inclusion/ Exclusion Criteria (except exclusion criterion 3)	X					
Exclusion Criterion 3		X				
Patient Information/ Informed Consent	X					
Demographics°	X					
Randomization			X			
Vital Signs (Heart Rate, Systolic/ Diastolic Blood Pressure)	X					
Physical Examination	X					
Safety Laboratory (CBC with Differential, Thyroid Hormones, Blood Glucose, CRP, ESR, Liver Enzymes)	X					
Urine Pregnancy Test	X					
Medical History and Concomitant Diseases	X					
Urine Drug Test	X	X	X	X	X	
Breath Alcohol Test	X	X	X	X	X	
Adverse Events (AEs)	X			X		
Concomitant Medications	X			X		
Investigational Treatment (Neurexan®/ Placebo)			X*	X	X	
Return of IMP					X	
Polysomnography (PSG) Parameters		X adaptation ('screening')	X	X adaptation	X	
Pittsburgh Sleep Quality Index (PSQI), Paper PRO		X				X
Sleep Diary°° (adapted from DGSM), ***AMS***-ePRO®	X	X	X	X	X	X
Insomnia Severity Index (ISI), ***AMS***-ePRO®	X	X		X		
Resting State EEG (rsEEG)		X		X		

	Run-In (9 to 29 days)			Treatment (14 days)		Follow-Up (14 days)
Procedure	**Screening Visit** Day -28 to -8	**Baseline 1** Day -2 to -1	**Baseline 2** Day -1 to 0	**Visit 1** Day 12 to 13 +2 days^	**Visit 2** Day 13 to 14 +2 days^	**Follow-Up Visit** Day 28 + 3 days^^
Resting Heart Rate (HR), Heart Rate Variability (HRV), and Electrodermal Activity (EDA)		X		X		
Daytime Performance Questionnaires, Level of Anxiety and Depression Paper PROs: BAI, BDI-II, WEIMuS, SF-36v2 ***AMS***-ePRO®: ESS		X		X BDI-II, ESS, WEIMuS		X BAI, SF-36v2
Actigraphy	X	X	X	X	X	X
Stress Questionnaires Paper PROs: PSS-10, SSQ-25 ***AMS***-ePRO®: PASA		X		X PASA		X PSS-10, SSQ-25
AMS-ePRO® Questionnaires to Measure Daily Stress Levels (EMAs)	X	X	X	X	X	X
Resting State Stress Response based on Saliva Biomarkers after Awakening and During the Day[#]		X	X	X	X	
Stress Responses induced by Cold Pressor Test (CPT)[1] as assessed with EEG, HR, HRV and EDA		X		X		
Neuropsychological Tests (TAP)		X		X		
End of Trial (EoT) Documentation[2]						X

Abbreviations: AE - Adverse Event, BAI - Beck's Anxiety Inventory (paper PRO), BDI-II - Beck's Depression Inventory revision II (paper PRO), CBC - Complete Blood Count, CPT - Cold Pressor Test, CRP - C-reactive protein, DGSM - Deutsche Gesellschaft für Schlafforschung und Schlafmedizin, DHEA-S - dehydroepiandrosterone sulfate, DSM-5 - Diagnostic and Statistical Manual of Mental Disorders, 5th edition, EDA - Electrodermal Activity, EEG – electro-encephalogram, EMAs - Ecological Momentary Assessments (used for daily stress assessment via ***AMS***-ePRO®), EoT - End of Trial, (e)PRO - (electronic) Patient-Reported Outcome, ESR - Erythrocyte Sedimentation Rate, ESS - Epworth Sleepiness Scale (***AMS***-ePRO®), HR - Heart Rate, HRV - Heart Rate Variability, IMP – Investigational Medicinal Product, ISI - Insomnia Severity Index (***AMS***-ePRO®), min - minutes, ml - milliliters, PASA - Primary Appraisal Secondary Appraisal (***AMS***-ePRO®), PRO - Patient- Reported Outcome, PSG - polysomnography; PSQI - Pittsburgh Sleep Quality Index (paper PRO), PSS-10 - Perceived Stress Scale-10 (paper PRO), rsEEG - resting state electroencephalography, SF-36v2 - Short Form-36 version 2.0 (paper PRO), SSQ-25 - Subclinical Stress Symptom Questionnaire-25 (paper PRO), TAP – Test of Attentional Performance, WEIMuS - Würzburg Fatigue Inventory (paper PRO).

^Visit 1 may be performed on Day 12 +2 days. In any case, Visit 1 and Visit 2 will be performed on two consecutive days.

^^Follow-up Visit may be performed on Day 28 +3 days.

°Demographics include sex, height and weight.

*Starting IMP treatment after having performed all Baseline 2 assessments and after randomization at midday Day 0 for 14 consecutive days (+ maximum 2 days allowance) until Visit 2 (Day 13).

°°Sleep diary will be completed daily; starting at Screening Visit until End of Trial

#Stress biomarkers in saliva: cortisol, alpha-amylase, dehydroepiandrosterone sulfate (DHEA-S) at 5 time points: after awakening from PSG nights at Baseline 1/ Day -1, Baseline 2/ Day 0, Visit 1/ Day 13 and Visit 2/ Day 14 collection of around 0.5 to 1 ml saliva at 0 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes (5 time points) for the assessment of Cortisol Awakening Response (CAR) and additionally at Baseline 1/ Day -1 and Visit 1/ Day 13 diurnal profile of stress biomarkers by collecting saliva samples at 3-hourly intervals over 12 hours (4 time points) synchronized to the awakening time.

1Including subjective pain rating and pain tolerance, blood sampling before CPT, 20 minutes and 50 minutes after CPT to assess biomarkers cortisol and DHEA-S, adrenalin, noradrenalin and inflammatory response (IL-6 and TNF-α), autonomic and cortical stress response (heart rate, HRV, electrodermal activity (EDA) and EEG).

2In case of premature discontinuation reason to be given.

3. Introduction

3.1 Background and Rationale

Healthy sleep is essential for a good daytime performance and a healthy life. However, the annual incidence of new onset or acute insomnia is almost one-third of the population (Ellis, Perlis et al. 2012, Perlis, Vargas et al. 2020). Short-term or acute insomnia is the most frequent type of insomnia and is defined as the subjective perception of difficulty with sleep initiation, duration, consolidation, or quality that occurs despite adequate opportunity for sleep, for at least 3 days per week and is present for less than 3 months. These sleep disturbances cause clinically significant distress or impairment in daytime functioning.

The diagnosis of insomnia is made based on the diagnostic criteria as defined in the recent version of the Diagnostic Statistical Manual of Mental Disorders, version 5 (DSM-5). Furthermore, based on the DSM-5 criteria it is essential to rule out other causes of insomnia, such as medical or mental disorders or the use of medication or stimulating substances. Patient-reported questionnaires such as the Insomnia Severity Index (ISI) and the Pittsburgh Sleep Quality Index (PSQI) are tools to aid in the diagnosis of insomnia. The gold standard to assess sleep disorders is polysomnography (PSG). In addition, actigraphy with wearable devices can deliver further useful information to monitor habitual sleep–wake patterns in the in-home setting.

The concept of hyperarousal as an important etiological factor in insomnia is used to explain the relationship between sleep disturbances and stress dysregulation.

Several studies using physiological markers of stress and arousal such as hypothalamic-pituitary-adrenal (HPA) axis provided support for the assumed relationship between stress and sleep. Thus, the summary of present literature indicates a dynamic and bidirectional relationship between stress and sleep. Acute insomnia is often triggered by stressful life events (such as major life events or daily hassles), and physiologically and cognitive-emotionally induced hyperarousal can interfere with sleep and lead to a chronicity of insomnia in a subset of the population.

Hyperarousal is described as a state of increased somatic, cortical, and cognitive activation at daytime and/or at night leading to increases in cortisol levels, body temperature, metabolic rate and heart rate (Kay and Buysse 2017, Vargas, Nguyen et al. 2020).

There is some evidence for the notion that patients with insomnia exhibit exaggerated maladaptive neurobiological and cognitive–emotional reactivity to stress. For example, patients with insomnia tend to respond differently to stressful events compared to normal sleepers (Morin, Rodrigue et al. 2003), which may lead to a hyperarousal state and interfere with sleep .

Stress leads to the activation of the hypothalamic-pituitary-adrenal axis (HPA axis) and the autonomic nervous system which are known to cause arousal and sleeplessness (McEwen, Bowles et al. 2015).

Furthermore, there is evidence for the direct relationship between stress and inflammatory response in the periphery and in the brain. Glucocorticoids as released during a stress response have an impact on the expression of several pro-inflammatory cytokines (e.g., interleukin-6 (IL-6)). Strong stressors could over-activate the immune system, leading to the imbalance of inflammation and anti-inflammation. Previous studies have reported associations between disrupted sleep and inflammatory responses. Although the physiological mechanisms underlying these relationships remain unclear, it is conceivable to assume this association is mediated by alteration in the stress processing and response.

Neurexan®, a medicinal product consisting of 3 herbal extracts (*Avena sativa, Coffea arabica, Passiflora incarnata*) and one mineral salt (*Zincum isovalerianicum*) in low ponderable concentrations, showed effects on stress response and sleep efficacy in various studies.

The efficacy of Neurexan® on psychological and neuroendocrine responses under acute stress was investigated in a placebo-controlled double-blind trial in healthy volunteers. A significantly decreased neuroendocrine response to an acute stressor (Trier Social Stress Test, TSST) after Neurexan® intake was observed. Neurexan® led to reduced salivary cortisol and plasma adrenaline levels after stress exposure, but did not affect the subjective stress ratings, heart rate and blood pressure (Doering, Wegner et al. 2016). In another placebo-controlled cross-over trial with mildly or moderately stressed healthy volunteers it could be shown that Neurexan® modulates the stress response. With functional magnetic resonance imaging (fMRI) a significantly reduced neuronal activation in the left amygdala was demonstrated after a single dose of Neurexan® compared to Placebo (Herrmann, Vicheva et al. 2020). In a study using electroencephalogram (EEG) to investigate Neurexan® efficiency on stress-induced changes, it was demonstrated that elevated frontotemporal beta2 power was ameliorated by intake of Neurexan®, indicating that Neurexan® effectively helps to cope with induced stress situations (Dimpfel 2019). Neurexan®'s effectiveness for patients with symptoms of nervousness and restlessness was assessed in an observational study with 777 participants. Compared to patients treated with valerian extracts patients treated with Neurexan® showed significantly decreased summary scores for nervousness/restlessness (Hübner, Von Haselen et al. 2009). Furthermore, in a very recent study, it was demonstrated that Neurexan® reduces plasma cortisol levels in high performance sled dogs after an exercise inducing stress response compared to placebo (Keller, Conradi et al. 2021).

A non-interventional study investigated the effect of Neurexan® in patients with insomnia compared to valerian extracts over a treatment period of 14 days. Sleep duration and latency were evaluated based on sleep diaries. The total duration of sleep increased and sleep latency decreased in both groups. Significantly more patients reported a lack of daytime fatigue with Neurexan® treatment than with valerian treatment (49% vs. 32%; $p < 0.05$) (Waldschütz and Klein 2008).

All above-mentioned previous studies with Neurexan® indicate a measurable effect on the stress response. Furthermore, there is some evidence corroborating an effect of Neurexan® on sleep parameters.

However, there is still a lack of evidence regarding the effect of Neurexan® on objective and subjective sleep parameters and daytime performance in insomnia patients based on randomized controlled trial (RCT) data as well as its relationship with assumed changes in the stress and inflammatory response.

The aim of this double-blind randomized controlled phase II trial is to investigate the effects of Neurexan® on sleep efficiency (SE) measured by polysomnography as well as other sleep related parameters in a specific sample of patients with short-term insomnia according to DSM-5, its impact on daytime performance, stress and inflammatory response as well as relationships between postulated changes in stress and inflammatory response and sleep patterns.

3.2 Investigational Drug

Neurexan® is a medicinal product sold over the counter in Germany that is marketed as tablets for oral use.

Neurexan® is a preparation of 4 diluted biological components. Active ingredients of Neurexan® are *Passiflora incarnata* (*P. incarnata*, purple passionflower) D2 0.6 mg, *Avena sativa* (*A. sativa*, common oats) D2 0.6 mg, Coffea arabica (*C. arabica*, coffee) D12 0.6 mg and *Zincum isovalerianicum* (isovalerate of zinc) D4 0.6 mg.

P. incarnata intake has been positively associated with several health benefits such as in treatment of insomnia, nervous tension and anxiety in healthy individuals and patients (Janda, Wojtkowska et al. 2020). Preparations from *A. sativa* are used as a nervous system restorative for acute and chronic anxiety and tension, stress and excitation as reported by the German Commission E, an independent scientific advisory board of the German Federal Institute for Drugs and Medical Devices. *C. arabica* is used for hedonistic and psychostimulant purpose (Harland 2000, Dórea and da Costa 2005).

Neurexan® has been investigated in post-marketing surveillance studies in patients with various stress associated complaints such as sleep disturbances, jitteriness, listlessness or nocturnal anxiety (Waldschütz and Klein 2008, Hübner, Von Haselen et al. 2009).

In a prospective non-randomized comparison with valerian compounds patients receiving Neurexan® reported a stronger decrease in nervousness than the group receiving valerian (Hübner, Von Haselen et al. 2009). Due to the observational design these results cannot provide conclusive evidence for the efficacy of Neurexan®.

In Table 1, the quantitative composition of the finished product per standard and maximum daily dose is presented. The 2 last columns show the amount of mother tincture per standard daily dose and maximum daily dose in adults. All doses can be regarded as safe in all age groups according to the Advisory Board on Homeopathy of the German Drug Regulatory Authority (Monograph Commission D). The potency of all components corresponds to the recommendations of Commission D. The recommended dose for adults and children from 12 years of age is one tablet to be dissolved in the mouth 3 times daily. In acute disorders, one tablet can be taken up to 12 times per day.

Table 1: Qualitative and quantitative composition of Neurexan®

Active substance (Scientific name of raw material)	**Used plant part/ Starting material/ Production method for the active substance/ Key specification parameter**	**Used potency (calculated potency corresponding to final dilution)**	**Mass of used potency per 1 tablet (mg)**	**Amount per daily standard dose (3 tablets)**	**Amount per maximum daily dose (12 tablets)**
Avena sativa mother tincture (Avena sativa L.)	Fresh, aerial parts harvested during flowering season Method 1.1.1, Ph. Eur. equivalent to Method 1a, GHP dry residue ≥ 2%	2 (4.7)	0.6	1.8 mg Dil D2 = 180 µg Dil D1 = 36 µg mother tincture (contains 18 µg expressed juice)	7.2 mg Dil D2 = 720 µg Dil D1 = 144 µg mother tincture (contains 72 µg expressed juice)
Coffea arabica mother tincture (Coffea arabica L.)	Ripe, dried, unroasted seeds deprived from the exocarp Method 1.1.8, Ph. Eur.	12 (14.7)	0.6	1.8 mg Dil D12 = 1.8×10^{-11} mg Dil D1 = mother tincture	7.2 mg Dil D12 = 7.2×10^{-11} mg Dil D1 = mother tincture

Active substance (Scientific name of raw material)	Used plant part/ Starting material/ Production method for the active substance/ Key specification parameter	Used potency (calculated potency corresponding to final dilution)	Mass of used potency per 1 tablet (mg)	Amount per daily standard dose (3 tablets)	Amount per maximum daily dose (12 tablets)
	equivalent to Method 4a, GHP; Ø ≥ 0.1% caffeine			with at least 1.8 x 10^{-14} mg alkaloids	with at least 7.2 x 10^{-14} mg alkaloids
Passiflora incarnata mother tincture (Passiflora incarnata L.)	Fresh aerial parts of *Passiflora incarnata L*. Method 1.1.5, Ph. Eur. equivalent to Method 3a, GHP dry residue ≥ 1.6%	2 (4.7)	0.6	1.8 mg Dil D2 = 180 µg Dil D1 = 54 µg mother tincture	7.2 mg Dil D2 = 720 µg Dil D1 = 216 µg mother tincture
Zincum isovalerianicum Dil D2 (Zincum isovalerianicum) *Zinc oxide + isovaleric acid* $Zn(C_5H_9O_2)_2 \times 2\ H_2O$	*Zincum isovalerianicum* (in German: Zinksalz der Isovaleriansäure) Method 3.1.1, Ph. Eur. equivalent to Method 5a, GHP Dil D2 = 0.93 – 1.08% substance	4 (6.7)	0.6	1.8 mg Dil D4 = 18 µg Dil D2 with ca. 0.18 µg substance	7.2 mg Dil D4 = 72 µg Dil D2 with ca. 0.72 µg substance

Calculation of dried plant material was done according to the Homeopathic Medicinal Products Working Group document "Points to consider on non-clinical safety of homeopathic medicinal products of botanical, mineral and chemical origin"

Pharmacology

The therapeutic dilemma in acute insomnia is its multicausality which implies a multi-target approach. Monocausal treatment approaches act only on a certain spectrum of the disease profile, which often results in only inadequate, partial improvement in the symptoms (e.g., short-acting benzodiazepines or non-benzodiazepine hypnotics). Neurexan® is a multi-component medication containing components with different actions to treat a multicausal disease. Multi-target is to be understood as involving action on different effectors, which produces a variety of effects. The precise molecular mode of action of multi-target / multi-component drugs is difficult to analyze, for Neurexan® it is still unknown. Nevertheless, many preclinical and clinical studies have demonstrated effects on stress associated complaints such as sleep disturbances, exercise-induced stress, amygdala activation, jitteriness, listlessness or nocturnal anxiety (Davies, Bignell et al. 2002, Waldschütz and Klein 2008, Hübner, Von Haselen et al. 2009, Doering, Wegner et al. 2016, Herrmann, Vicheva et al. 2020, Keller, Conradi et al. 2021).

Pharmacokinetics

As we do not have pharmacokinetics (PK) data due to the nature of our products (unclear which compound / which ingredient affects what – and there are numerous per product) and there are no linear dose-dependent effects, there is no information available on the pharmacokinetic profile as related to pharmacology. Therefore, the documentation and discussion of bibliographic or other data on the pharmacokinetic profile is not considered.

Toxicology

Experimental toxicological studies with the medicinal product Neurexan® are not available. No systematic toxicological studies with the active substances of Neurexan® have been conducted so far.

The assessment of the non-clinical safety of Neurexan® is based mostly on results of experimental *in vitro* and *in vivo* studies, which have been published within recent decades, either on the starting materials or diluted plant ingredients. The study designs as well as the measured parameters in general are adequate for the assessment of the preclinical safety of Neurexan®.

The assessment of pharmacological and toxicological properties of Neurexan® is based on the review of relevant experimental data and evidence as published in the scientific literature on the starting materials of the active substances. Specifically, the aspects of genotoxicity, carcinogenicity, reproductive and developmental toxicity are considered explicitly.

Integrated Overview and Conclusions

Neurexan® is a medicinal product in accordance with the European Commission directive 2001/83/EC as amended. It contains several single remedies as active substances (see Table 1).

The toxicological profile of the active substances and ingredients of the considered product is well or sufficiently described and allows final conclusions on the safety of Neurexan®. No significant risks concerning acute and chronic toxicity, genotoxicity and mutagenicity, developmental and reproductive toxicity, immunotoxicity and local tolerability have become apparent. It is unlikely or highly impossible, that the active constituents pose any drug-associated risks at the given concentration in the finished product and the given posology.

The reviewed data as well as the calculated daily quantity of the single active substances revealed that toxic or adverse effects are not expected following an oral administration in adults as well as in children and adolescents below 18 years of age or the elderly.

Moreover, the long-term clinical experiences as well as the post-marketing experience in many countries with the active ingredients in this combination generally support Neurexan® to be a combination without any toxic risks.

3.3 Risk-Benefit Evaluation

Expected Benefit / Efficacy

The intake of Neurexan® has been associated with improved sleep and lower nervousness / restlessness. Waldschütz and Klein investigated sleep quality in 409 individuals suffering from insomnia by comparing Neurexan® treatment to valerian treatment (Waldschütz and Klein 2008). Both treatments resulted in improved sleep quality. Moreover, individuals receiving Neurexan® reported lower daytime fatigue than those with a valerian therapy. In addition, Neurexan® may lower symptoms of nervousness / restlessness such as excitability / jitteriness, hyperactivity, sleep disturbances, nocturnal anxiety, difficulties with concentration / forgetfulness, fatigue, listlessness, moroseness, gastrointestinal disturbances and headache / pressure (Hübner, Von Haselen et al. 2009). Furthermore, preliminary results indicate that the intake of Neurexan® changes electrical brain activity as compared to placebo under stress. In a double-blind, randomized, placebo-controlled trial with cross-over design, different arithmetic tasks were used to induce stress experimentally (Dimpfel 2019). The trial was conducted on 30 healthy participants aged 30-60 who received 4 Neurexan® or 4 Placebo tablets. β2 power was used as a surrogate marker for stress-induced anxiety. The stress test resulted in elevated β2 power in both experimental groups. However, a smaller increase in β2 power was observed in participants receiving Neurexan® as compared to the Placebo group. This was interpreted in terms of better coping abilities under stressful challenges. Doering and colleagues reported diminished salivary cortisol and plasma adrenaline levels in verum relative to placebo group. Although no reduced subjective stress rating

was found as an effect of Neurexan® intake, significant neuroendocrine changes were present (Doering, Wegner et al. 2016).

Possible Risks / Safety

In a large-scale observational study with 553 participants receiving Neurexan® two adverse events with a possible relationship to study treatment were reported, nystagmus and increased restlessness (Hübner, Von Haselen et al. 2009). Furthermore, a mild caffeine intolerance associated with Neurexan® intake has been reported (reported by 1/156 study participants) (Waldschütz and Klein 2008). Adverse reactions from post-marketing spontaneous reporting concern hypersensitivity reactions, such as transient skin reactions.

There are no safety concerns for the use of Neurexan® in children and adolescents below 18 years of age. There are also no safety concerns regarding the use in elderly or any other especially sensitive patient groups. This is due to the low quantity of each substance (stock) administered, which are significantly below any toxic dosages.

Specific product-related data on the use during pregnancy and lactation is not available. Considering the available - scarce - data on the developmental and reproductive toxicity and considering the characteristics of each active substance used in the product, there are no concerns or apparent risks in this patient group.

Moreover, the long-term clinical experience as well as the post-marketing experience in many countries with the active ingredients in this combination generally supports Neurexan® to be a combination without any safety concerns.

4. Trial Objectives and Endpoints

This double-blind randomized controlled trial aims to investigate the effects of Neurexan® on sleep efficiency measured by polysomnography as well as on other sleep related parameters in patients with short-term insomnia according to DSM-5. In addition, Neurexan®'s impact on daytime performance, stress and the inflammatory response as well as the relationship between postulated changes in the stress and inflammatory response and sleep patterns are to be investigated.

The objective of this clinical trial is to demonstrate the effects of daily treatment with Neurexan® on improvements of objective and subjective measures of sleep quality, daytime performance and stress responsiveness in patients with short-term insomnia patients according to DSM-5 (Diagnostic and Statistical Manual of Mental Disorders, 5th edition).

4.1 Primary Objective and Endpoint

The primary objective is to demonstrate an improvement of sleep efficiency (SE) measured by polysomnography on daily treatment with Neurexan® in short-term insomnia.

The primary endpoint of this trial is to assess Neurexan® related changes in SE at Visit 2 compared to Baseline 2 as revealed by polysomnography (PSG).

4.2 Secondary Objectives and Endpoints

The secondary objectives are to assess further PSG-based objective as well as subjective sleep parameters, daytime performance and stress processing in patients with short-term insomnia treated daily with Neurexan® compared to Placebo.

Secondary endpoints are described in the following.

Sleep Pattern by

- Further objective sleep parameters revealed by polysomnography (PSG)

  To be assessed as change at Visit 2 compared to Baseline 2

  - Sleep Onset Latency (SOL): changes in SOL compared to Baseline 2
  - Wake After Sleep Onset (WASO): changes in WASO compared to Baseline 2
  - Total Sleep Time (TST): changes in TST compared to Baseline 2
  - Number of awakenings (NWAK): changes in NWAK compared to Baseline 2
  - Number of arousals during (Arousal Index; ArI) changes in Arousal Index compared to Baseline 2
  - Latencies to sleeping stages S1, S2, S3, and Rapid Eye Movement (REM): changes in these latencies compared to Baseline 2.
- Objective in-home assessment of sleep pattern revealed by actigraphy (24/7)
  - A portable device, i.e., an actigraphy watch will be used to continuously monitor movement information over the whole clinical trial duration for actimetric sleep–wake detection to investigate habitual sleep–wake patterns and daily activity.
- Subjective sleep pattern assessed using sleep questionnaires and sleep diary (adapted from Deutsche Gesellschaft für Schlafforschung und Schlafmedizin, DGSM), to be recorded on ***AMS***-ePRO®
  - Insomnia Severity Index (ISI): changes in ISI at Visit 1 compared to Baseline 1.
  - Pittsburgh Sleep Quality Index (PSQI): changes in PSQI at Follow-Up Visit compared to Baseline 1.
  - Sleep diary parameters: changes in subjective SE, SOL, WASO, TST, and NWAK compared to baseline.

Daytime Performance by

- Resting state EEG:
  - Changes in time course of EEG vigilance fluctuations during wakefulness assessed by Vigilance Algorithm Leipzig (VIGALL) version 2.1 (validated with the Multiple Sleep Latency Test (MSLT) by (Olbrich, Pawlowski et al. 2015) at Visit 1 compared to Baseline 1
  - Changes in EEG asymmetry index at Visit 1 compared to Baseline 1
  - Regional changes in spectral characteristics of EEG using relative power in delta-theta-alpha-beta frequency ranges at Visit 1 compared to Baseline 1
- Resting state heart rate (HR) and heart rate variability (HRV) and electrodermal activity (EDA) at Visit 1 compared to Baseline 1
- Questionnaires: Epworth Sleepiness Scale (ESS), Würzburg Fatigue Inventory (WEIMuS) and Beck's Depression Inventory-II (BDI-II) at Visit 1 compared to Baseline 1, and Quality of Life (QoL) by QoL questionnaire Short Form-36 version 2.0 (SF-36v2) and Beck's Anxiety Inventory (BAI) at Follow-Up Visit compared to Baseline 1
- Neuropsychological tests assessing general cognitive functioning, alertness, vigilance, and sustained attention: three subtests from electronic Tests of Attentional Performance (TAP) test battery at Visit 1 compared to Baseline 1.

Stress Processing

Given the reported HPA axis dysregulation in the form of an altered cortisol secretion pattern in insomnia patients, a secondary outcome is to demonstrate changes in decreased resting state stress parameters in saliva as well as stress reactivity to physiological challenge using the Cold Pressor Test (CPT) in terms of changes in serum levels of cortisol in short-term insomnia patients treated with Neurexan®.

- Resting state stress response by
  - Changes in stress biomarkers in saliva compared to baseline
    - Cortisol
    - Alpha-amylase
    - Dehydroepiandrosterone sulfate (DHEA-S)
    - Time course of stress biomarkers release synchronized to the awakening time (5 time points after awakening: 0 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes for the assessment of Cortisol Awakening Response (CAR))
    - Diurnal profile of stress biomarkers by collecting saliva samples at 3-hourly intervals over 12 hours (4 time points) synchronized to the awakening time.
- Stress response induced by CPT
  - Changes in subjective pain rating and pain tolerance at Visit 1 compared to Baseline 1

Blood sampling

- Changes in the cortisol time course: Visit 1 before CPT, 20 minutes, and 50 minutes after CPT compared to Baseline 1
- Changes in dehydroepiandrosterone sulfate (DHEA-S) time course: Visit 1 before CPT, 20 minutes, and 50 minutes after CPT; Visit 1 compared to Baseline 1
- Changes in the time course of adrenalin, noradrenalin and the inflammatory response (IL-6 and TNF-α): Visit 1 before CPT, 20 minutes, and 50 minutes after CPT compared to Baseline 1.

Autonomic stress response:

- Changes in heart rate and associated HRV measures, electrodermal activity (EDA); Visit 1 compared to Baseline 1.

EEG stress response:

- Changes in time course of EEG vigilance fluctuations during wakefulness assessed by VIGALL version 2.1 (validated with the Multiple Sleep Latency Test (MSLT) by (Olbrich, Pawlowski et al. 2015) at Visit 1 compared to Baseline 1
- Changes in EEG asymmetry index at Visit 1 compared to Baseline 1
- Regional changes in spectral characteristics of EEG using relative power indices in delta-theta-alpha-beta frequency ranges at Visit 1 compared to Baseline 1.

- Stress questionnaires

  Changes in subjective stress levels compared to Baseline 1 as assessed by
  - Subclinical Stress Symptom Questionnaire-25 (SSQ-25) at Follow-Up Visit
  - Primary Appraisal Secondary Appraisal (PASA) at Visit 1
  - Perceived Stress Scale-10 (PSS-10) at Follow-Up Visit

- Continuous (over the whole trial period) daily stress assessment using AMS-ePRO® functionality and Ecological Momentary Assessments (EMAs).

Further secondary objectives are to assess correlations between:

- Changes in sleep parameters, stress parameters, and daytime performance
- Changes in sleep parameters, stress parameters, and inflammatory parameters

5. Trial Design

5.1 Trial Type and Design Features

This monocentric exploratory clinical trial is designed as a prospective, double-blind, placebo-controlled, randomized trial to evaluate the effects of Neurexan® regarding sleep, daytime performance, stress and inflammatory response in a patient cohort with short-term insomnia.

It is planned to randomize a total of 80 patients with short-term insomnia. Eligible patients are to be allocated in a 1:1 randomization to either Neurexan® or Placebo treatment.

5.2 Investigational Treatment

The trial treatment consists of 2 tablets taken sublingually 3 times daily for a period of 14 days. The trial treatment is either Neurexan® or Placebo, which is randomly assigned. The trial treatment should be taken at approximately midday, evening and bedtime and not to be taken with meals.

5.3 Trial Duration

The First Patient First Visit (FPFV) was in June 2023. A total of 80 patients will be randomized within approximately 32 months. The Last Patient Last Visit (LPLV) is planned to be in January 2026.

The total trial duration for each patient is 37 to maximum 60 days (Table 3). Patients start with a Screening Visit 8 to 28 days prior to first investigational treatment (on Day 0), followed by two baseline visits for a PSG adaptation ('screening') night at Baseline 1/ Day -2 to -1 and the primary outcome relevant PSG night at Day -1 to Day 0, respectively. Patients are randomized to their investigational treatment after completion of visit Baseline 2 / Day 0. Patients will take their investigational treatment for 14 consecutive days (starting at Day 0 to Day 13). Patients will visit the investigational site at Day 12 +2 for a PSG adaptation night and at Day 13 +2 for the primary outcome relevant PSG night. On Day 28 +3 a Follow-up Visit will take place to collect paper-based patient-reported outcome data of BAI, PSQI, PSS-10, SF-36v2 and SSQ-25 questionnaires and the End of Trial (EoT) documentation is to be completed.

5.4 Discussion of Trial Design

This double-blind placebo-controlled randomized trial is well suited to address the lack of RCT data on Neurexan® efficiency on objective sleep parameters using PSG, on daytime performance and on its relationship with assumed changes to stress and inflammatory response.

5.5 Trial Administrative Structure

5.5.1 Sponsor

The Sponsor of trial C2104 is Biologische Heilmittel Heel GmbH (Dr.-Reckeweg-Straße 2-4, 76532 Baden-Baden, Germany).

5.5.2 Contract Research Organization (CRO)

The Sponsor delegates the conduct of the trial ('full service') to the CRO *AMS* Advanced Medical Services GmbH (Mannheim, Germany).

5.5.3 Central Laboratories

The local routine laboratory of the investigational site (Institut für Klinische Chemie und Laboratoriumsdiagnostik IKCL, University Hospital Jena) will analyze safety laboratory blood samples (see section 9.2.4) as well as blood biomarker samples (see section 9.1.8) and will provide frozen interim storage of saliva samples in their biobank. Saliva biomarker samples (9.1.2) will be analyzed at Dresden LabService GmbH.

5.5.4 Participating Site

This is a monocentric trial conducted at the Department of Psychiatry and Psychotherapy of the Jena University Hospital (Philosophenweg 3, 07743 Jena, Germany).

5.5.5 Trial Coordinating Investigator

The Coordinating and Principal Investigator of trial C2104 is Prof Dr Martin Walter, chair of the Department of Psychiatry and Psychotherapy of the Jena University Hospital.

5.5.6 Prerequisites for the Start of the Trial

The trial may start as soon as all applicable approvals of both the competent Ethics Committee and the Competent Authority (Bundesinstitut für Arzneimittel und Medizinprodukte, BfArM) have been obtained. The investigational site may start trial activities no sooner than all applicable approvals are obtained and when investigational medicinal products (IMPs) are available at the site.

6. Patient Screening, Randomization, Discontinuation and Withdrawal

Randomization of 80 patients suffering from short-term insomnia is planned for the trial. If a patient fulfils all of the inclusion criteria and none of the exclusion criteria, his/her eligibility will be assessed during the Screening Visit and he/she will be randomly assigned to Neurexan® or Placebo treatment with 1:1 allocation and stratification by sex.

6.1 Inclusion Criteria

Eligible patients have to meet all of the following inclusion criteria at Screening Visit (Day -28 to -8):

1. Insomnia definition according to DSM-5 criteria; episode duration less than 3 months
2. Short-term insomnia with moderate symptoms according to ISI of at least 8 and below 22 being present for at least one week, but no longer than 3 months prior to Screening Visit
3. Reports habitual bedtime, defined as the time the participant attempts to sleep, between 21:00 and 01:00

4. Reports regular time spent in bed, either sleeping or trying to sleep, between 6 and 9 hours
5. ≥18 years of age, not older than 65 years
6. Legally competent male or female patient
7. Signed Informed Consent
8. Females of childbearing potential must agree to maintain highly effective or acceptable birth control throughout the trial (CTFG 2020).

   Highly effective (failure rate of less than 1% per year)

   - **Combined** (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal
   - **Progestogen-only** hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable
   - **Intrauterine** device
   - **Intrauterine** hormone-releasing system
   - Bilateral **tubal** occlusion
   - **Vasectomized** partner (provided partner is sole sexual partner and if vasectomized partner has received medical assessment of the surgical success)
   - Sexual **abstinence** (only if defined as refraining from heterosexual intercourse during the entire period of risk associated with investigational treatment)

   Acceptable birth control methods which may not be considered as highly effective (failure rate of more than 1% per year)

   - **Progestogen-only** oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
   - Male or female **condom** with or without spermicide
   - **Cap, diaphragm** or **sponge** with spermicide
   - **Combination** of male condom with either cap, diaphragm or sponge with spermicide (**double barrier** methods)
9. Body Mass Index (BMI) between 18.5 and 29.9 kg/m^2 at Screening Visit
10. Use of digital device e.g., smartphone, tablet or laptop
11. German speaking and reading.

6.2 Exclusion Criteria

Potential trial patients will be excluded if at least one of the following exclusion criteria is present:

1. Patients with insomnia symptoms present longer than 90 days prior to Screening Visit
2. Based on the diagnostic interview, reported history (within 2 years) of other sleep disorders (e.g., chronic insomnia, circadian rhythm sleep disorders, restless legs syndrome (RLS), obstructive sleep apnea (OSA)), i.e., STOPBang (SBQ) questionnaire score ≥5, International Restless Legs Scale score ≥16)
3. Based on the first polysomnographic screening night at Baseline 1, insomnia due to sleep apnea or periodic limb movement disorder (PLMD): OSA (Apnea Hypopnea Index of >5 events/ hour), PLMD (Periodic Limb Movement Index (PLMI) >15 events/ hour)
4. Rotating shift work with overnight shifts
5. History of psychiatric disorders within the last 6 months prior to Screening Visit according to the Structured Clinical Interview for DSM-5® Disorders – Clinician Version (SCID-5-CV)
6. History of sensitivity to any component of Neurexan®
7. Unwilling or unable to comply with all the requirements of the clinical trial protocol
8. Cognitive impairment (cut-off of 24 points in the Montreal Cognitive Assessment [MoCA]; Thomann, Berres et al. 2020) at Screening Visit
9. Any history of or current abuse of alcohol and/or amphetamines, benzodiazepines, cocaine, marijuana, methaqualone, methadone, opioids, propoxyphene, barbiturates, phencyclidine; or expected to take during trial participation (urine drug screening at Screening Visit and adaptation nights)
10. Current use of medication affecting sleep, i.e., antidepressants, antipsychotics, diuretics, blood pressure drugs, anti-dementia drugs (e.g., piracetam), herbal and homeopathic medicine, hormone preparations (e.g., thyroxine) with the exception of hormonal contraceptives
11. Use of Neurexan® within the last two weeks from Screening Visit
12. Non-pharmacological insomnia therapies (e.g., cognitive behavioral therapy within the last 6 months of Screening Visit, sleep restriction therapy, complementary and alternative therapies as meditation, Traditional Chinese Medicine, aromatherapy)
13. Excessive consumption of xanthine-containing beverages (more than 7 cups daily of coffee or tea or other beverages containing xanthines)
14. Use of nicotine during the last 6 months prior to Screening Visit
15. Participation in any interventional clinical study within the past 30 days prior to Screening Visit
16. Any relationship of dependence with the Sponsor or with the Investigator
17. Active infection/ disease (C-Reactive Protein [CRP] >5 mg/l)
18. Hypertension defined as systolic blood pressure ≥140 mmHg (Burnier, 2018)
19. History of neurological, rheumatic, chronic pain, immune, cardiovascular, pulmonary, liver/ kidney, or metabolic disorder within the last 6 months prior to Screening Visit

20. Nocturia
21. Pregnancy (as proven by positive urine pregnancy test at Screening Visit) or breastfeeding
22. Patients with moderate to severe skin allergies and/or eczema
23. Raynaud's disease
24. Donation of blood or platelets 3 months prior to or in-between in-hospital visits.

Patient screening will include blood tests for complete blood count (CBC) and differentials, as well as thyroid hormone and blood glucose; urinary toxicology screens will also be conducted (exclusion criterion number 9). The eligibility of patients based on safety laboratory parameters (except CRP) are at the discretion of the investigator.

The medical history and physical examination will be conducted by a trial physician.

6.3 Patient Premature Withdrawal from Trial/ Withdrawal Criteria

Possible reasons for a premature discontinuation of randomized patient participation in this trial are:

- AE(s) that endanger the patients' safety, making it ethically unacceptable to continue
- Deterioration of the patient's clinical condition(s) that requires appropriate therapy/ treatment during the trial period
- Insufficient treatment compliance as specified in the protocol (see section 11.3.2)
- Intake of prohibited concomitant medication (see section 7.6.1) that might influence the trial procedures or the results
- Patient's unwillingness or inability to adhere to trial requirements (lack of patient's compliance)
- Patients requiring quarantine due to acute infections such as SARS-CoV2
- Positive test for SARS-CoV2 (not part of trial procedures)
- Positive test for drugs, alcohol (breath alcohol test and urine drug screening)
- Daytime napping at PSG days
- Pregnancy
- Withdrawal of Informed Consent
- Lost to follow-up
- Death.

If possible, a remotely prematurely withdrawn patient should be invited for a last onsite visit to return remaining IMP and the Actiwatch. The procedures of the Follow-Up Visit should be followed.

Prematurely withdrawn patients ('Drop Outs') will not be replaced.

6.4 Premature Termination of the Trial by the Sponsor

The Sponsor reserves the right to prematurely terminate the trial at any time, after discussion with the Coordinating/ Principal Investigator. The Sponsor may terminate the trial if

- Drug-related AEs occur that constitute a serious risk to the health of the participating patients,
- The risk-benefit assessment becomes negative.

In any case of a termination/ suspension of the trial the Investigator, the Ethics Committee and the regulatory authority have to be informed within the legally defined timelines of the termination/suspension and of the reason(s).

The Sponsor reserves the right to prematurely terminate the trial at the trial site if:

- The trial site does not comply with the requirements settled in the trial protocol
- The trial site does not adhere to the standards of Good Clinical Practice (GCP)
- The time schedule settled for the recruitment of patients is not complied with.

7. Trial Treatment

7.1 Investigational Medicinal Product (IMP)

Biologische Heilmittel Heel GmbH will supply the Investigational Medicinal Product (IMP), Neurexan® and matching Placebo, directly to the trial site.

Neurexan®

The active ingredients are *Passiflora incarnata* Dil. D2 (0.6 mg / tablet), *Avena sativa* Dil. D2 (0.6 mg / tablet), *Coffea arabica* Dil. D12 (0.6 mg/tablet) and *Zincum isovalerianicum* Dil. D4 (0.6 mg / tablet). Inactive excipients are lactose monohydrate and magnesium stearate.

Placebo:
Contains lactose monohydrate, magnesium stearate and looks identically in terms of taste, size, color and labelling.

Information on substance identity is provided in

Table 2.

Table 2: Substance information

	Neurexan®	Placebo
Dosage form and strength	tablets	tablets
Manufacturer	Biologische Heilmittel Heel GmbH	Biologische Heilmittel Heel GmbH

7.2 Dosage and Administration

The trial treatment consists of 2 tablets taken sublingually 3 times daily (a total of 6 tablets/ day) for a period of 14 consecutive days (+ up to 2 days allowance, see flow chart at section 2.2 and Table 3). The trial treatment should be taken at approximately midday, evening and bedtime and not to be taken with meals.

7.3 Blinding

As this is a double-blind, randomized trial, all involved personnel at the investigational site, the Sponsor (except production unit) and the CRO (except independent biostatistician for performing the randomization) will be blinded during the trial. The Investigator keeps the sealed treatment code envelopes throughout the course of the trial for the individual patients and must not break the code without a valid reason (e.g., in case of emergency).

Emergency unblinding is to be done only when absolutely necessary for the management of an individual patient and where stopping the blinded medication is not sufficient in the judgement of the Investigator. The justification for the unblinding has preferably to be discussed upfront by

telephone with the Sponsor to ensure whether unblinding is necessary and that appropriate steps for patient management are taken. The unblinding has to be documented by the Investigator. Details of the unblinding procedure will be explained in the Safety Reporting Plan for this trial.

7.4 Manufacturing, Packaging and Labelling

All IMP in this trial will be prepared, packed and labelled under the responsibility of a qualified person of the Sponsor in accordance with Good Manufacturing Practice (GMP) and all applicable local laws and regulations.

Neurexan® and Placebo will be packed in white cylindrical containers with 100 tablets, labelled including tamper-proof seal and released by the Sponsor.

Labelling implements the use of the computer-generated randomization plan in a treatment ratio of 1:1 and stratification by sex. IMP containers for males and for females are provided, distinguishable by randomization numbers and IMP label color.

All IMP must be kept in a secure place (locked room and/or locked cupboard with restricted access). Based on available stability date no special storage conditions are required for the medication.

7.5 Storage and Dispensing of Trial Medication(s)

The Investigator is responsible for ensuring IMP accountability, including reconciliation of drugs and maintenance of drug records.

- Upon receipt of IMP, the Investigator (or pharmacist) will check for accurate delivery and acknowledge receipt by signing and dating the documentation provided by the Sponsor. A copy of each document will be filed in the Trial Master File and another copy be retained for the Investigator Site File.
- The dispensing of the IMP will be carefully recorded at the site on the appropriate drug accountability forms provided by the Sponsor and an accurate accounting will be available for verification by the monitor at each monitoring visit.
- IMP accountability records will include:
  - Confirmation of IMP delivery to the trial site
  - The inventory of IMP at the site provided by the Sponsor
  - The use of each dose by each patient
  - The return of unused IMP
  - Dates, quantities, batch numbers, expiry dates and the patient IDs assigned.
- The Investigator should maintain records that adequately document:
  - The patients were provided the amount of IMP specified in the protocol/ amendment(s)
  - All IMP provided by the Sponsor were fully reconciled.

Patients should be instructed to bring with them to Visit 1 their IMP container with remaining tablets in order to allow the assessment of compliance with trial treatment (“pill count”).

IMP that has been dispensed to a patient must not be re dispensed to a different patient. Unused IMP must not be discarded or used for any purpose other than the present trial. The monitor will collect the IMP accountability forms and check all IMP returns (both unopened and opened containers) prior to making arrangements for their return to the Sponsor authorized according to Sponsor instructions.

7.6 Concomitant Medications/ Treatment

7.6.1 Prohibited Medications/ Substances/ Treatments During the Trial

- Abuse of alcohol and/or use of amphetamines, benzodiazepines, cocaine, marijuana, methaqualone, methadone, opioids, propoxyphene, barbiturates, phencyclidine
- Use of nicotine
- Medication affecting sleep, i.e., antidepressants, antipsychotics, diuretics, blood pressure drugs, anti-dementia drugs (e.g., piracetam), herbal medicine, hormone and homeopathic preparations (e.g., thyroxine) with the exception of contraceptives
- Use of Neurexan® different than IMP
- Non-pharmacological insomnia therapies (i.e., cognitive behavioral therapy, sleep restriction therapy, complementary and alternative therapies as meditation, Traditional Chinese Medicine, aromatherapy)
- Excessive consumption of xanthine-containing beverages (more than 7 cups daily of coffee or tea or other beverages containing xanthines).

8. Trial Procedures

8.1 Visit Schedule

During the trial 6 regular trial visits are scheduled.

A **Screening Visit** (Day -28 to -8) will be performed to decide about the patient's eligibility for trial participation. Once the patient gave his/her informed consent he/she will complete the Insomnia Severity Index (ISI) questionnaire on ***AMS***-ePRO®. A urine and breath alcohol test will be conducted on drugs and alcohol.

The following procedures will be performed:

- Obtain written informed consent from the patient (section 12.4)
- Completion of ISI on ***AMS***-ePRO® (section 9.1.14)
- Diagnosis of short-term insomnia based on DSM-5
- Check if patient agrees to continuously (day and night) wear the actimetric device on the wrist (section 9.1.5)
- Check applicable eligibility criteria (inclusion and exclusion criteria, section 6.1 and 6.2)
- Documentation of demographics
- Documentation of medical history and concomitant diseases
- Documentation of concomitant medication
- Physical examination (section 9.2.3)
- Assessment of vital signs (heart rate, systolic/ diastolic blood pressure) (section 9.2.2)
- Collection of 8.4 ml blood for safety laboratory analyses (complete blood count (CBC) with differential, thyroid hormones, blood glucose, C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), liver enzymes (aspartate aminotransferase [ASAT], alanine transaminase [ALAT], gamma-glutamyl transferase [gamma GT], alkaline phosphatase), (section 9.2.4)
- Urine pregnancy test in women with childbearing potential (section 9.1.12)
- Urine collection for test on drugs (section 9.1.11)
- Breath alcohol test (section 9.1.11)

- Adverse Event recording from Screening Visit onwards (starting with written informed consent) until End of Trial (section 9.2.1).
- Instruction in the use of the actigraphy watch, i.e., Actiwatch Spectrum Plus, and its handing over.

From Screening Visit onwards daily until the End of Trial (Follow-up Visit/ Day 28+3) patients must wear an actigraphy watch recording their sleeping and activity cycles, complete a sleep diary and answer digital questionnaires regarding stress level (***AMS***-ePRO®).

At **Baseline 1 (**Day -2 to Day -1) patients will have their first night at the trial site for PSG adaptation and screening for exclusion criterion 3. Patients complete their electronic sleep diary (adapted from DGSM, on ***AMS***-ePRO®) to be kept until the End of Trial (Follow-up Visit/ Day 28+3) and questionnaires on sleep, mood, stress and quality of life. In the evening before the Baseline 1 night urine and breath alcohol tests will be conducted on drugs and alcohol, respectively. After awakening (Day -1) resting state stress parameters in saliva are to be collected (at 5 time points) and every 3 hours (at 4 time points). In the course of the morning, patients will perform neuropsychological tests from the TAP battery. An rsEEG and measurement of resting heart rate and HRV will be performed at approximately 15 minutes prior CPT. Around 12:00 at Day -1 a CPT will be conducted, before and afterwards blood samples will be collected. Patients will complete a subjective pain and stress rating during CPT test.

The following procedures will be performed:

- Urine collection for test on drugs in the evening at Day -2 (section 9.1.11)
- Breath alcohol test in the evening at Day -2 (section 9.1.11)
- Completion of the following questionnaires:
    - a) on paper: BAI, BDI-II, WEIMuS, PSQI, PSS-10, SF-36v2, SSQ-25
    - b) on ***AMS***-ePRO®: ESS, ISI, PASA (section 9.1.14)
- PSG for screening of exclusion criterion 3 and for adaptation to the unfamiliar sleep lab environment in the night between Day -2 and Day -1 (section 9.1.1)
- Check exclusion criterion 3 at Day -1
- Collection of 0.5 to 1 ml saliva to measure cortisol, DHEA-S and alpha-amylase at awakening (0 minutes), and after 15 minutes, 30 minutes, 45 minutes, 60 minutes, 3 hours, 6 hours, 9 hours and 12 hours after awakening at Day -1 (section 9.1.2)
- Neuropsychological tests (TAP) at Day -1 (section 9.1.10)
- Resting state EEG, resting state HR and HRV to measure daytime performance at Day -1 (section 9.1.3)
- CPT around 12:00 including subjective pain rating and pain tolerance, heart rate and associated HRV measures, electrodermal activity, and EEG at Day -1 (section 9.1.7)
- Collection of approximately 12.5 ml blood for measuring cortisol, DHEA-S, adrenaline, noradrenaline, inflammation markers (IL-6 and TNF-α) prior to CPT, 20 minutes and 50 minutes after CPT at Day -1 (section 0)
- Adverse events at Day -2 (section 10)
- Changes in concomitant medication at Day -2
- Complete sleep diary and stress assessment (in ***AMS***-ePRO®).

At **Baseline 2** (Day -1 to Day 0) patients will stay at the site for a second night for PSG. After awakening (Day 0) resting state stress parameters in saliva are to be collected (at 5 time points). Patients will be randomly assigned to treatment group Neurexan® or Placebo with a 1:1 allocation and stratification by sex.

The following procedures will be performed:

- Urine collection for test on drugs in the evening at Day -1 (section 9.1.11)
- Breath alcohol test in the evening at Day -1 (section 9.1.11)
- Baseline 2 PSG night to measure primary, i.e., SE and secondary endpoints, i.e., SOL, WASO, TST, NWAK, and ArI during sleep in the night between Day -1 and Day 0 (section 9.1.1)
- Collection of 0.5 to 1 ml saliva to measure cortisol, DHEA-S and alpha-amylase at awakening (0 minutes), and at 15 minutes, 30 minutes, 45 minutes, 60 minutes after awakening at Day 0 (section 9.1.2)
- Adverse events at Day -1 and Day 0 (section 9.2.1)
- Changes in concomitant medication at Day -1 and Day 0
- Randomization to treatment group at Day 0 (section 11.2)
- Distribution of IMP and instruction regarding use of IMP at Day 0
- Starting IMP treatment after having performed all Baseline 2 assessments at midday Day 0 (section 7.1)
- Complete sleep diary and stress assessment (in ***AMS***-ePRO®).

Patients will continue to take their trial treatment for 14 consecutive days (Day 0 - Day 13).

At **Visit 1** (Day 12 to Day 13; +2 days) patients return to the trial site for another PSG adaptation night. A urine and breath alcohol test will be conducted on drugs and alcohol, respectively. After awakening (Day 13) resting state stress parameters in saliva are to be collected (at 5 time points) and every 3-hours (at 4 time points). In the course of the morning, patients will perform neuropsychological tests from the TAP battery. An rsEEG and measurement of resting heart rate and HRV and EDA will be performed at approximately 15 minutes prior CPT. Around 12:00 at Day 13 a CPT will be performed, blood samples will be collected (before CPT and 20 minutes and 50 minutes after CPT) and patients will complete a subjective pain and stress rating. During CPT an EEG will be performed and heart rate and HRV will be measured. Additionally, patients will complete questionnaires on sleep, stress and quality of life on paper (BDI-II, WEIMuS) and on ***AMS***-ePRO® (ESS, ISI, PASA).

The following procedures will be performed:

- Urine collection for test on drugs in the evening at Day 12 (section 9.1.11)
- Breath alcohol test in the evening at Day 12 (section 9.1.11)
- PSG night, again for adaptation to the unfamiliar sleep lab environment between Day 12 and Day 13 (section 9.1.1)
- Collection of 0.5 to 1 ml saliva to measure cortisol, DHEA-S and alpha-amylase at awakening (0 minutes), and at 15 minutes, 30 minutes, 45 minutes, 60 minutes, 3 hours, 6 hours, 9 hours and 12 hours after awakening at Day 13 (section 9.1.2)
- Neuropsychological tests (TAP) at Day 13 (section 9.1.10)

- Resting state EEG, resting state HR and HRV to measure daytime performance at Day 13 (section 9.1.3)
- CPT around 12:00 including subjective pain and stress rating, heart rate and associated HRV measures, electrodermal activity, and EEG at Day 13 (section 9.1.7)
- Collection of approximately 12.5 ml blood for measuring cortisol, DHEA-S, adrenaline, noradrenaline, inflammation markers (IL-6 and TNF-α) prior to CPT, 20 minutes and 50 minutes after CPT at Day 13 (section 9.1.8)
- Completion of the following questionnaires: ESS, ISI, PASA (***AMS***-ePRO®); BDI-II and WEIMuS (both paper-based; section 9.1.14)
- Adverse events at Day 12 (section 9.2.1)
- Changes in concomitant medication at Day 12
- Complete sleep diary and stress assessment (in ***AMS***-ePRO®).

At **Visit 2** (Day 13 to Day 14; + 2 days but on the next day after Visit 1) patients will stay at the site for a second night for PSG. After awakening (Day 14) resting state stress parameters in saliva are to be collected (at 5 time points).

The following procedures will be performed:

- Urine collection for test on drugs in the evening at Day 13 (section 9.1.11)
- Breath alcohol test in the evening at Day 13 (section 9.1.11)
- Last IMP intake in the night of Day 13 prior to sleep
- PSG recording to measure SOL, WASO, TST, NWAK, and arousal indices during sleep in the night between Day 13 and Day 14 (section 9.1.1)
- Collection of 0.5 to 1 ml saliva to measure cortisol, DHEA-S and alpha-amylase at awakening (0 minutes), and at 15 minutes, 30 minutes, 45 minutes, 60 minutes after awakening at Day 14 (section 9.1.2)
- Adverse events at Day 13 and Day 14 (section 9.2.1)
- Changes in concomitant medication at Day 13 and Day 14
- Complete sleep diary and stress assessment (in ***AMS***-ePRO®)
- Return of IMP.

At **Follow-up Visit** (Day 28; + 3 days) patients will have one last Visit at the trial site to complete questionnaires on sleep, stress and quality of life (BAI, PSQI, PSS-10, SF-36v2, SSQ-25, based on paper). Patients will complete their electronic sleep diary (***AMS***-ePRO®) and return their portable actimetric device.

- Completion of the following paper-based questionnaires (BAI, PSQI, PSS-10, SF-36v2, SSQ-25)
- Completion of the last daily sleep diary and stress assessment (***AMS***-ePRO®)
- Return of portable actimetric device
- Adverse events between Visit 2 and until Follow-up Visit (section 9.2.1)
- Changes in concomitant medication between Visit 2 and Follow-up Visit.

Following all above-mentioned procedures including the Follow-up Visit (Day 28 + 3 days) to collect patient-reported outcomes on questionnaire, the End of Trial (EoT) documentation will be completed.

The EoT documentation is also to be completed in the electronic Case Report Form (eCRF) in the event of premature termination/ discontinuation, with reasons to be given (section 6.3).

Table 3: Flowchart of Trial Procedures

	Run-In (9 to 29 days)			Treatment (14 days)		Follow-Up (14 days)
Procedure	**Screening Visit** Day -28 to -8	**Baseline 1** Day -2 to -1	**Baseline 2** Day -1 to 0	**Visit 1** Day 12 to 13 +2 days^	**Visit 2** Day 13 to 14 +2 days^	**Follow-Up Visit** Day 28 + 3 days^^
Diagnosis according to DSM-5 (short-term insomnia)	X					
Inclusion/ Exclusion Criteria (except exclusion criterion 3)	X					
Exclusion Criterion 3		X				
Patient Information/ Informed Consent	X					
Demographics°	X					
Randomization			X			
Vital Signs (heart rate, systolic/ diastolic blood pressure)	X					
Physical Examination	X					
Safety Laboratory (CBC with differential, thyroid hormones, blood glucose, CRP, ESR, liver enzymes)	X					
Urine Pregnancy Test	X					
Medical History and Concomitant Diseases	X					
Urine Drug Test	X	X	X	X	X	
Breath Alcohol Test	X	X	X	X	X	
Adverse Events (AEs)	X	X				
Concomitant Medications	X	X				
Investigational Treatment (Neurexan®/ Placebo)			X*	X	X	
Return of IMP					X	
Polysomnography (PSG) Parameters		X adaptation ('screening')	X	X adaptation	X	
Pittsburgh Sleep Quality Index (PSQI), Paper PRO		X				X
Sleep Diary°° (adapted from DGSM), ***AMS***-ePRO®	X	X	X	X	X	X
Insomnia Severity Index (ISI), ***AMS***-ePRO®	X	X		X		
Resting State EEG (rsEEG)		X		X		

	Run-In (9 to 29 days)			Treatment (14 days)		Follow-Up (14 days)
Procedure	**Screening Visit** Day -28 to -8	**Baseline 1** Day -2 to -1	**Baseline 2** Day -1 to 0	**Visit 1** Day 12 to 13 +2 days^	**Visit 2** Day 13 to 14 +2 days^	**Follow-Up Visit** Day 28 + 3 days^^
Resting Heart Rate (HR) and Heart Rate Variability (HRV), and Electrodermal Activity (EDA)		X		X		
Daytime Performance Questionnaires, Level of Anxiety and Depression Paper PROs: BAI, BDI-II, WEIMuS, SF-36v2 ***AMS***-ePRO®: ESS		X		X BDI-II, ESS, WEIMuS		X BAI, SF-36v2
Actigraphy	X	X	X	X	X	X
Stress Questionnaires Paper PROs: PSS-10, SSQ-25 ***AMS***-ePRO®: PASA		X		X PASA		X PSS-10, SSQ-25
AMS-ePRO® Questionnaires to measure daily stress levels (EMAs)	X	X	X	X	X	X
Resting State Stress Response based on Saliva Biomarkers after Awakening and During the Day[#]		X	X	X	X	
Stress Responses induced by Cold Pressor Test (CPT)[1] as assessed with EEG, HR, HRV and EDA		X		X		
Neuropsychological Tests (TAP)		X		X		
End of Trial (EoT) Documentation[2]						X

Abbreviations: AE - Adverse Event, BAI - Beck's Anxiety Inventory (paper PRO), BDI-II - Beck's Depression Inventory revision II (paper PRO), CBC - Complete Blood Count, CPT - Cold Pressor Test, CRP - C-reactive protein, DGSM - Deutsche Gesellschaft für Schlafforschung und Schlafmedizin, DHEA-S - dehydroepiandrosterone sulfate, DSM-5 - Diagnostic and Statistical Manual of Mental Disorders, 5th edition, EDA - Electrodermal Activity, EEG – electro-encephalogram, EMAs - Ecological Momentary Assessments (used for daily stress assessment via ***AMS***-ePRO®), EoT - End of Trial, (e)PRO - (electronic) Patient-Reported Outcome, ESR - Erythrocyte Sedimentation Rate, ESS - Epworth Sleepiness Scale (***AMS***-ePRO®), HR - Heart Rate, HRV - Heart Rate Variability, IMP – Investigational Medicinal Product, ISI - Insomnia Severity Index (***AMS***-ePRO®), min - minutes, ml - milliliters, PASA - Primary Appraisal Secondary Appraisal (***AMS***-ePRO®), PRO - Patient-Reported Outcome, PSG - polysomnography; PSQI - Pittsburgh Sleep Quality Index (paper PRO), PSS-10 - Perceived Stress Scale-10 (paper PRO), rsEEG - resting state electroencephalography, SF-36v2 - Short Form-36 version 2.0 (paper PRO), SSQ-25 - Subclinical Stress Symptom Questionnaire-25 (paper PRO), TAP – Test of Attentional Performance, WEIMuS - Würzburg Fatigue Inventory (paper PRO).

^Visit 1 may be performed on Day 12+2 days. In any case, Visit 1 and Visit 2 will be performed on two consecutive days.

^^Follow-up Visit may be performed on Day 28 +3 days.

°Demographics include sex, height and weight.

*Starting IMP treatment after having performed all Baseline 2 assessments and after randomization at midday Day 0 for 14 consecutive days (+ maximum 2 days allowance) until Visit 2 (Day 13).

°°Sleep diary will be completed daily; starting at Screening Visit until End of Trial

#Stress biomarkers in saliva: cortisol, alpha-amylase, dehydroepiandrosterone sulfate (DHEA-S) at 5 time points: after awakening from PSG nights at Baseline 1/ Day -1, Baseline 2/ Day 0, Visit 1/ Day 13 and Visit 2/ Day 14 collection of around 0.5 to 1 ml saliva at 0 minutes, 15 minutes, 30 minutes, 45 minutes, 60 minutes (5 time points) for the assessment of Cortisol Awakening Response (CAR) and additionally at Baseline 1/ Day -1 and Visit 1/ Day 13 diurnal profile of stress biomarkers by collecting saliva samples at 3-hourly intervals over 12 hours (4 time points) synchronized to the awakening time.

1Including subjective pain rating and pain tolerance, blood sampling before CPT, 20 minutes and 50 minutes after CPT to assess biomarkers cortisol and DHEA-S, adrenalin, noradrenalin and inflammatory response (IL-6 and TNF-α), autonomic and cortical stress response (heart rate, HRV, electrodermal activity (EDA) and EEG).

2In case of premature discontinuation reason to be given.

9. Efficacy and Safety Parameters

9.1 Efficacy Parameters/ Assessments

9.1.1 Polysomnography (PSG)

PSG is the gold standard for the objective assessment of sleep parameters. The Interdisciplinary Center for Sleep and Ventilatory Medicine at the Jena University Hospital is certified by the German Sleep Medicine Society (DGSM) and follows the recommended diagnostic and therapeutic standards.

During PSG brain activity (electroencephalogram, EEG), eye movement (electro-oculogram), muscle activity (electromyogram) and cardiac activity (electrocardiogram) as well as respiratory parameters (airflow, respiratory effort, and oximetry) will be recorded.

Sleep efficiency (SE) is calculated as the ratio of total sleep time (TST) to time in bed (TIB) (i.e., both sleeping and attempting to fall asleep or fall back asleep). Sleep onset latency (SOL) is the time in minutes from 'lights out' to the first sequence scored as sleep. The sleep time in minutes on each sleep phase S1, S2, S3 and rapid eye movement (REM) are recorded. Total sleep time (TST) is the total amount of sleep time summarized of different sleep stages during the total recording time (time in bed, TIB). Periods of wakefulness occurring after the onset of sleep are defined as wake after sleep onset (WASO). The number of awakenings (NWAK) are counted.

Patients will spend 2 nights each onsite in the sleep laboratory for PSG adaptation (Baseline 1 and Visit 1) and for PSG (Baseline 2 and Visit 2). PSG measurements recorded during adaptation nights will not be used for primary and secondary endpoints but analyzed on exploratory basis only.

Daytime napping is not allowed before and after PSG nights. With urine and breath alcohol tests, patients will be screened for drug abuse and alcohol at Screening Visit and prior to PSG nights at Baseline 1, Baseline 2, Visit 1 and Visit 2.

9.1.2 Resting State Stress Response

Resting state stress response will be assessed based on the stress biomarkers cortisol, alpha-amylase and dehydroepiandrosterone sulfate (DHEA-S) in saliva samples.

Around 0.5 to 1 ml of saliva are to be collected at each of 5 time points starting immediately after awakening from PSG adaptation nights (Baseline 1/ Day -1 and Visit 1/ Day 13) and PSG nights (Baseline 2/ Day 0 and Visit 2/ Day 14) followed by sampling at 15 minutes, 30 minutes, 45 minutes and 60 minutes after awakening for the assessment of Cortisol Awakening Response (CAR).

To assess a diurnal profile of stress markers 4 additional saliva samples (around 0.5 to 1 ml/ sample) are to be collected at 4 time points at 3-hourly intervals over 12 hours at Baseline 1/ Day -1 and Visit 1/ Day 13 (Table 4).

Table 4: Resting state saliva sampling

Cortisol Awakening Response					Diurnal Profile*			
After awaken-ing	+15 min	+30 min	+45 min	+60 min	+3 h	+6 h	+9 h	+12 h
X	X	X	X	X	X	X	X	X

*Only at Baseline 1 and Visit 1.

Saliva samples are collected in 2 ml SaliCap sampling tubes (TECAN/ IBL International, Hamburg, Germany, ref. no. RE69985) using short drinking straws. One sample of 0.5 to 1 ml saliva is sufficient to analyze cortisol, alpha-amylase and DHEA-S.

After instruction by the site personnel, samples are collected independently by each trial participant. Salivary samples are stored frozen at -80°C (+/- 10°C fluctuation) for interim storage (approximately 3 months) at the biobank of the local laboratory of the investigational site (Institut für Klinische Chemie und Laboratoriumsdiagnostik, IKCL, Jena University Hospital) before shipping to the saliva biomarker laboratory.

Stress parameter levels in saliva will be analyzed at Dresden LabService GmbH, Dresden, Germany, using commercial immunoassays from IBL International, Hamburg, Germany (i.e., Cortisol Saliva Luminescence ImmunoAssay (CLIA); and DHEA-S Saliva Enzyme-Linked Immunosorbent Assay (ELISA) following manufacturer's instructions. Saliva alpha-amylase will be analyzed following the method of Rohleder and colleagues (Rohleder, Nater et al. 2004).

9.1.3 Electroencephalography (EEG)

Electroencephalography (EEG) is a widely used technique to investigate human brain functioning.

In addition to EEG recordings during PSG nights, patients will undergo continuous EEG measurements at Baseline 1 (Day -1) and Visit 1 (Day 13). These include a resting-state EEG before the CPT, an EEG recording during the CPT, and a resting-state EEG after the CPT.

Changes in time course of EEG vigilance fluctuations during wakefulness will be assessed by VIGALL version 2.1 (validated with the Multiple Sleep Latency Test (MSLT) by Olbrich et al. 2015) at Visit 1 compared to Baseline 1. EEG asymmetry index results from the subtraction of the alpha's power natural logarithm of the right hemisphere from that of the left hemisphere and represents a robust stress feature (Giannakakis, Grigoriadis et al. 2015). Changes in the EEG asymmetry index at Visit 1 will be compared to Baseline 1.

Regional changes in spectral characteristics of EEG using relative power indices in delta-theta-alpha-beta frequency ranges are assessed.

9.1.4 Heart Rate (HR), Heart Rate Variability (HRV) and Electrodermal Activity (EDA)

The two peripheral branches of the autonomic nervous system (ANS), the parasympathetic and the sympathetic system, modulate the intrinsic activity of the cardiac pacemaker cells in the sinoatrial node. While the parasympathetic or vagal activity reducing energy expenditure is anabolic and health promoting, the sympathetic branch is needed for an adequate stress response. Thus, the heart rate and its variability mirror the resulting homeostasis of an organism influenced by physical as well as by psychological variables, such as stress in a certain environment.

The control of cardiovascular function is critical to adaptive behavior and can be measured by heart rate variability (HRV) reflecting dynamic interactions between excitatory and inhibitory autonomic control mechanism. The following HRV parameters will be selected:

- RMSSD (Root Mean Square of Successive Differences) as an index of parasympathetic activity in the time domain
- Baevsky's stress index (SI) as an index of sympathetic activity

- LF/HF (low frequency/ high frequency power) ratio as a frequency-based feature for sympathovagal balance.

Electrodermal activity (EDA) is a measure of changes in conductance at the skin surface due to sweat production, which is sympathetically controlled. It is therefore considered a sensitive measure of stress by reflecting activity within the sympathetic branch of the ANS.

HR, HRV and EDA will be assessed in resting state during rsEEG and under stress response during CPT as well as during sleep in the PSG (only HR and HRV indices).

9.1.5 Actigraphy

After patients have given their informed consent, they will receive and wear continuously (day and night except during PSG) an actigraphy watch (Actiwatch Spectrum Plus, Philips GmbH), which will continuously monitor movement information over the whole trial duration (Screening Visit/ Day -28 to -8 until Follow-up Visit/ Day 28) to assess SE, SOL, WASO, TST and NWAK as well as to track daily activity to investigate habitual sleep–wake patterns. At Screening Visit patients will be asked whether they will accept to continuously (day and night) wear the actigraphy watch around their wrist. Actiwatch is a water-resistant medical device that continuously records motion and light.

9.1.6 ePRO Designed Stress Questionnaires (EMAs)

Patients perceived stress levels (using Ecological Momentary Assessments, EMAs) will be reported by the patients inside the ***AMS***-ePRO® which can be accessed via the patients' own smartphone, tablet or laptop (Bring Your Own Device = BYOD). Patients will report their stress levels daily over the whole clinical trial duration (Screening Visit until Follow-up Visit/ Day 28).

The site staff will assist patients with the installation of the app and give instructions for use.

9.1.7 Cold Pressor Test (CPT)

The CPT is used to induce a stressor and to assess the patients' stress response. CPT involves placing a hand or forearm in cold water (~0°C), a stimulus that induces stress response as pain is slowly increasing from mild to moderate intensity and is terminated by the patient by voluntary withdrawal of the limb.

CPT will be performed twice with each patient during this trial, following PSG adaptation nights at Baseline 1 (Day -1) and at Visit 1 (Day 13).

During CPT an EEG is performed to assess changes in EEG vigilance fluctuations, in the EEG asymmetry index and spectral characteristics of EEG due to stress induction. The patients' heart rate, HRV and EDA are measured during CPT to assess changes in the autonomic stress response.

Before conduction of the CPT (baseline), 20 minutes and 50 minutes after CPT blood (12.5 ml/ blood sample) will be taken to assess cortisol and DHEA-S, adrenalin and noradrenalin and inflammatory response (IL-6 and TNF-α).

Patients will complete a subjective pain rating questionnaire to measure the individual pain level during CPT on a numeric rating scale (0 to 10). Pain tolerance will be measured by the amount of time the patient spent with the hand or forearm in ice water.

9.1.8 Blood Samples for Stress Response

At Baseline 1 (Day -1) and Visit 1 (Day 13) patients will have repeated blood sampling.

Blood samples will be collected totally 6 times (3 times at each CPT) to assess stress response based on the assessment of biomarkers cortisol, DHEA-S, adrenaline and noradrenaline levels as well as to assess the inflammatory response based on IL-6 and TNF-α.

Each blood sample consists of 12.5 ml blood that will be split up in 3 monovettes (all from Sarstedt, Nümbrecht, Germany) as following:

- 4.9 ml blood will be collected in an S-Monovette® Serum Gel (ref no 04.1935.001) for the analysis of cortisol, DHEA-S and TNF-α,
- 4.9 ml blood will be collected in an S-Monovette® K3 EDTA (ref no 04.1931.001) for the analysis of adrenalin and noradrenalin, and
- 2.7 ml blood will be collected in an S-Monovette® Lithium-heparin gel (ref no. 04.1928.001) for the analysis of IL-6.

Blood biomarker samples will be analyzed at the local laboratory of the investigational site (Institut für Klinische Chemie und Laboratoriumsdiagnostik IKCL, University Hospital Jena).

9.1.9 Sleep Diary

After patients have given their informed consent, they will receive access to an electronic sleep diary (adapted from DGSM - Deutsche Gesellschaft für Schlafforschung und Schlafmedizin, on ***AMS***-ePRO®), which they are asked to complete every day starting at Screening Visit (Day -28 to -8) in the evening until End of Trial (Follow-up Visit/ Day 28). Beside subjective sleep parameters patients will also report their daily intake of IMP, adverse events and concomitant medication, if any.

9.1.10 Neuropsychological Tests (TAP)

With neuropsychological tests the Investigator assesses the patients' alertness, vigilance and sustained attention. These tests will be performed at Baseline 1 and at Visit 1 and last approximately 60 minutes. The electronic Tests of Attentional Performance® (TAP; Zimmermann and Fimm 2002) version 2.3.1 will be used to investigate changes in the alertness, vigilance and sustained attention (three subtests of the TAP).

9.1.11 Urine Test and Alcohol Breath Test

Urine drug screening test and an alcohol breath test will be performed at Screening Visit (Day -28 to -8) and prior to the PSG nights (Baseline 1, Baseline 2, Visit 1 and Visit 2).

9.1.12 Pregnancy Test

Urine pregnancy test will be performed at Screening Visit/ Day -28 to -8 in women with childbearing potential.

9.1.13 Blood Pressure

Blood pressure will be measured at Screening Visit to assess the patients' eligibility. The blood pressure will be measured at rest (patient should be at rest for at least 20 minutes). Measurement will be repeated 3 times with a break of 1 minute in between each measurement. The last measured systolic value will be taken to assess eligibility (see exclusion criterion no. 18).

Blood pressure measurements for the assessment of vital signs will be performed correspondingly.

9.1.14 Patient Questionnaires

Patient-Reported Outcomes (PROs) will be used in this trial to assess insomnia, the patients' perceived stress level and the patients' quality of life. Patients will complete the following questionnaires twice, at Baseline 1 (either in the evening prior to Baseline 1 PSG night or next morning following Baseline 1 PSG night) and either at Visit 1 (either in the evening prior to Visit 1 PSG night or next morning following Visit 1 PSG night), or at Follow-up Visit.

9.1.14.1 Pittsburgh Sleep Quality Index (PSQI)

The Pittsburgh Sleep Quality Index (PSQI) is a self-rated questionnaire which assesses sleep quality and disturbances. Nineteen individual items generate 7 component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction. The PSQI is well suited for measuring change in insomnia and will be completed on paper at Baseline 1 and at Follow-up Visit.

9.1.14.2 Insomnia Severity Index (ISI)

The ISI is a questionnaire widely used to assess nature, severity and impact of insomnia. It has 7 questions, which are summarized to a total score. The ISI will be completed electronically at Screening Visit, Baseline 1 and at Visit 1 (***AMS***-ePRO®).

9.1.14.3 Epworth Sleepiness Scale (ESS)

The ESS is a validated measure of the patient's general level of daytime sleepiness. The patient rates the chances to fall asleep in 8 different situations commonly encountered in daily life. The total ESS score is based on a scale of 0 to 24. The higher the score, the higher the patient's level of daytime sleepiness. The ESS will be completed electronically at Baseline 1 and at Visit 1 (***AMS***-ePRO®).

9.1.14.4 Würzburg Fatigue Inventory (WEIMuS)

The WEIMuS is a 17-item scale designed to assess fatigue as a symptom of a variety of different chronic conditions and disorders. The scale addresses fatigue's effects on daily functioning, querying its relationship to motivation, physical activity, work, family, and social life, and asking respondents to rate the ease with which they are fatigued and the degree to which the symptom poses a problem for them. The WEIMuS will be completed on paper at Baseline 1 and at Visit 1.

9.1.14.5 Short Form-36 Health Survey Version 2 (SF-36v2)

The SF-36v2 questionnaire (Short Form Health 36, version 2.0) questionnaire is a globally established, validated and frequently used questionnaire regarding the quality of life. It consists of 36 questions on general health. The SF-36v2 will be completed on paper at Baseline 1 and at Follow-up Visit.

9.1.14.6 Subclinical Stress Symptom Questionnaire-25 (SSQ-25)

The Subclinical Stress Symptom Questionnaire (SSQ-25) is a comprehensive, reliable, and valid instrument that allows a valid assessment and differentiation of subclinical stress symptoms. This questionnaire consists of 25 questions regarding symptoms following stressful events and will be completed on paper at Baseline 1 and at Follow-up Visit.

9.1.14.7 Primary Appraisal Secondary Appraisal (PASA)

Primary Appraisal Secondary Appraisal (PASA) assesses 4 anticipatory cognitive appraisal processes, threat, challenge, self-concept of own abilities and control expectancy. Each of these primary PASA scales comprises 4 items that are rated on a 6-point Likert scale ranging from strongly

disagree to strongly agree. The PASA will be completed electronically at Baseline 1 and at Visit 1 (***AMS***-ePRO®).

9.1.14.8 Perceived Stress Scale-10 (PSS-10)

The PSS (Cohen, Kamarck et al. 1983) is a widely used psychological instrument for measuring stress perception. It measures how unpredictable, uncontrollable, and overloaded individuals judge their lives within the last month. The English version of the PSS-10 comprises 10 items that are answered on a five-point rating scale ranging from 0 = 'never' to 4 = 'very often' and will be completed on paper at Baseline 1 and at Follow-up Visit. A German translation of the English PSS-10 version is used.

9.1.14.9 Beck's Depression Inventory-II (BDI-II)

The BDI-II is a 21-item, self-report rating inventory that measures characteristic attitudes and symptoms of depression. The BDI-II is a new revised edition of BDI and includes items intending to index symptoms of severe depression, which would require hospitalization. It will be completed on paper at Baseline 1 and at Visit 1.

9.1.14.10 Beck's Anxiety Inventory (BAI)

The BAI is a 21-item multiple-choice self-report inventory that measures the severity of anxiety in adults and adolescents. Because the items in the BAI describe the emotional, physiological, and cognitive symptoms of anxiety but not depression, it can discriminate anxiety from depression. The BAI will be completed on paper at Baseline 1 and at Follow-up Visit.

9.2 Safety Parameters

9.2.1 Adverse Events

Definition, collection and reporting of adverse events are described in section 10.

9.2.2 Vital Signs

Vital signs (heart rate, systolic/ diastolic blood pressure) will be collected at Screening Visit.

9.2.3 Physical Examination

At Screening Visit (Day -28 to -8) a physical examination will be performed. Physical status of cardiovascular, respiratory, musculoskeletal and gastrointestinal systems, head and eyes and other abnormalities, if applicable, will be recorded in the eCRF.

9.2.4 Safety Laboratory

Safety laboratory blood samples will be collected once at Screening Visit (and up to 2 working days later) using S-Monovette® Lithium-Heparin Gel (2.7 ml for CRP, liver enzymes and thyroid hormones), S-Monovette® GlucoEXACT (3.1 ml for blood glucose), S-Monovette® K3 EDTA (2.6 ml for complete blood count [CBC] with differential and Erythrocyte Sedimentation Rate [ESR]), totally 8.4 ml. Samples will be consecutively analyzed at the local routine laboratory of the investigational site, the Institut für Klinische Chemie und Laboratoriumsdiagnostik (IKCL), Jena University Hospital.

The following parameters will be analyzed:

- Complete Blood Count (CBC with differential)
- Thyroid hormones
- Blood glucose
- C-Reactive Protein (CRP)

- Erythrocyte Sedimentation Rate (ESR)
- Liver enzymes (ASAT, ALAT, gamma GT, alkaline phosphatase).

9.3 Appropriateness of Measurements

All clinical procedures that will be used in this trial are standard and generally accepted.

10. Safety Reporting

10.1 Definitions

10.1.1 Adverse Event (AE)

According to ICH E2A an Adverse Event (AE) is defined as:

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether or not considered related to the IMP.

10.1.2 Treatment-Emergent Adverse Events (TEAE)

A treatment-emergent adverse event (TEAE) is defined as any event not present prior to the initiation of the treatment or any AE already present that worsens in either intensity or frequency following exposure to the treatment.

All AEs collected from visit Baseline 2 (Day -1 to 0) until 1 day (24 hours) following last IMP intake are considered as TEAEs in this clinical trial.

10.1.3 Adverse Drug Reaction (ADR)

An Adverse Drug Reaction (ADR) is any untoward and unintended response to an IMP that is considered to have a reasonable possibility of a causal relationship with the treatment, i.e., the relationship cannot be ruled out.

10.1.4 Serious Adverse Event/ Serious Adverse Drug Reaction (SAE/ SADR)

A Serious Adverse Event (SAE) or Serious Adverse Reaction (SADR) is any untoward medical occurrence or effect that at any dose:

- Results in death
- Is life-threatening (note: the term “life-threatening” refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically may cause death if it is more severe)
- Requires inpatient hospitalization (overnight stay) or prolongation of existing hospitalization
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect
- Is of special interest at the Investigator’s discretion
- Or other medically important condition.

Other medically important conditions are defined as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one or more of the outcomes listed in the definition above.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsion that does not result in hospitalization, or development of drug dependency or drug abuse.

However, medical judgement has to be exercised in deciding whether an event is serious in any other situations considered medically relevant.

The evaluation of the AE as being serious or not-serious is made independently of any attribution of causality. Events requiring treatment on an emergency outpatient basis (not resulting in admission to hospital) and not fulfilling any of the definitions of seriousness given above are NOT considered to be SAEs.

10.1.5 Medical Events not to be Considered AEs or SAEs

The following events should not be recorded as an AE/ SAE if recorded at the screening period and/or the basic examination of a clinical trial:

- A pre-planned procedure, e.g., surgery and invasive procedures, unless the condition for which the procedure was planned has worsened since Baseline 1.
- Complications to pre-planned procedures should be recorded as AEs.
- A pre-existing condition found as a result of screening procedures.
- Pregnancy is not considered to be an AE.

Note: Any worsening in severity or frequency of a concomitant illness or any new illness diagnosed in the trial period has to be regarded as AEs/ SAEs.

10.1.6 Unexpected Adverse Reaction (UAR)

An Unexpected Adverse Reaction (UAR) is an ADR, the nature or severity of which is not consistent with the information available on the IMP reference safety information.

10.1.7 Suspected Unexpected Serious Adverse Reaction (SUSAR)

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is a serious ADR, the nature or severity of which is not consistent with the applicable reference safety information.

10.2 Criteria for Clinically Relevant Abnormal Findings

The assessment whether abnormal laboratory findings are of clinical relevance will be done by the Investigator and documented in the electronic Case Report Form (eCRF).

10.3 Recording of AEs

All AEs that occur during the entire course of the trial after the patient has signed the informed consent are to be collected and reported in the eCRF, regardless of whether they are reported by the patient, elicited by Investigator questioning, detected through physical examination, or by other means.

For the case that AEs are still ongoing at the end of the trial, Investigators should make a reasonable effort to obtain a final status of the AE and, where possible, an end date even if the end date is later than the final trial-specific visit for the patient concerned. Information dated after the end of the trial for the patient concerned will not be part of the data collection in the eCRF.

10.4 Evaluation of AEs

As far as possible, each AE is described by:

- Duration (start and end dates)
- Start/ end of (investigational) medication

- Intensity (mild, moderate, severe)
- Investigator assessed causality (relationship to the IMP)
- Action(s) taken (change of investigational medication, treatment of AE etc.) including start and end of respective action
- Concomitant diseases and respective medication in general
- Outcome.

10.4.1 AE Intensity

AE intensity determined by the Investigator on the basis of his/her direct observations or the patient's reporting:

- Mild: causes no limitation of usual activities; the patient may experience slight discomfort
- Moderate: causes some limitation of usual activities; the patient may experience annoying discomfort
- Severe: causes inability to carry out usual activities; the patient may experience intolerable discomfort or pain.

10.4.2 AE Causality

Any AE has to be judged for causality (relationship to investigational medication and relationship to trial procedures). The Investigator will assess causal relationship between the IMP and all AEs whether there is a reasonable possibility that the drug caused the event.

The assessment will be based on the question 'Do you consider that there is a reasonable possibility that the AE may have been caused by the IMP?'. The evaluated answer will be `yes` or `no`.

In case a reasonable possibility cannot be excluded, reasonable possibility of causal relationship should be considered until further evidence will be available to exclude it.

For AEs the causal relationship to other medications and trial procedures will also be assessed. In case of reasonable possibility that the AE may have been caused by trial procedures or other medication the evaluated answer will be `yes`.

Data collection will be set up in a way that allows to distinguish between IMP relatedness and procedure relatedness.

Emerging information from procedure-related AEs, if any, will be taken into account for continuous risk-management and re-assessment if the intended procedures remain adequate. Procedures will be revised where there is evidence that risk or burden for the trial participants is not acceptable.

10.4.3 Handling of AEs

If an AE occurs, appropriate diagnostic and therapeutic measures are to be taken and the IMP has to be discontinued if appropriate. Follow-up evaluations of the patient are to be performed until the patient recovers or until the Investigator considers the situation to be no longer clinically significant or until the degree of a permanent disability can be assessed.

Investigators monitor and register all AEs on the AE form of the eCRF at each visit.

For any AE a specific diagnosis rather than signs or symptoms have to be given in the eCRF. In absence of a specific diagnosis, an individual AE form has to be filled in for each sign or symptom to allow proper coding according to Medical Dictionary for Regulatory Activities (MedDRA).

MedDRA coding will be based on the latest version available at trial start throughout the trial to achieve consistency.

Persistent AEs will be entered once in the eCRF until they are resolved or if a new event has to be documented due to deterioration. These AEs will be carefully monitored; further details of monitoring of persistent AEs will be provided in the Monitoring Plan. If an AE is still not resolved at the end of the trial for the individual patient, this will be documented as ongoing at trial completion.

For recurrent AEs, i.e., AEs of the same nature, but with a different date of onset, an individual AE form has to be completed for each of them.

AEs occurring after the termination of the trial individually and/or of the trial in total are to be reported to Biologische Heilmittel Heel GmbH even after the clinical trial has been completed if, in the judgement of the Investigator, there is an association between the event and the previous use of the product under investigation.

10.5 Reporting of Serious Adverse Events (SAEs)

10.5.1 Responsibility of Investigator

If the AE is classified as serious, the Investigator has to complete and also sign the SAE module in the eCRF. It is the responsibility of the Investigator to send the SAE report out of the eCRF within 24 hours after becoming aware of it and print the report out of the eCRF and file it in the Investigator Site File. At the earliest possible date, the SAE report form has to be followed by a detailed report and any documentation that might have become available, e.g., hospital records, autopsy reports, and/or other pertinent documents.

All the above documents will be sent by fax or scan via email to the Drug Safety Department of the designated CRO within 24 hours (see contact details below) and the original of the form will be retained in the patient file (keeping a photocopy in the Investigator Site File). Personal data of the patient have to be deleted or redacted, the patient number and trial code have to be used for patient identification.
For reporting SAEs in the event of technical failure of eCRF, a paper form of the SAE report has to be completed and faxed or emailed as scan within 24 hours using the following contact:
AMS Advanced Medical Services GmbH Safety Department
Fax no.: +49 621700 95 950
Email: safety-C2104@ams-europe.com

At the earliest possible date, the SAE report form has to be completed in the eCRF and procedures described in the first paragraph of this section should be followed. A detailed follow-up report and any documentation that may be available, e.g., hospital case records, autopsy reports, and/or other pertinent documents will be sent via fax or scan via email.

The primary person responsible for Safety at the Pharmacovigilance Department of Biologische Heilmittel Heel GmbH is:

[redacted]
Biologische Heilmittel Heel GmbH
Dr.-Reckeweg-Straße 2-4
76532 Baden-Baden, Germany
Phone: [redacted]
Fax: +49 7221 501-3099
Email: drugsafety@heel.com

The Qualified Person for Pharmacovigilance (QPPV) at Biologische Heilmittel Heel GmbH is:

[redacted]
Biologische Heilmittel Heel GmbH
Dr.-Reckeweg-Straße 2-4
76532 Baden-Baden, Germany
Phone: [redacted]
Fax: +49 7221 501-3099

Availability in emergency patient safety situation (24 hours/day and 7 days/week)
Mobile: +49 160 88 29 373

10.5.2 Responsibility of Sponsor

The Sponsor will assess if an SAE is in line with the IMP's existing reference safety information (thus 'expected') or if the SAE is not in line with the existing reference safety information (thus 'unexpected').

All Suspected Unexpected Serious Adverse Reactions (SUSARs) will be reported to the Competent Authority(ies) concerned and to the Ethics Committee(s) concerned as soon as possible but within a maximum of **15 days** (fatal or life-threatening SUSARs within a maximum of **7 days**) of first knowledge by the Sponsor.

Relevant follow-up information of fatal or life-threatening SUSARs will be communicated subsequently within an additional 8 days.

Any circumstances which require a review of the risk-benefit assessment of the IMP will be reported to the Competent Authority(ies) and to the Ethics Committee(s) as soon as possible but within a maximum of 15 days.

Throughout the clinical trial, the Sponsor shall provide the Competent Authority(ies) and the Ethics Committee(s) with yearly safety assessment reports, Development Safety Update Reports (DSURs), which list Suspected Serious Adverse Reactions (expected and unexpected).

The Sponsor shall within 15 days notify the Competent Authority(ies) concerned and the Ethics Committee(s) concerned when the clinical trial has been suspended or interrupted by the Sponsor, giving the reasons for the suspension or interruption.

10.6 Follow-up of AEs

Regardless of the duration of the trial, every patient suffering from an AE must be monitored by the Investigator and/or the Sponsor independently from trial termination at least for SAEs until its complete resolution or until its cause has been established.

10.7 Pregnancy

Females who are pregnant or breastfeeding are not allowed to participate in this clinical trial. Urine pregnancy test will be performed for women of childbearing potential at Screening Visit before first IMP administration to exclude pregnancy. Women are considered of childbearing potential, i.e., fertile, following menarche and until becoming postmenopausal unless permanently sterile. Permanent sterilization methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

Women of childbearing potential must agree to maintain highly effective or acceptable birth control throughout the trial (CTFG 2020).

Highly effective (failure rate of less than 1% per year)

- **Combined** (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: oral, intravaginal, transdermal
- **Progestogen-only** hormonal contraception associated with inhibition of ovulation: oral, injectable, implantable
- **Intrauterine** device
- **Intrauterine** hormone-releasing system
- Bilateral **tubal** occlusion
- **Vasectomized** partner (provided partner is sole sexual partner and if vasectomized partner has received medical assessment of the surgical success)
- Sexual **abstinence** (only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments)

Acceptable birth control methods which may not be considered as highly effective (failure rate of more than 1% per year)

- **Progestogen-only** oral hormonal contraception, where inhibition of ovulation is not the primary mode of action
- Male or female **condom** with or without spermicide
- **Cap, diaphragm** or **sponge** with spermicide
- **Combination** of male condom with either cap, diaphragm or sponge with spermicide (**double barrier** methods)

All pregnancies observed during the course of the trial and their outcomes should be reported to the CRO and/or the Sponsor. If a patient becomes pregnant the investigational product should be discontinued immediately and the patient should be withdrawn from the trial. The Investigator or other site personnel will have to inform the CRO and/or the Sponsor within 24 hours of when he or she becomes aware of the pregnancy.

A Pregnancy Report Form should be used for reporting and sending via fax or scan via email to:

AMS Advanced Medical Services GmbH Safety Department
Fax no.: +49-(0)621700 95 950
Email: safety-C2104@ams-europe.com
Contact details of Drug Safety at Heel see in section 10.5.1 above.

The designated CRO and/or Sponsor representative liaise with the Investigator to ensure that all relevant information is provided within 24 hours in case of SAEs and within 30 days for pregnancies without any complications.

Pregnancy itself is not considered as AE, but any complications e.g., congenital abnormalities, birth defects, and spontaneous miscarriages should be handled and reported as SAEs.

11. Statistical Methods and Data Analysis

11.1 Sample Size Estimation

Estimation of sample size is based on the demonstration of improved sleep efficiency (SE) in short-term insomnia patients treated with Neurexan® as assessed by polysomnography. The standard deviation (SD) of SE is estimated at 10%. With α= 5%, two-sided and an expected mean difference of 6.7% between patients treated with Neurexan® and Placebo, a power of 80% is achieved with 72 patients. Expecting a dropout rate of 10%, the total number of patients needed is 80.

11.2 Randomization

Patients eligible for treatment will be randomized to one of two treatment arms according to a computer-generated randomization plan in a ratio of 1:1 and stratification by sex. Placebo tablets will be identical in appearance, packaging and labelling to Neurexan®. The randomization schedule will be 'blocked' to avoid temporal bias.

Patients' allocation to treatment will take place after completion of all baseline assessments prior to beginning of trial treatment.

The randomization list will be generated according to the appropriate Standard Operating Procedures (SOPs). Sealed copies will be stored by the Trial Statistician and the Sponsor/ Drug Safety and will not be opened prior to termination of the double-blinded trial, i.e., after data base lock.

11.3 Statistical Planning

A detailed methodology for summary and statistical analysis of the data collected in this trial will be documented in a Statistical Analysis Plan (SAP). This has to be finalized at the latest prior to database lock. This document may modify the plans outlined in the protocol; however, any major modification of the primary endpoint definition and/or its analysis would also be reflected in a protocol amendment to be submitted to the Competent Authorities and Independent Ethics Committee for endorsement.

All programming of tables, figures, listings and statistical analyses will be performed using the statistical software package SAS® version 9.4 or higher.

The statistical planning and evaluation of the trial will be carried out by a qualified biostatistician in accordance with the ICH guidelines and adequate biostatistical SOPs.

Definition of Baseline

The last available information at or prior to Baseline 2/ Day 0 collected before randomization and subsequent first IMP intake will be defined as baseline value. For PSG derived parameters the baseline is defined as the night from Day -1 to Day 0 (Baseline 2).

Subgroup Analyses

The following subgroups will be defined:

- Age
- Sex (female/ male)
- Neurexan® responder vs. non-responder
- Duration of short-term insomnia (≤ 1 month, > 1 month)
- Cortisol response influenced by hormonal contraceptives

Other subgroups may be specified in the SAP or during the Blind Data Review Meeting (BDRM).

Descriptive Analyses

All variables will be described by treatment group using descriptive statistics.

For continuous variables the number of observations (n), arithmetic mean, standard deviation (SD), minimum, median, 25%-, 75%-quantile, maximum, 95% confidence interval (CI) (2-sided), and the number of missing data will be presented.

For categorical variables n, frequency and percentage, 95% CI (where applicable), and number of missing data will be presented.

11.3.1 Hypothesis

The null and alternative hypotheses for the comparison of Neurexan® versus Placebo can be formulated as follows:

$$H_0: \mu_N = \mu_P$$
$$H_A: \mu_N \neq \mu_P$$

H_0: Null-hypothesis; H_A: Alternative Hypothesis; N: Neurexan®; P: Placebo Treatment; μ: mean/ rate of population

The statistical null hypothesis of no difference between the 2 treatment groups (Neurexan® versus Placebo) in terms of the change of sleep efficiency measured by polysomnography from Baseline 2 to Visit 2 will be tested in a confirmatory sense based on the Intention-To-Treat (ITT) population. The test of the null hypothesis will be carried out two-sided. The null hypothesis will be rejected and the superiority of a treatment (Neurexan® or Placebo) will be claimed if the p-value will be equal or less than 0.05.

Sensitivity Analyses

Efficacy analyses will be performed with the ITT population and additionally with the Per-Protocol Population (PPP) to explore the robustness of the findings. The decision to perform a Per-Protocol (PP) analysis will be based on the number of patients excluded from the PPP. If this number is sufficiently low (less than 10% of the ITT population), no PP analysis will be performed. This decision will be taken prior to database lock.

Another sensitivity analysis will compare observed cases and data imputed with the last observation carried forward (LOCF) method (Section 11.4.3).

Further on, robustness analyses will include the Wilcoxon rank-sum test. Difference from baseline will be used, as appropriate.

11.3.2 Analysis Populations

Analyses will be based on the safety population, ITT population, and the Per-Protocol Population (PPP).

The screening population is defined as all patients who signed informed consent.

The safety population is defined as all patients who were randomized and received at least one dose of investigational medication. All safety analyses will be based on the treatment actually taken by the patient (‘as treated’).

The ITT population is defined as patients who were randomized and received at least one dose of investigational medication and had at least one post-dose assessment.

The Per-Protocol Population (PPP) is defined as all patients who received investigational treatment and fulfil the following criteria:

- The absence of any important major protocol deviations
- No technical difficulties during PSG
- Patients who were correctly stratified and received the correct treatment
- Sufficient treatment compliance of 80%-120%.

Treatment compliance will be defined via the amount of IMP that has been taken between first intake at Baseline 2/ second day and last intake at Visit 2/ first day in relation to the amount that should have been taken by a patient.

Protocol deviations will be collected during the entire trial and will be classified as minor or major at the BDRM. Protocol deviations that may affect the efficacy outcome of patients will be classified as major. The decision about major protocol deviations and assignments to populations will be taken in a blinded fashion and will be documented in the BDRM meeting minutes prior to unblinding of the trial.

11.4 Efficacy Analysis

11.4.1 Primary Efficacy

The primary efficacy variable for changes in sleep pattern will be sleep efficiency (SE) measured by polysomnography at Baseline 2 and Visit 2, calculated as the ratio of total sleep time (TST) to time in bed (TIB), as difference at Visit 2 to Baseline 2. Analysis of Covariance (ANCOVA) will be used to test the hypothesis of section 11.3.1. The model will include treatment and the stratification factor sex and will account for sleep efficiency at baseline as covariate. The test will be performed using a Type I error level of 5%, two-sided.

11.4.2 Secondary Efficacy

For all secondary variables, exploratory statistics comparing the 2 treatment groups will be performed. Difference from baseline will be used, as appropriate. Group comparisons of continuous numeric parameters will be tested using an ANCOVA including treatment, the stratification factor sex, and the baseline value of continuous numeric parameters as covariate; comparisons of dichotomous variables are specified by using the Fisher’s Exact Test.

Secondary analyses on efficacy will be exploratory in nature without adjustment for multiplicity. All secondary analyses will be performed with two-sided hypotheses on superiority using a Type I error level of 5%.

Secondary efficacy variables are the following:

Polysomnography at Baseline 2 and Visit 2

- SOL
- WASO
- TST
- NWAK
- Latencies to S1, S2, S3, REM
- Arousal Index (ArI).

Biochemical parameters

- Resting state cortisol and other stress-related parameters in saliva
- Serum cortisol levels and other stress-related parameters in response to the stress induction by CPT
- Stress test induced inflammation parameters in blood serum.

Actigraphy

- Continuous in-home assessment of sleep
- Daytime activity.

Patient Questionnaires

- Subclinical Stress Symptom Questionnaire-25 (SSQ-25)
- Primary Appraisal Secondary Appraisal (PASA)
- Perceived Stress Scale-10 (PSS-10)
- Epworth Sleepiness Scale (ESS)
- Würzburg Fatigue Inventory (WEIMuS)
- Short Form-36 (SF-36v2)
- Insomnia Severity Index (ISI)
- Pittsburgh Sleep Quality Index (PSQI)
- Beck's Depression Inventory-II (BDI-II)
- Beck's Anxiety Inventory (BAI).

Sleep diary (adapted from DGSM) data: subjective SE, SOL, WASO, TST, NWAK

Continuous digital stress assessments

Neurexan® response

Exploratory Efficacy Variables

- EEG based vigilance fluctuations during resting state
- EEG based vigilance fluctuations during CPT
- EEG based asymmetry index during resting state
- EEG based vigilance fluctuations during CPT
- Regional changes in spectral characteristics of EEG during resting state
- Regional changes in spectral characteristics of EEG during CPT
- Cognitive performance in three attentional tasks

- PSG sleep parameters derived from the two adaptation nights at Baseline 1 and Visit 1.

Correlations between

- Changes in sleep parameters, stress parameters, and daytime performance
- Changes in sleep parameters, stress parameters, and inflammatory parameters

may be analyzed using Spearman's rank correlation coefficient or other appropriate method.

More details for analyzing the secondary efficacy parameters will be given in the SAP.

11.4.3 Handling of Missing Data

In general, for the patient data listings, no imputation of missing or incomplete dates will be applied. Data will be presented as collected.

The last observation carried forward (LOCF) method will impute missing data values using the last observation observed prior to the missing value, i.e., the last observation prior to the missing value is carried forward to the future missing time point(s). Only on-treatment, post-Baseline 2 observations will be used in the LOCF procedure, i.e., baseline values will not be used to impute values.

A more detailed description of the handling of missing data will be provided in the SAP.

11.5 Safety Analysis

Clinical safety will be addressed by assessing AEs, TEAEs, physical examinations, vital signs and as needed laboratory assessments in a descriptive manner. AEs and TEAEs will be presented by MedDRA Preferred Term and System Organ Class in different categories. Safety analyses will be performed with the safety population. All events will be listed.

Safety variables are:

- AEs
- TEAEs
- Other observations related to safety (physical examinations, vital signs, laboratory assessments).

12. Ethical and Regulatory Requirements

12.1 Independent Ethics Committee (IEC)

The trial may start as soon as the availability of the consent of the respective Independent Ethics Committee (IEC) is available.

The clinical trial protocol (including all substantial amendments) together with the written informed consent form and informed consent updates, patient recruitment procedures (e.g., advertisements), any other written information to be provided to patients, and any other documents needed by the IEC will be submitted for approval to the IEC which is in charge according to local regulatory requirements. Written approval of the trial needs to be obtained from the IEC prior to the start of the trial.

12.2 Competent Authorities

In accordance with national legal requirements, all documents required to assess and approve the trial will be submitted prior to the start of the trial to the German Competent Authority 'Bundesinstitut für Arzneimittel und Medizinprodukte' (BfArM) for approval.

Additionally, all notifications to the local authority of the federal state Thuringia (German: Bundesland Thüringen) will be made as laid down in the relevant national regulations. The Investigator will receive copies of the substantial submissions and notifications for information.

An End of Trial notification will be forwarded to the Competent Authorities and the Ethics Committee within 90 days after the trial has been completed (defined as Last Patient Last Visit) or in the event of premature termination of the trial within 15 days.

The Competent Authorities and the IEC will be informed of all subsequent protocol amendments and of all SUSARs occurring during the trial and all other events that have an impact on the safety of the patients or the conduct of the trial.

Irrespective of the outcome of the clinical trial, the trial results are to be reported within one year from the end of the clinical trial to regulatory authorities and published in international databases.

A summary of the results understandable to laypersons will be made available in the EU database irrespective of the outcome of the trial.

12.3 Ethical Conduct of the Trial

The trial will be conducted in accordance with the EU Regulation 536/2014, the ICH guideline for Good Clinical Practice (GCP) last adopted December 2016 and the ethical principles laid down in the current version of the Declaration of Helsinki. The trial was originally submitted under the EU Clinical Trials Directive (EC) No. 2001/20/EC and transitioned in 2024 to the EU Clinical Trials Regulation (EU CTR No. 536/2014). Current national regulations and guidelines will also be followed.

12.4 Patient Information and Consent

The patients will be informed about the nature and importance of the trial; they will receive a detailed description of the foreseeable risks and discomforts and of the procedures to be followed. The patients will be informed that they are free to withdraw from the trial at any time without any disadvantages. Prior to the start of the trial the patients will agree to the participation in the trial by dating and signing the informed consent form.

The patients will also agree that data and documents gained through their participation in the trial can be checked by clinical monitors or other personnel by the Sponsor as well as by the responsible authorities.

The patient information/ informed consent form must be approved (along with the protocol) by the Ethics Committee. Consent must be in a language fully comprehensible to the prospective trial participant. Voluntary written informed consent will be obtained from each participant at Screening Visit prior to any trial-related procedures. The consent will be signed and dated by the patient and by the Investigator who conducted the informed consent interview. All patients will be fully informed about the meaning, aim and conduct of the trial. This will take place under conditions where the patient has adequate time to consider the risks and benefits associated with his/her participation in the trial. The patients will have the possibility to ask all of their questions.

It is the responsibility of the Investigator to assure that a written informed consent is obtained from each trial participant in accordance with International Conference on Harmonisation guidelines on Good Clinical Practice (ICH GCP) and local regulations. The signed informed consent will be retained onsite with the trial records. Each trial participant will receive a copy of the signed informed consent.

The Investigator should maintain a log of all patients who signed the informed consent form and indicate if the patient received investigational drug or, if not, the reason why. The patient's medical records should also document that and when the informed consent form was signed and dated prior to any trial-related procedures being performed.

The eCRF contains a section on which the Investigator will confirm having given proper explanation and obtaining the patient's consent to take part in the trial.

12.5 Amendments to the Protocol

The procedures described in this protocol must be adhered to during the trial. Subsequent amendments must be produced in writing and justified. They are subject to the same approval process as the protocol itself. Amendments will be distributed with appropriate instructions to all persons/ institutions who have received the protocol. For each amendment it is necessary to check whether additional explanation to the patient is needed, and whether this qualifies to set up a revised patient information/ informed consent form to be submitted to the relevant Ethics Committee for its opinion.

12.6 Patient Insurance

In accordance with the relevant national regulations, the Sponsor has taken out a patient liability insurance for all patients who have given their consent to participate in the clinical trial. This cover is designed for the event that a fatality, physical injury, or damage to health occur during the clinical trial's execution.

The Sponsor has taken out this patient liability insurance valid for Germany:

HDI Global SE, HDI-Platz 1, D-30659 Hannover, policy no. 70-509683-03087/390

The policies regarding compensation for injury for patients are described in the compensation information leaflet and are available upon request. A copy of the compensation information leaflet will be given to the patient upon request in accordance with the country specific requirements.

12.7 Confidentiality

Adequate records have to be maintained for the trial, including patient medical records, eCRFs, laboratory reports, worksheets, nursing notes, signed informed consent forms, investigational product forms, SAE paper forms (to be used in case an eCRF is not available), and information regarding patient premature discontinuation (drop out) and reasons for discontinuation. The confidentiality of each record with unique patient identification is to be guaranteed by the Investigator.

This clinical trial protocol and other trial documents contain trade secrets and commercial information that is privileged and confidential. Such information is not to be disclosed unless required by laws or regulations. The Investigator agrees to use this information only in conducting this trial and is not allowed to use it for any other purposes without written consent from the Sponsor. Results obtained from this trial are the property of the Sponsor.

12.8 Serious Breaches

A serious breach is any deviation of the approved protocol version or the Clinical Trials Regulation that is likely to affect the safety, rights of trial participants and/or data reliability and robustness to a significant degree in a clinical trial.

Serious breaches require immediate attention of the Investigator. Any suspected serious breaches must be reported to the Sponsor within 24 hours after becoming aware of it using the following contact details:

Emails: C2104@heel.com, drugsafety@heel.com

Phone: +49 160 88 29 373

13. Data from Patients and Sites: Handling and Record Keeping

Detailed procedures will be separately provided in the Data Management, Monitoring, and Quality Plans.

13.1 Data Handling

All data obtained during this clinical trial will be captured electronically in a project-specific programmed Electronic Data Capture (EDC) application, also referred to as electronic Case Report Form (eCRF). All patient data have to be reported in the eCRF in a pseudonymized form. Patients are identified by a unique 7-digit patient identification number starting with 4901- and being generated in the eCRF.

The Investigator will be responsible for the completeness, accuracy, and legibility of the information in the eCRF and other trial documents. For documents other than eCRF, only black ballpoint pen is to be used and any change of data is to be done by striking out the incorrect data with a single line and dating and initialing the changes made.

The clinical monitors have to verify the eCRF entries according to the source documents. The frequency, extent and responsibilities of the monitoring methods are defined in the Monitoring Plan.

The Investigator must ensure that eCRFs are completed in a timely manner to ensure quality of data and to have oversight on patient safety. The Investigator has to electronically sign it off.

A copy of the eCRF including audit trail is to be archived by the Investigator together with the trial documents, source data, and laboratory records (if applicable) for the time required by relevant guidelines and national regulations. Any evaluations documented on paper will be entered into the eCRF. The original entries on paper documents, including those completed by the patient, will be considered source data.

13.2 Monitoring

To ensure compliance an onsite visit will be held prior to initiation of patient recruitment (Site Initiation Visit). The protocol, eCRFs, trial treatment supplies, handling of trial medication, and other trial procedures will be explained in detail.

A clinical monitor assigned by the designated CRO will conduct regular onsite visits for the purpose of clinical monitoring to ensure that the rights and wellbeing of patients are protected; that trial data are accurate, complete, and verifiable with source data; and that the trial is conducted in compliance with the protocol, GCP, and the applicable regulatory requirements.

The Investigator must agree to allow the appointed monitor and other authorized representatives of the designated CRO and/or the Sponsor to inspect all eCRFs and corresponding source documents (e.g., original medical records, patient records and other relevant records); to allow access to the investigational product and other clinical supplies, dispensing, and storage areas; and to agree to assist with their activities, if requested. The Investigator should provide adequate time and facilities for monitoring visits.

The monitor will query any missing or spurious data with the Investigator, which should be resolved in a timely manner. A monitoring log will be maintained to record each visit of the monitor, the reason for the visit, the monitor's signature, and the Investigator's or designee's confirmation signature.

During the Site Initiation Visit the monitor explains to the Investigator all the documents and procedures related to this trial. A detailed description of monitoring is defined in the Standard Operating Procedures (SOPs) of the designated CRO and in the Monitoring Plan.

Regular onsite monitoring visits are carried out at appropriate intervals in order to clarify questions and to review all eCRFs in terms of completeness and plausibility and to perform Source Data Verification (SDV). Remote monitoring might be performed if appropriate. Details as frequency, extent and responsibilities of the monitoring methods are defined in the Monitoring Plan.

The Investigator has to maintain these data up to date and well documented in the medical files ('source').

Personnel changes at the Investigator's site and changes in responsibilities must be notified immediately.

The following is also constantly monitored: the trial's logistic workflow, compliance with regulations and the trial medication(s) handling.

The monitor is the Investigator's permanent contact person. Unusual incidents will be documented immediately and forwarded to the Sponsor Project Manager.

The remaining trial medication will be returned to the Sponsor whenever requested by the Sponsor, but at the latest on or shortly after the clinical trial's completion.

13.3 Data Management

The designated CRO will perform the activities associated with the Data Management of this trial, including the production of an eCRF, setting up a relevant clinical database, along with appropriate validation of data and resolution of queries. All data will be entered into the eCRF. Automated and manual checks will be made against the data to ensure completeness and consistency. Resolution of queries will be implemented in the clinical database.

13.3.1 Source Data

For every patient, the original patient's file ("source") should clearly document at least:

- Last and first name
- Date of birth
- Sex
- Date of the consent form signature
- Date of entry in the trial (Screening Visit)
- Trial protocol number (C2104)
- Diagnosis of short-term insomnia
- Concomitant diseases and medication taken at entry in the trial (Screening Visit)
- Concomitant medication during the trial
- Date of each trial visit
- Date and time of all trial-related measurements
- Date and time of IMP distribution (Baseline 2/ Day 0)
- Paper-based patient-reported outcomes (PROs, completed questionnaires)
- AEs and changes in the concomitant therapy throughout the trial
- End of Trial Treatment and End of Trial status
- Date of the trial termination (Follow-up Visit/ Day 28, in case of premature withdrawal/ Drop Out the reason must be indicated).

The Investigator will permit trial-related monitoring, audits, and regulatory inspections, providing direct access to all source data/ documents. All persons involved are bound to maintain strict confidentiality concerning the identification of the patients.

13.3.2 Electronic Case Report Form (eCRF)

All data obtained during this clinical trial will be captured electronically in a project-specific programmed EDC application, also referred to as electronic Case Report Form (eCRF). The eCRF is specifically designed for the collection of the clinical data detailed in this trial protocol. The EDC application used for this trial is the Clinical Data Management System Clincase® (supplied by Quadratek Data Solutions Ltd (Clincase®), which is fully validated and compliant with United States Food and Drug Administration (FDA) 21 Code of Federal Regulations (CFR) part 11). The project-specific eCRF will be subjected to system validation according to Good Automated Manufacturing Practice (GAMP) 5 prior to go live. Only authorized staff will have access to the EDC system via a secure website (Secure Sockets Layer [SSL] encryption), using unique user name and password. Data will be entered into eCRFs in accordance with instructions from the Sponsor and/or designee.

Data captured on standardized paper questionnaires, obtained in interviews (e.g., anamnestic data, concomitant diseases, concomitant medications, adverse events, etc) or laboratory results from safety laboratory assessments will be entered into eCRFs by the Investigator or authorized site

staff. Each Investigator is responsible for ensuring that accurate data are entered into the EDC system in a timely manner and are assigned to the correct patient. The Investigator confirms this by electronically signing the documentation, equivalent to a traditional handwritten signature. Each initial data entry, each data modification (incl. reason for change), as well as all actions in the eCRF are tracked in an audit trail, including username, date and time.

13.3.3 Direct Entries

The eCRF itself will be the source for the following data (Direct Entry) as defined by the Monitoring Plan, e.g.:

- Pregnancy test results
- Breath alcohol and urine drug test results
- Physical examination results
- Vital signs.

13.3.4 Coding

AEs will be standardized for terminology and classification, using MedDRA (the latest available version at trial start will be used for the entire duration of the trial).

Concomitant medical conditions and medical history terms will be standardized for terminology and classification, using the most current MedDRA Dictionary Version at First Patient First Visit (Version 26.0).

Concomitant drugs will be standardized for terminology and classification using World Health Organization (WHO)-Drug Dictionary (version B3 Global of March 2023). Both coding dictionaries will be updated to the recent version prior to final analysis.

13.3.5 Confidentiality of Personal Data

The Investigators, the designated CRO, and the Sponsor will preserve the confidentiality of all patients taking part in the trial, in accordance with ICH GCP and local regulations. During this trial, all clinical data will be collected and stored only pseudonymously.

The confidentiality of all patient identities will be maintained except during onsite Source Data Verification (SDV), when clinical monitors, auditors, and other authorized agents of the Sponsor or its designee, as well as any other applicable regulatory authorities, will be granted direct access to the trial patients' original medical records.

No material bearing a patient’s name will be kept on file by the designated CRO or Sponsor. The data retained from this trial will be protected in accordance with all applicable legal requirements.

13.4 Record Keeping

The Investigator agrees to retain copies of the eCRF, the Investigator Site File and other relevant trial-related documents (e.g., the protocol and any protocol amendments, the IMP reference safety information, IEC approval, signed patient informed consent forms, and source documents for each patient in the trial) in a secure place as long as needed to comply with national and international regulations.

The Investigator has to archive all essential records and documents for 25 years as specified by EU regulation 536/2014. According to ICH GCP, these documents have to be retained until at least

2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the trial medication (ICH GCP section 4.9.5).

The Investigator will be informed by the Sponsor when the documents need not to be retained any longer. The Investigator will inform the Sponsor of the storage location of the essential documents and must contact the Sponsor for approval before disposing of any. The Investigator should take measures to prevent accidental or premature destruction of these documents.

In case the Investigator retires, relocates, or for any other reason withdraws from the responsibility for maintaining records for the period of time required, custody of the records has to be transferred to any other person who accepts responsibility for the records, e.g., another Investigator, or the Sponsor. Notice of such transfer has to be given in writing to the Sponsor.

13.4.1 Investigator Site File (ISF)

The Investigator will be provided with an *Investigator Site File* (ISF). In the ISF the Investigator will store those documents necessary for the clinical trial. As part of the monitoring procedures, the ISF will be checked for up to date information and completeness in accordance with the national and international regulations.

The Investigator records in the *Patient Identification Log* the following details for all persons giving their consent to participate in the trial: name (first and surname), date of birth and the assigned unique 7-digit trial patient number. The Screening Visit date must also be documented. This *Patient Identification Log* allows patients identification and remains onsite with the Investigator.

All trial documentation, including the *Patient Identification Log*, will be properly archived in accordance with the Sponsor's instructions.

14. General Regulations, Agreements and Organizational Procedures

14.1 Responsibilities of the Investigator (Among Others)

The Investigator agrees to carry out the trial in accordance with the guidelines and procedures outlined in this clinical trial protocol. The Investigator especially consents to strictly adhere to the ethical principles (see section 12 of this protocol).

The Investigator knows that he/she must, according to professional regulations for physicians, obtain the approval of the Ethics Committee.

Any deviation from the trial protocol must, before its implementation, be agreed by the Sponsor in writing, and by the Ethics Committee initially consulted.

Changes to the protocol require a written 'amendment to the protocol' and written approval by the Coordinating/ Principal Investigator. Changes are allowed only if they are ethically justifiable. The amendment must be passed on to all participating Investigators with the obligation to adhere to its provisions. If warranted, the patient information has to be changed accordingly.

It is within the responsibility of the Investigator that an eCRF is completed consecutively and signed after the patient has finished the trial for each patient participating in the trial. All data that the Investigator provides to the Sponsor or its delegate(s) will be verified and signed by the Investigator

or his adequately qualified Deputy. Verification and signature will take place on an ongoing basis for each relevant data section (e.g., newly completed visit in a given patient).

All entries on paper must be made in black ballpoint pen; corrections are made by placing a single horizontal line through the incorrect entry, so that it can still be seen, and placing the revised entry beside it. The revised entry must be filled in, initialed and dated by a member of the Investigator's research team authorized to make paper entries. Correction fluid must not be used.

Upon Sponsor's request or latest at the conclusion of the trial, the Investigator will return all partly used, unused and empty drug containers to the Sponsor.

By signing this protocol, the Investigator confirms that he/she has read the entire trial protocol, agrees to its procedures, and will comply strictly with the formulated guidelines.

14.2 Audits and Inspections

In the interests of quality assurance, independent experts appointed by the Sponsor may carry out audits during the clinical trial's implementation phase and after its completion. In conjunction with the audit it may also be examined whether the planning, implementation and analysis of the clinical trial meets the relevant statutory regulations and the requirements of GCP. This includes a review of data maintenance and organization at the investigational site, inspection of equipment and laboratories and of the source documents. The Investigator will permit direct access to all trial documents, drug accountability records, medical records, and source data.

Regulatory Authorities may perform an inspection of the trial, even up to several years after its completion. If an inspection is announced, the Sponsor must be informed immediately.

14.3 Final Report and Publication/ Registry

An integrated Clinical Trial Report in accordance with the ICH Harmonized Tripartite Guideline (E3) (Structure and content of clinical study reports) will be developed.

14.3.1 Confidentiality

Any information provided by the Sponsor related to this trial and all data and records generated in the course of the trial conduct, in particular the objectives and contents of this clinical trial as well as its results are to be treated as confidential and may not be made accessible to third parties. All aforementioned information, data or records shall not be used for any other purpose than conducting the trial without prior written approval of the Sponsor. If confidential information will be disclosed to employees participating in the trial, the disclosing person shall ensure that the respective employees are bound by this confidentiality obligation.

14.3.2 Data Ownership

All data and records provided by the Sponsor or generated during the trial (other than patient's medical records) and all data and inventions discovered in the course of conducting the trial, whether patentable or not, are the sole and exclusive property of the Sponsor to the extent legally possible.

14.3.3 Publications

Any publication of the results, either in part or in total, will require the prior written agreement of the Coordinating/ Principal Investigator and the Sponsor. With this written agreement the Coordinating/

Principal Investigator and Sponsor will engage to form a Publication Steering Committee to the extent it is necessary, composed of all relevant stakeholders, the Coordinating/ Principal Investigator, Sponsor and other parties as needed and publish the primary clinical trial manuscript within 18 months from trial completion. All parties to follow Good Publication Practice and (CONsolidated Standards of Reporting Trials) CONSORT guidelines in planning, writing and approving publications. The Publication Steering Committee will determine the publication strategy and plan, and will also invite relevant stakeholders including Sponsor to author publications according International Committee of Medical Journal Editors (ICMJE) authorship criteria. Publication authors will draft and approve the congress abstracts, manuscripts and/or other publications. Professional medical writers may be involved to support authors.

15. References

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16. Appendices

None