Protocol Title:

A Randomized, Double-Blind, Vehicle-Controlled, Multicenter, Parallel-Design, Phase 2 Study to Assess the Efficacy and Safety of CLE-400 Topical Gel for the Treatment of Chronic Pruritus in Adult Subjects with Notalgia Paresthetica

NCT06262607

22 July 2024

Certain portions of this protocol have been redacted in order to protect personally identifiable information (PII) and company confidential information (CCI). Redacted content may include, but is not limited to:

- Names of study personnel, investigators, or institutions.
- Proprietary instruments, scales, coding systems, or other methodologies designated as confidential.
- Additional information considered necessary to protect the confidentiality of Clexio Biosciences,
- safeguard personal privacy, or maintain the scientific integrity of the clinical study.

A RANDOMIZED, DOUBLE-BLIND, VEHICLE-CONTROLLED, MULTICENTER, PARALLEL-DESIGN, PHASE 2 STUDY TO ASSESS THE EFFICACY AND SAFETY OF CLE-400 TOPICAL GEL FOR THE TREATMENT OF CHRONIC PRURITUS IN ADULT SUBJECTS WITH NOTALGIA PARESTHETICA

PROTOCOL NUMBER: CLE400-NP-201

Sponsor:	Clexio Biosciences Ltd. [redacted]
[redacted]	[redacted]

Protocol Version 1.0: 28-NOV-2022

[redacted]

Protocol Amendment Version 2.0: 22-Jul 2024

STATEMENT OF COMPLIANCE

The trial will be conducted in accordance with the protocol, International Council for Harmonisation (ICH) Good Clinical Practice (GCP), and applicable local regulations. The principal investigator will assure that no planned deviation from, or changes to the protocol will take place without prior agreement from the sponsor and documented approval from the institutional review board (IRB)/research ethics board (REB), except where necessary to eliminate an immediate hazard(s) to the trial subjects. All personnel involved in the conduct of this study have completed ICH GCP training.

The protocol, informed consent form(s), recruitment materials, and all subject materials will be submitted to the IRB/REB for review and approval. Approval of both the protocol and the consent form must be obtained before any subject is enrolled. Any amendment to the protocol will require review and approval by the IRB/REB before the changes are implemented to the study. All changes to the consent form will be IRB/REB approved.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and provides the necessary assurances that this trial will be conducted according to this protocol, applicable local regulations, and ICH GCP guidelines.

[redacted]

PRINCIPAL/QUALIFIED INVESTIGATOR SIGNATURE PAGE

[redacted]

I have carefully read and understand the foregoing protocol and agree it contains all the necessary information for conducting this study safely.

I will agree to personally conduct this study in strict accordance with this protocol, International Conference on Harmonization (ICH) guidelines for Good Clinical Practice (GCP), the Code of Federal Regulations, local regulatory and ethical principles that have their origin in the Declaration of Helsinki. I will attempt to complete the study within the time designated.

I will ensure the rights, safety, and welfare of subjects under my care are protected. I will ensure control of the drugs under investigation in this study.

I will provide access to the protocol and all other study-related information supplied by the sponsor to all personnel who participate in the study. I will discuss this information with them to assure that they are adequately informed regarding the drug and conduct of the study.

I agree to keep records on all Subject information (case report forms, shipment and drug return forms and all other information collected during the study) and drug disposition in accordance with Food and Drug Administration (FDA) regulations. I agree to retain and maintain strict accountability of the Investigational Medicinal Products supplied to the study site.

I will not enroll any subjects into this study, until Institutional Review Board/Research Ethics Board (IRB/REB) approval and sponsor's approval are obtained for the protocol.

LIST OF PROTOCOL SUMMARY

1.1 Synopsis

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

Title of Study:

A Randomized, Double-blind, Vehicle-controlled, Multicenter, Parallel-Design, Phase 2 Study to Assess the Efficacy and Safety of CLE-400 Topical Gel for the Treatment of Chronic Pruritus in Adult Subjects with Notalgia Paresthetica

Phase of Development:

Phase 2

Study Centers:

This study will be conducted at [redacted] study centers located in the United States.

Number of Subjects (planned):

Approximately 54 subjects are planned to be randomized in this study (approximately 27 subjects/arm).

Duration of Study:

The maximum study duration per subject is approximately 11 weeks, including up to 37 days for the screening period (including a 7-day run-in period), 4 weeks for the treatment period, and 2 weeks for the follow-up period.

Study Treatments, Dosage, and Mode of Administration:

CLE-400 gel will be provided at a concentration of 0.28% (w/w). Matching vehicle gel (active ingredient -free vehicle) will also be provided. [redacted]

Subjects will be randomized in a 1:1 ratio on Day 1 to CLE-400 gel 0.28% or vehicle.

CLE-400/vehicle gel will be administered topically once daily (QD) for 4 weeks on the affected skin area located in the scapular/mid back region (application by a caregiver or self-application). Study treatment should be applied in the morning (at approximately the same time every day ± 1 hour, except for applications during on-site visits). During study visits conducted on-site, the study treatment will be administered under the supervision of the study staff.

The maximal amount of study treatment to be applied will be determined on Day 1 based on the size of the affected skin area, and the same area will be treated with the study treatment throughout the treatment period. [redacted]

Name of Sponsor/Company:	Name of Study Treatment:	Name of Active Ingredient:
Clexio Biosciences Ltd.	CLE-400 or Vehicle	Detomidine HCl monohydrate

[redacted]

Objectives and Endpoints:

OBJECTIVES	ENDPOINTS
Primary Objective	
To evaluate the efficacy of CLE-400 for the treatment of chronic pruritus in subjects with NP	Primary Efficacy Endpoint: • Percent change from baseline in weekly mean of the daily 24-hour WI-NRS score at Week 4.
	Secondary Efficacy Endpoints: • Proportion of subjects achieving ≥ 4-point decrease from baseline in weekly mean of the daily 24-hour WI-NRS score at Week 4. • PGI-C ratings at Week 4.
	Exploratory Efficacy Endpoints: • Percent change from baseline in weekly mean of the daily 24-hour WI-NRS at Week 2. • Change from baseline in weekly mean of the daily 24-hour WI-NRS at Week 4. • Proportion of subjects achieving ≥ 3-point decrease from baseline in weekly mean of the daily 24-hour WI-NRS score at Week 4. • Change in PGI-S ratings from baseline to Week 4. • Proportion of subjects achieving improvement ("Much improved" or "Very much improved") according to the PGI-C at Week 4. • Change from baseline in weekly mean of daily sleep quality NRS at Week 4. • Change from baseline in weekly mean of daily pain NRS at Week 4. • Change from baseline in ItchyQoL at Week 4.
Secondary Objectives	
To evaluate the safety and local tolerability of CLE-400 in subjects with chronic pruritus associated with NP	Secondary Safety Endpoints: • Incidence of TEAEs. • Change from baseline in vital signs, clinical laboratory parameters, ECGs, and ESS. • Local Tolerability Assessment (LTA).

Name of Sponsor/Company:	Name of Study Treatment:	Name of Active Ingredient:
Clexio Biosciences Ltd.	CLE-400 or Vehicle	Detomidine HCl monohydrate

To evaluate the plasma concentration of detomidine in subjects with chronic pruritus associated with NP	Secondary PK Endpoints: • Measurement of plasma concentrations of detomidine in subjects receiving active treatment.

Abbreviations: TEAE, Treatment Emergent Adverse Event; ECG, Electrocardiogram; ESS, Epworth Sleepiness Scale; ItchyQoL, Itching Quality of Life; LTA, Local Tolerability Assessment; NP, Notalgia Paresthetica; NRS, Numeric Rating Scale; PGI-C, Patient Global Impression of Itch Change; PGI-S, Patient Global Impression of Itch Severity; WI-NRS, Worst Itch Numeric Rating Scale.

Study Design:

This is a Phase 2, multicenter, randomized, vehicle-controlled, double-blind, parallel-group study to assess the efficacy, safety, and tolerability of CLE-400 for the treatment of chronic pruritus in adult subjects with notalgia paresthetica (NP). This study will also evaluate the plasma concentrations of detomidine in subjects with chronic pruritus associated with NP.

This study will include approximately 54 male and female subjects, aged 18 to 80 years (inclusive), with the following disease characteristics:

- Diagnosis of NP based on localized pruritus in a circumscribed, unilateral area in the scapular/mid-back region, with or without pigmentation changes.
- Chronic pruritus for ≥ 3 months prior to the screening visit.
- [redacted]
- Weekly mean of the daily 24-hour Worst Itch-Numeric Rating Scale (WI-NRS) score ≥ 5.0 and ≤ 9.5 and a WI-NRS score ≥ 5 on at least 3 days over the 7-day run-in period.

The study will consist of an up to 37-day screening period (including a 7-day run-in period), a 4-week treatment period, and a 2-week follow-up period. For scheduled visits, subjects will come to the study center on up to 9 occasions: screening, Day 1, Day 3, Week 1, Week 2, Week 3, Week 4, and follow-up at Week 5 and Week 6 (Week 6 visit may be remote if there is no safety concern).

All subjects will read and sign an informed consent form (ICF) prior to any screening procedures being performed and will then undergo screening for study eligibility. To be eligible to participate in the study, subjects will also complete a 7-day run-in period (from Day -7 to Day -1) during which they will be required to complete daily WI-NRS assessments in an electronic diary (e-diary). For consistency, subjects will be requested to complete the WI-NRS at a similar time each evening. Subjects will also record the pain Numeric Rating Scale (NRS; each evening) and the sleep quality NRS (each morning) in their e-diary daily during the run-in period.

After a screening period of no more than 37 days (including the 7-day run-in period), eligible subjects will be randomized in a 1:1 ratio at Day 1 to treatment with CLE-400 gel 0.28% or

Name of Sponsor/Company:	Name of Study Treatment:	Name of Active Ingredient:
Clexio Biosciences Ltd.	CLE-400 or Vehicle	Detomidine HCl monohydrate

vehicle, administered topically QD for 4 weeks. During the 4-week treatment period, the study treatment will be applied QD to the affected skin area. For each subject, the site staff will determine the amount of gel to be applied based on the size of the affected skin area. The site staff will inform the subject on the amount of gel to apply daily. [redacted] Study treatment should be applied in the morning (at approximately the same time every day ± 1 hour, except for applications during on-site visits). During study visits within the treatment period, the study treatment will be administered under the supervision of the study staff [redacted]

Subjects will be instructed to refrain from driving or operating heavy machinery if they observe an effect of the study treatment on their ability to drive or operate heavy machinery. When ESS is assessed at home, subjects will be instructed to report to the site staff if the total score is ≥16. Subjects will also be instructed on recognition of symptoms that may be related to changes in blood pressure (BP) and heart rate (HR), fall precautions (preventive precautions and guidance on pre-fall symptoms), and on contacting the site as relevant.

Efficacy will be assessed using WI-NRS, pain NRS, sleep quality NRS, Itching Quality of Life (ItchyQoL), Patient Global Impression of Itch Change (PGI-C), and Patient Global Impression of Itch Severity (PGI-S). Subjects will report their WI-NRS over the previous 24 hours every day from the start of the run-in period through the 4-week treatment period. The pain NRS and sleep quality NRS will be reported by the subject every day during the run--in period and during the 4th week of treatment. The Patient Health Questionnaire 8 (PHQ-8) will be assessed at the screening visit only as part of eligibility evaluation. A Study Participant Survey will be completed at Week 5.

Safety will be assessed by collecting adverse events (AEs), performing local tolerability assessments (LTAs), recording vital signs, performing physical examinations and electrocardiograms (ECGs), evaluating clinical laboratory results, and collecting Epworth Sleepiness Scale (ESS) scores. Assessments of BP, pulse, and ECGs will be performed pre-dose at every study visit. On Day 1, BP (systolic and diastolic) and pulse will also be recorded post-dose before the subject is discharged. BP and pulse measurements at rest will be performed in triplicate and will be followed by a measurement of orthostatic changes. Electrocardiograms will be assessed in triplicate. ESS scores will be reported by study subjects at each specified visit (predose), as well as at home in the first week on day 5, and in the following treatment weeks once at mid-week.

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate
Blood samples will be collected from all subjects to evaluate pre-dose plasma concentrations of detomidine on Day 1, Day 3, Week 1, Week 2, and Week 3, and at any time during the visits on Week 4 (or early termination [ET], if applicable), Week 5, and Week 6 (if onsite).		

Inclusion/Exclusion Criteria:

Inclusion Criteria:

In order to be eligible to participate in this study, a subject must meet all of the following criteria, either at the screening and Day 1 visits or only at one of the specified visits (screening or Day 1) as noted in the criterion:

1. Adult male or female subject aged 18 to 80 years, inclusive, at the time of consent.
2. Subject has a confirmed diagnosis of NP as per the investigator (board certified dermatologist) based on localized pruritus in a circumscribed, mostly unilateral area in the scapular/mid-back region, with or without pigmentation changes.
3. Subject is suffering from chronic pruritus at the relevant skin area for ≥ 3 months (information obtained from medical chart or subject's physician, or directly from the subject) prior to the screening visit.
4. [redacted]
5. Subject has a caregiver who can apply the gel on the affected skin area daily in the mornings, or subject is able to self-apply the gel on the affected skin area (confirmation of ability to appropriately self-apply the gel should be done at screening).
6. Subject is willing and able to complete a daily e-diary for the duration of the study and was compliant in reporting WI-NRS via the e-diary during the 7-day run-in period (i.e., reported WI-NRS at least 4 out of the 7 days during the 7-day run-in period).
7. [redacted]
8. For a subject who performs strengthening and stretching exercises specific for NP, subject has been performing these exercises for a minimum period of 14 days prior to the run-in period and agrees not to change his/her routine (type and frequency) throughout the study.
9. For a subject who is having massages, and/or physical therapy, and/or is using cervical soft collars for the treatment of NP, subject must have been using any of these therapies for a minimum period of 14 days prior to the run-in period and agrees not to change his/her routine throughout the study.

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

10. For female subject of childbearing potential involved in any sexual intercourse that could lead to pregnancy: the subject must agree to use a highly effective contraceptive method from at least 4 weeks prior to Day 1 until at least 4 weeks after the last study treatment administration. Highly effective contraceptive methods include oral, intravaginal, or transdermal combined estrogen and progesterone, oral, injectable, or implantable progesterone-only, intrauterine devices or intrauterine hormone-releasing system, double barrier methods of contraception (e.g., male condom with cervical cap, male condom with diaphragm) in conjunction with spermicide, vasectomized partner(s) (provided his vasectomy was performed ≥ 4 months prior to Screening), and bilateral tubal occlusion.

    Note: Subjects using hormonal contraceptives must have been on a stable dose for at least 4 weeks before Day 1.

    Note: The above list of contraceptive methods does not apply to subjects who are abstinent for at least 4 weeks before Day 1 and will continue to be abstinent from penile-vaginal intercourse throughout the study. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (calendar, symptothermal, post-ovulation methods) is not acceptable.

    Note: A female subject of nonchildbearing potential is defined as follows:

    - Female subject who has had surgical sterilization (hysterectomy, bilateral oophorectomy, or bilateral salpingectomy).
    - Female subject who has had a cessation of menses for at least 12 months prior to the screening visit without an alternative medical cause, and a follicle-stimulating hormone (FSH) test confirming nonchildbearing potential (refer to laboratory reference ranges for confirmatory levels).

    Note: Female subjects must agree to not have egg retrieval throughout the study and for 4 weeks after the last study treatment application.

11. For male subject involved in any sexual intercourse that could lead to pregnancy, subject must agree to use a condom throughout the study (and for at least 2 weeks after the last study treatment application).

    Note: Subjects must agree to refrain from donating sperm throughout the study and for 2 weeks after the last study treatment application.

12. Subject is willing to participate and is capable of giving informed consent.

    Note: Consent must be obtained prior to any study-related procedures.

13. Subjects must be willing to comply with all study procedures and must be available for the duration of the study.

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

Exclusion Criteria:

A subject who meets any of the following criteria at the screening and/or Day 1 visits, as applicable, will be excluded from participation in this study:

1. Subject has chronic pruritus that is related to a condition other than NP, and is either generalized, widespread on a large area, or is located in the trunk/arms, and/or it is assessed by the investigator that the subject is not able to differentiate it from the pruritus of NP (for example, chronic pruritus associated with dermatologic diseases, allergic conditions, systemic diseases, psychogenic or medication-induced pruritus).
2. Subject has acute pruritus at any area other than the predefined typical areas of the disease (as per Inclusion Criterion 2).
3. Subject with previous finding of macular amyloidosis or lichen amyloidosis in skin biopsy of the affected area (if done).
4. Subject has used gabapentin and/or pregabalin, systemic corticosteroids, or systemic sedative antihistamines within 4 weeks prior to the run-in period (Day -7).

   Note: Oral non-sedative H1 antihistaminic will be permitted during the study only if the subject has been on a stable dose for at least 2 weeks prior to run-in and continues to use the same agent at the same frequency throughout the study.
5. Subject has received the following treatments for NP:
   - Capsaicin patch on the affected skin area within 12 weeks prior to the run-in period (Day -7).
   - Topical corticosteroids or capsaicin cream on the affected skin area within 2 weeks prior to the run-in period (Day -7).
   - Treatment with any other topical medication for pruritus on the affected skin area (including emollients containing active components such as urea, polidocanol, lidocaine, pramoxine, amitriptyline, ketamine, menthol, camphor, cannabidiol [CBD] oils), topical calcineurin inhibitors, phosphodiesterase-4 [PDE-4] and Janus Kinase [JAK] / signal transducer and activator of transcription [STAT] inhibitors, or cold/hot packs) within 1 week prior to the run-in period (Day -7).

     Note: Application of emollients without active components on the affected skin area should be avoided during the study starting at Day -7, unless regularly applied for at least 4 weeks before screening and subject continues to apply at the same frequency throughout the study.
   - Subject has used thalidomide for the treatment of NP within 6 weeks prior to the run-in period (Day -7).

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

- Subject has had cryolipolysis, or cervical or thoracic spine surgery (e.g., discectomy with or without fusion, spinal fusion, etc.) for the treatment of NP within 6 months prior to the run-in period (Day -7).
- Subject has received transcutaneous electrical nerve stimulation (TENS), electrical muscle stimulation (EMS), spinal manipulation, osteopathic manipulative therapy, or acupuncture for the treatment of NP within 2 weeks prior to the run-in period (Day -7).
- Subject has used intralesional corticosteroids, has received paravertebral local anesthetic block or trigger point injections, or has had cervical traction for the treatment of NP within 4 weeks prior to the run-in period (Day -7).
- Subject has used botulinum toxin injections for the treatment of NP within 12 weeks prior to the run-in period (Day -7).
- Subject has received biological agent (e.g., or dupilumab) within 12 weeks or 5 half-lives (whichever is longer) prior to the run-in period (Day -7).
- Subject has undergone surgical decompression for the treatment of NP within 12 weeks prior to the run-in period (Day -7).
- Subject has had phototherapy (including tanning beds), laser therapy, or psoralen-UV-A (PUVA) treatment to the back to treat NP within 4 weeks prior to the run-in period (Day -7).

6. Subject has a clinically significant skin disorder affecting the planned area of application or within 3 cm from it (e.g., atopic dermatitis, lymphedema, immature scar) that is chronic or currently active, or may interfere with the study assessments, including open wounds or sores or otherwise non-intact skin. Subjects with only typical pigmentation changes or scratch lesions may be included.
7. Subject has received hair removal treatments such as waxing, epilation, or laser treatments at the affected skin area within 2 weeks prior to the run-in period (Day -7).

   Note: Subject should not remove hair throughout the study in the affected skin area.
8. Subject has clinical signs of skin atrophy (parchment-like skin appearance and/or visible telangiectasia) on the planned area of application (e.g., in subjects on long-term treatment with corticosteroids).
9. Subject has received treatment with or nonmedical use of any of the below within 4 weeks or 5 half-lives (whichever is longer) prior to the run-in period (Day -7):
   - α2-adrenoreceptor (AR) antagonists (e.g., mirtazapine);
   - Other α2-AR agonists (e.g., clonidine, guanabenz, methyldopa);
   - Sedating medications including sedative antihistamines, sedatives (benzodiazepines, benzodiazepine-like drugs, barbiturates), hypnotics, opioid-receptor agonists, and

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

over-the-counter (OTC) products with sedating properties (e.g., Benadryl [Diphenhydramine], Doxilamine [Unisom], Melatonin, Valerian supplement, CBD, or cannabis).

- Medications that are known to prolong the QT interval (e.g., antimicrobial agents such as fluoroquinolones, macrolides, and antifungal agents; antipsychotic agents that prolong QT; antidepressants such as tricyclic antidepressants [TCAs] and selective serotonin reuptake inhibitors [SSRIs]).

10. Subject has a known hypersensitivity to detomidine, or any other α2-AR agonists, or to any of the excipients in CLE-400.
11. Subject has been diagnosed with hypotension or is at risk of hypotension based on investigator judgement (e.g., has experienced repeated episodes of symptomatic hypotension, repeated drops in systolic blood pressure [SBP] below 90 mmHg or in diastolic blood pressure [DBP] below 60 mmHg, repeated episodes of orthostatic hypotension, fainting spells, or blackouts in the past).
12. Subject has SBP < 90 mmHg or > 160 mmHg or bradycardia with heart rate (HR) < 55 bpm at screening (physically fit subjects with a HR < 55 bpm, but no less than 45 bpm, may be enrolled with sponsor approval in cases where clinically significant bradycardia can be ruled out); symptomatic hypotension during screening period, or orthostatic hypotension at screening defined as a SBP decrease ≥ 20 mmHg or DBP decrease ≥ 10 mmHg when standing up from the seated or supine position.
13. Subject has received treatment with HR lowering medications (e.g., amiodarone, beta-blockers, non-dihydropyridines calcium channel-blockers [diltiazem and verapamil], digoxin) within 2 weeks prior to the run-in period (Day -7).
14. Subject is receiving an anti-hypertensive treatment which has not been stable for at least 4 weeks prior to screening.
15. Subject has a history of bradycardia with HR < 55 bpm and no reasonable explanation, or a history of sick sinus syndrome or sinoatrial (SA) block unless treated with pacemaker, or evidence of sick sinus syndrome or SA block in ECG at screening.
16. Subject has any clinically significant abnormality identified on the screening 12-lead ECG: prolonged QRS duration (QRS > 120 ms), QTcF > 480 ms, first degree atrioventricular (AV) block with PQ > 240 ms (PR where there is no Q wave), second- or third-degree AV block.
17. Subject has a current angina pectoris, or history of stable or unstable angina or myocardial infarction (MI) in the past 6 months, and/or heart failure with reduced ejection fraction (EF ≤ 45%) and/or symptomatic heart failure (New York Heart Association Criteria Class ≥ III) at screening.

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

18. Subject has clinically significant peripheral vascular disease (as evidenced by a history of intermittent claudication, limb ischemia, or ischemic limb ulcers), active Raynaud's phenomenon, thromboangiitis obliterans, scleroderma, or Sjögren's syndrome.
19. Subject has uncontrolled diabetes mellitus with hemoglobin A1c (HbA1C) > 10% or repeated episodes of symptomatic hypoglycemia in the last 6 months, or evidence of severe Diabetic Neuropathy.
20. Subject has a history of cerebrovascular disorders: stroke of any kind, transient ischemic attack (TIA) unless subject had revascularization for internal carotid artery stenosis with no TIA since revascularization or known hemodynamically significant carotid artery stenosis.
21. Subject has a current major depression episode or history of significant uncontrolled psychiatric disorders in the last year prior to the run-in period (Day -7).
22. Subject has a known history of clinically significant drug or alcohol abuse in the last year prior to the run-in period (including recreational substance use judged by the investigator not to be compatible with study participation).
23. Subject has current symptoms of depression with a PHQ-8 score ≥ 10 at screening.
24. Subject has an active malignancy or history of malignancy within 5 years prior to screening, with the exception of adequately treated localized carcinoma in situ of the cervix, and of successfully resected basal cell carcinoma or skin squamous cell carcinoma not located in the affected skin area or < 10 cm away.
25. Subject has a current thoracic spinal tumor or lesion.
26. Subject has aspartate transaminase (AST) or alanine transaminase (ALT) or total bilirubin ≥ 2.5 times the upper limit of normal or known moderate-severe hepatic insufficiency.
27. Subject has estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73m^2 or known chronic kidney disease (CKD) stage ≥ 3.
28. Subject has a known history of human immunodeficiency virus (HIV), hepatitis B virus (HBV), or hepatitis C virus (HCV; however, a subject with documented proof of cure from HCV can be enrolled).
29. Subject has a known active COVID-19 infection during the screening period (rescreening may occur 10 days or greater after the onset of COVID-19, provided that symptoms have resolved).
30. Subject is a female who is breastfeeding, pregnant, or who is planning to become pregnant during the study.
31. Subject with known or suspected history of a clinically significant systemic disease, unstable medical disorders, life-threatening disease, including

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

physical/laboratory/ECG/vital signs abnormality that would, in the opinion of the investigator, put the subject at undue risk or interfere with interpretation of study results.

32. Subject has received a nonbiological investigational product or device within 4 weeks prior to the run-in period (Day -7) or has received any investigational biological agent within 12 weeks or 5 half-lives (whichever is longer) prior to the run-in period (Day -7).
33. Planned major surgery during the clinical study.
34. Sponsor or CRO employees or employees under the direct supervision of the investigator and/or involved directly in the trial, or their family members/subjects in same household.
35. In the opinion of the investigator, the subject is unsuitable for participating in the study.

Statistical methods:

The significance level of this study will be 0.05, 2-sided.

A fixed sequence gatekeeping (hierarchical) approach will be applied to adjust for multiplicity and to control type I error due to multiple efficacy endpoints testing of the primary and secondary endpoints. The sequence order is according to the defined order of the primary and secondary endpoints. If the primary analysis is statistically significant at the 2-sided 0.05 level, the secondary endpoints will be analyzed sequentially according to the order of the secondary endpoints as listed above. A secondary endpoint will only be considered statistically significant at the 2-sided 0.05 level if the previous secondary or primary endpoint in the hierarchy is significant at the 2-sided 0.05 level.

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Analysis sets:

The Intent-To-Treat (ITT) Analysis Set will include all randomized subjects. In this analysis set, treatment is assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually received.

The Full Analysis Set (FAS), which is a subset of the ITT Analysis Set, will include all randomized subjects who received at least 1 dose of study treatment and have both baseline and at least one postbaseline WI-NRS evaluation. In this analysis set, treatment is assigned based on the treatment to which subjects were randomized, regardless of which treatment they actually received.

The Safety Analysis Set includes all subjects who receive at least 1 dose of study treatment.

The ITT Analysis Set will be used for summaries of subject demographics and baseline variables.

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

The FAS Analysis Set will serve as the principal analysis set for efficacy inference.

The Safety Analysis Sets will be used for analyses of safety variables.

Efficacy Analyses:

Efficacy will be evaluated on the basis of the FAS analysis set.

For all WI-NRS endpoints (% change and change from baseline and proportion of subjects), the weekly mean of the 24-hour WI-NRS will be defined as the sum of the daily 24-hour WI-NRS score reported during a specific week of the study (e.g., Days 1 to 7, Days 8 to 14, Days 15 to 21, etc.) divided by the number of days with nonmissing scores for that week. For all WI-NRS endpoints, the baseline score will be defined as the average of all nonmissing daily 24-hour WI-NRS scores over the run-in period (Day -7 to Day -1); at least 4 completed WI-NRS assessments will be required during the run-in period.

For the primary efficacy endpoint, the percent change from baseline in weekly mean of daily 24-hour WI-NRS, the statistical model to be used for inference will be the Mixed Models Repeated Measures (MMRM) analysis using the SAS® MIXED procedure with the REPEATED subcommand. The model will include terms for treatment, categorical Week in study (1, 2, 3, and 4), treatment by week interaction, pooled sites, Baseline weekly mean WI-NRS and Baseline weekly mean WI-NRS by week interaction. The percent change from baseline in the weekly mean of daily 24-hour WI-NRS score will be the response variable in the model. The model derived least squares means of percent changes at Week 4 will be compared between the CLE-400 group and the vehicle group. The model will use the unstructured covariance matrix, the Restricted Maximum Likelihood (REML) estimation method, and the Kenward Roger adjustment method for the degrees of freedom. In the case that the model does not converge, the Maximum-Likelihood estimation method will be used instead of the default REML. If the model still does not converge, then a simpler covariance structure with fewer parameters will be used according to the following order: heterogeneous autoregressive (1), heterogeneous compound symmetry, autoregressive (1), and compound symmetry.

Safety Analyses:

Safety analyses will include TEAEs, LTA, laboratory tests, vital signs, ESS scores, and ECGs.

No inferential statistics will be done on safety variables.

Pharmacokinetic Analyses:

Detomidine concentration data will be listed per subject and summarized by visit, dose and nominal timepoints using descriptive statistics.

Name of Sponsor/Company: Clexio Biosciences Ltd.	**Name of Study Treatment:** CLE-400 or Vehicle	**Name of Active Ingredient:** Detomidine HCl monohydrate

Sample Size Consideration:

The sample size was determined based on the following assumptions: a difference of 24% in the percent change from baseline to Week 4 in WI-NRS between CLE-400 and vehicle, a randomization ratio of 1:1, a standard deviation (SD) of 30%, a power of 81%, a 2-sided alpha level of 0.05, and an overall drop-out rate of 5%.

Under the above assumptions, the estimated number of subjects required for this study is 54 subjects (27 subjects per treatment arm).

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