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History of changes

Version	Date	Modification(s)
1.1	December 20, 2021	First complete version
1.2	January 6, 2022	Updated definition of V1, V2 and wording on screening in Section 5.4, clarified text on "AEs leading to discontinuation", removed table for schedule of events, and edits to Section 9 on Safety Analysis to align better with language in the protocol
1.3	January 15, 2022	Added: additional information in preface, analysis of PGIC, and likability defined the safety set and other minor edits
1.4	March 9, 2022	Edits to address feedback from the January 19, 2022 DSMB meeting, including mediation analysis for rescue medication use
1.5	April 19, 2022	Edits for updates for C-SSRS
1.6	June 6, 2022	Removed Inclusion/Exclusion criteria since that is in the ISA, changed multiple imputation to last observation carried forward for prohibited medication use for primary analysis and indicate that the proposed estimands provide supporting information for primary analysis
1.7	April 11, 2023	Reduced sample size from 260 (130 per arm) to 122 (61 per arm) based on an effect size of 0.52 instead of 0.35, Added section discussing the reduction of sample size (current Sec 6.1), Removed Inclusion/Exclusion criteria about female of childbearing potential, Removed blinded criteria about SD does not exceed 1.2
1.8	June 20, 2023	Updated visit schedules as in Table 1 ISA
1.9	Sept 14, 2023	Fixed numbering issues about EOs on Sections 4.3 and 10.5. They are now aligned.
2.0	Apr 15, 2024	Updated Appendix B inclusion criteria. Specifically, 1) the score changed to ≥ 4 points and < 10 from ≥ 5,≤ 9. 2) the criteria about completion of the daily pain assessment on at least out of the 7 days, has been moved to the PP inclusion criteria. 3) the criteria about not having ≥ 1 score of 10 points of the 24-average pain NRS has been deleted. See Amendment 4.8.24 and Appendix B version 3.0 of 4.8.24 for details. Clarified some of the "allowed medications". Specifically, in Section 5.3 Concomitant Medications, removed 1)"for insomnia" and "for depression" 2) removed SSRI for anxiety (the dose must have been stable for 8 weeks).

Version	Date	Modification(s)
2.1	Apr 26, 2024	Added EO 17 sec. 10.9.6 Sec 3.2 changed screening phase language with "A defined 7-day screening period and a defined 7-day baseline period" changed Figure 1 Updated "Visit Schedules" on Sec 3.4 Revised whole Sec 5.4 - It now matches the one from the ISA Revised Sec 4.2.1 Primary Safety Endpoint Updated sec 4.3.6 with EO17
2.2	May 21, 2024	Double check and aligned dates with version 8.0 of ISA Updated Figure 1 - better resolution
2.3	Jan 21, 2025	Updated Section 7.3 to reflect the change in score (from version 2.0)
2.4	Apr 07, 2025	Added a sentence at the beginning of the Section 3 saying that the SAP will exclusively analyze subjects randomized to treatment within the ISA (NRD) protocol, with both the Platform and ISA protocols comprehensively described in the Clinical Study Report. Section 3: removed PK sub-study paragraph. Updated Section 4.1.1 adding info on primary WAP Week 12 endpoint, this will be computed by simply averaging available NRS's from week 12. Removed sections 4.3.5-4.3.7 regarding EO 10-17 and PK analysis. Updated the last paragraph of Section 7 regarding the primary analysis, this will be conducted using an ITT principle: all randomized participants within the ISA. Updated the first sentence of Section 10.1 changing from mITT to ITT as in Section 7. Removed mentioning of gatekeeping. Section 7.2: Removed mentioning regarding number of participants screened. Section 10.1: Removed mentions about FDA not allowing continuation. Replaced from Section 10.4.2 the likelihood ratio test with the Wald test. Removed from Section 10.4.3, the adjusted analysis using logistic regression –because we are not sure how many people will have occurrence of 30% or 50% reduction in WAP from baseline. Also added Fisher's Exact Test in case of small frequencies. Removed the gatekeeping approach of testing from Section 10.4.3. Updated Section 10.5.1: 1) included analysis at discrete time points 2) replaced likelihood ratio test with Wald test 3) removed text about multiple comparisons 4) change language about superior/positive effect to different

Version	Date	Modification(s)
2.4 (cont'd)	Apr 07, 2025	Updated Section 10.5.2: 1) EO 3a removed mixed effects model, included analysis at discrete time points. 2) added a comment about independent missingness (EO 3b); 3) changed language about superior to different (EO 3c) 4) removed generalized linear mixed effect model and changed to difference in proportions (EO 3c); 4) Removed NNT. 5) Changed Cox model to Kaplan-Meier estimator Section 10.5.3: 1) Removed EO5 (mediation analysis) 2) Changed generalized linear mixed-model with mixed-effect model and analysis at discrete time points. 3) Changed Cox model to Kaplan-Meier estimator Removed Section 10.9 Other Objectives (EO 12-16) Removed Section 10.10 PK/PD Analyses
2.5	Jun 12, 2025	Section 10.5.1 removed TCNS and mTCNS from the list of neuropathy-specific instruments. Removed Section 10.8 describing objectives 10-11. Section 7: changed back to mITT from ITT with mITT description. Removed Section 10.4.4, Number-Needed-to-Treat (NNT) analysis for secondary efficacy endpoints. Section 10.5.2, EO3b: revised the language as in the protocol. "the change from the last 7 days of DB treatment to SBPWOP with respect to WAP and WWP"

Version	Date	Modification(s)
2.5 (cont'd)	Jun 12, 2025	Current Section 10.8: Added back old EO12 Exploratory Objective Related Likability, now EO10. We had previously removed EO12-16 due to limited resources and the secondary nature of these objectives. However, we added back old EO12 (likability) now EO10, because considered important by CCC during protocol development. Section 10.1: 1) added some language for the non-compliance definition; 2) added some language for multiple NRS same day; Section 4.2.2: 1) added "Exposure to study drug" as an additional safety endpoint in 2. and 3. 2) added "AEs in relation to study drug" as an additional safety endpoint in 2. and 3. Section 3.1: 1) Removed 4th paragraph due to repetitions 2) change to 127 participants from 122. Added DSIS: Daily Sleep Interference Score to table of Abbreviations and Definitions. Section 3.3: 1) Bullet point 1.: Clarified that the start of double blind period is day 1 (the day of randomization to treatment within ISA). 2) Bullet point 2.: Changed 0-6 to 1-7 3) Bullet point 3.: Added definition of Week 1, 1-7 days. Section 3.4: 1) V3: Changed Day 0 to Day 1. V9: added comment about "on-time" Section 4.3.3: added list of prohibited medication with input from the Medical Monitor. Section 5.3: changed NEForm app to Medaple app. Section 5.4: Updated n=122 to n=127. Section 7.3: removed site as a conditioning variable. Section 10.4.1: revised the proposed model. Added Section 10.2.2: multiple imputation details. Removed "site" as a random effect from modeling. Cleaned up modeling details.

Version	Date	Modification(s)
2.5	Jun 25, 2025	Removed exploratory objective to evaluate natural and inderect effects of treatment,
		Minor edits to fix typos
2.6	Sep 05, 2025	Added back EO11-17 and PK/PD analysis
		Added back NNT analysis and EO5
		Addressed DSMB comments 10.4.1-10.5.3.
		Polished statistical methods for exploratory endpoints.
		Polished estimand for sensitivity analysis (MNAR across all weeks).

1 SAP Signatures

I give my approval of the attached SAP entitled "EN21-01: A MULTICENTER, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY TO ASSESS THE SAFETY AND EFFICACY OF 80 MG DAILY OF NRD135S.E1 VERSUS PLACEBO IN ADULT AND ELDERLY PARTICIPANTS WITH PAINFUL DIABETIC PERIPHERAL NEUROPATHY (SERENDIPITY-1)"

Dated: _____

Protocol Principal Investigator

Name: _____

Signature: _____

Date: _____

CCC Protocol Principal Investigators

Names: _____

Signature: _____

Date: _____

DCC Protocol co-Principal Investigator

Names: _____

Signature: _____

Date: _____

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2 Abbreviations and Definitions

API	Active Pharmaceutical Ingredient
AUC	Differences in dose-normalized exposure
CGIC	Clinician Global Impression of Change
CI	Confidence interval
DRL	Dr. Reddy's Laboratories Ltd.
DSIS	Daily Sleep Interference Score
EOS	End of study
ET	Early Termination
IE	Intercurrent event
IP	Investigational Product
ISA	Intervention Specific Appendix
IXRS	Interactive Web Response System
MAR	Missing-at-Random
MI	Multiple imputation
mITT	Modified-Intent-to-Treat
MNAR	Missing-Not-at-Random
NNT	Number-Needed-to-Treat
NOAEL	No-Observed-Adverse-Effect-Level
NSAID	Non-Steroidal Anti-Inflammatory Drug
PGIC	Patient Global Impression of Change
PK	Pharmacokinetics
SAP	Statistical Analysis Plan
SB	Single-Blind
SBPWOP	Single-Blind-Placebo-Washout-Phase
SMQ	Standard MedDRA Query
VPK	PK Sub-Study Visit
WAP	Weekly Average Pain, defined as the mean over a 7-day period of the ratings on the "average daily pain" on NRS
WWP	Weekly Worst Pain, defined as the mean over a 7-day period of the ratings on the "worst daily pain" on NRS

3 Introduction

The Statistical Analysis Plan (SAP) will exclusively analyze subjects randomized to treatment within the ISA (NRD) protocol, with both the Platform and ISA protocols comprehensively described in the Clinical Study Report.

3.1 Preface

This Statistical Analysis Plan (SAP) describes all planned analyses for a 12-week, double-blind multicenter, Phase II study of the safety, tolerability, and effect of NRD135S.E1 (80mg/day). The content of this SAP is based on the final version of the EPPIC-Net platform protocol for painful diabetic peripheral neuropathy (PDPN) and the Intervention Specific Appendix (ISA) for NRD135S.E1, EN21-01.

People with diabetes are at risk for various forms of peripheral neuropathy, the most common of which is a length-dependent, axonal, primarily sensory polyneuropathy. This form of diabetic peripheral neuropathy is characterized by sensory loss which typically begins in the feet, which is fairly symmetric and may progress to involve more proximal parts of the lower limbs as well as the hands. This study focuses on a subset of patients reporting pain due to their diabetic peripheral neuropathy, referred to hereafter as Painful Diabetic Peripheral Neuropathy (PDPN). This pain may be experienced as burning, aching, hypersensitivity to touch, or simply as pain. It is important to distinguish patients with neuropathic pain from those with uncomfortable numbness in isolation because the latter is unlikely to respond to symptomatic treatments without neuroregenerative properties.

NRD135S.E1 is identified as a potential treatment for PDPN. It is a fully synthetic derivative of a bacterium. It has been optimized for its anti-nociceptive effects in multiple animal models of acute and chronic pain. The molecular target of NRD135S.E1 is not known, though it does not appear to work through any of the opioid receptors or molecular pathways currently targeted by pain relief. NRD135S.E1 has been well tolerated at all dose levels tested in single-dose (up to 1,200 mg in healthy volunteers) and repeat-dose regimens (up to 300 mg/day over 5 days in healthy volunteers or 150 mg over 3 weeks in subjects with PDPN), and it has been shown to have predictable pharmacokinetics with dose-dependent increases in exposure. The study NRD135S.E1-201 in subjects with PDPN, both the 40-mg and 150-mg daily doses induced a clinically relevant reduction in pain.

This trial utilized a 1:1 randomization to NRD135S.E1 (administered in the morning orally) versus matching placebo with the intent of randomizing a total of 127 participants in order to have at least 80% power to detect a standardized effect size of 0.52.

The PK sub-study will be described in a separate SAP.

3.2 Scope of the Analyses

Using a parallel trial design, to study the safety and efficacy of NRD135S.E1 (80mg/day) for the treatment of PDPN.

Figure 1 illustrates the overall design for this study. This schema shows 2 arms: NRD135S.E1 and placebo with the following phases:

- A defined 7-day screening period and a defined 7-day baseline period
- A double-blind treatment phase (12 weeks),
- A single-blind 1-week placebo washout phase (to assess for withdrawal symptoms),
- A safety follow-up phase (30 days from the end of the double-blind treatment phase).

Information to be collected daily includes: time of IP dose, use/timing of any rescue medication, 11-point Numeric Rating Scale (NRS) for average and worst pain, and DSIS. On days the study drug is taken at site, the time of drug intake will also be recorded by the site personnel in the eCRF.

3.3 General Definitions

The following conventions are used to define several of the analysis endpoints described in this SAP and in the protocol/ISA:

1. Start of double blind period is day 1 (the day of randomization to treatment within ISA).

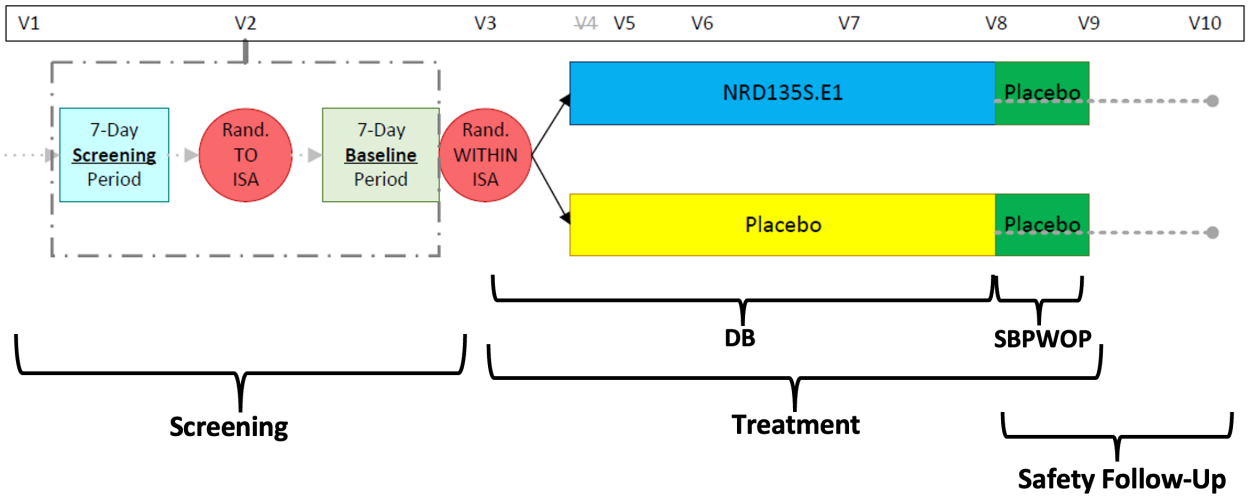

Figure 1: Schema for the parallel arm trial

2. The week number is the next highest integer after the study day divided by 7 (e.g., days 1-7 are week 1). The numbering of the weeks is independent of the visits.
3. Week 1 is days 1-7. Week 12 is the interval starting on Day 78 and ending on Day 84.
4. Week 0 is the baseline week and includes the last 7 days before randomization; it is used as the baseline for the computation of the change in weekly NRS pain measures from baseline. For values obtained once on the randomization day (e.g., specific safety measures), this randomization-day value will be the baseline value.
5. EoDB is reached on the morning of Visit 8 (V8; Day 84), i.e., the day after the last dose of DB study treatment, or earlier in the case of premature treatment discontinuation.
6. The Safety follow-up phase starts at EoDB and lasts 30 days.
7. The SBPWOP (Single-Blind Placebo Washout Phase; considered a withdrawal phase) starts at the EoDB (V8) with the first dose of single-blind placebo treatment after DB and lasts 7 days.
8. EoT (End of Treatment) is reached after all assessments have been performed on the day of V9.
9. End of Study (EoS) is the final check-in which can be performed by audio or video call to assess the participant for any intervening adverse events. Following this call, study participation is complete.

3.4 Visit Schedules

The following notation is used to denote timing of visits:

V1	Visit 1	Screening visit (single day) between -60 to -8
V2	Visit 2	Screening visits, variable duration between -59 to -1
V3	Day 1	IP initiation
V4		Visit 4 has been removed from the study
V5	Day 7 (+/- 1 day)	Early safety
V6	Day 28 (+/- 4 days)	Full follow-up visit (week 4)
V7	Day 56 (+/- 4 days)	Full follow-up visit (week 8)
V8/ET	Day 84 (+/- 4 days)	EoDB, Full follow-up visit (week 12)
VPK	Single Day (between 14-77)	PK Sub-study
V9	Day 91* (+ 3 days)	EoT, Withdrawal check.
V10/EOS	Day 114 (+ 7 days)	Final Safety Check

* An "on-time" subject would be in the SBWOP on days 85-91.

4 Study Objectives and Endpoints

The objectives of this study are categorized as Primary, Safety and Exploratory.

4.1 Primary Objective

To demonstrate that NRD135S.E1 80 mg daily is superior to placebo in relieving neuropathic pain associated with PDPN after 12 weeks' treatment.

4.1.1 Primary Efficacy Endpoint

Change from the baseline to Week 12 in the weekly mean of the daily 24-hour average pain measured by NRS (abbreviated herein as WAP for weekly average pain). WAP endpoint for a given week will be computed by simply averaging available NRS's from that week. If more than one NRS on the same day, we only use the first NRS of the day.

4.1.2 Secondary Efficacy Endpoints

a) Occurrence of 30% reduction of WAP from baseline to Week 12.

b) Occurrence of 50% reduction of WAP from baseline to Week 12.

4.2 Safety Objectives

To assess the safety and tolerability of oral administration of NRD135S.E1 80mg/daily in participants with PDPN.

4.2.1 Primary Safety Endpoint

The frequency (i.e. number of participants) with treatment emergent adverse events (TEAEs) reported in the time period defined by first administration of IP (V3) until 7 days after the last dose of DB IP (EoDB), i.e., the V3/Initiation of IP visit through the V9/Withdrawal Check or ET visit.

4.2.2 Additional Safety Endpoints

1. The frequency during the screening period of:

- AEs
- SAEs
- AEs leading to discontinuation
- ECG abnormalities
- Marked Laboratory Abnormalities (MLAs)

2. The frequency during the double-blind treatment period of:
   - TEAEs
   - SAEs
   - AEs leading to discontinuation
   - Treatment-emergent ECG abnormalities
   - Treatment-emergent MLAs
   - Exposure to study drug
   - AEs in relation to study drug.
3. The frequency during the single-blind wash out period of:
   - TEAEs
   - SAEs
   - AE's leading to discontinuation
   - Treatment-emergent ECG abnormalities
   - Treatment-emergent MLAs
   - Exposure to study drug
   - AEs in relation to study drug.
4. The frequency during safety follow-up of:
   - Occurrence of SAEs
5. Change from baseline to the last value and change from baseline to the worst value (defined as the value resulting in the largest unfavorable change from baseline) up to and including EoDB and EoT in:
   - MNSI
   - Vital signs (systolic and diastolic blood pressures, pulse rate, respiratory rate, and body temperature)
   - Body weight
   - ECG parameters
   - Laboratory variables
6. For the C-SSRS, the number and percentage of subjects with suicidal ideation or suicidal behavior during the study will be tabulated.

4.3 Exploratory Objectives (EO) and Endpoints

4.3.1 Objectives Related to General Neuropathy and Pain Outcome Measures

1. To assess the effect of NRD135S.E1 in comparison to placebo with respect to Core HEAL CDEs:
   a) Pain, Enjoyment, and General Activity Scale (PEG)
   b) Pain Catastrophizing Scale - Short Form 6 (PCS-SF6)
   c) (PROMIS) Physical Functioning Short Form 6b
   d) Patient Health Questionnaire (PHQ-2)
   e) General Anxiety Disorder Scale (GAD-2)
   f) Patient Global Impression of Change (PGIC) (Kroenke *et al.*, 2019)
   g) Tobacco, Alcohol Prescription Medication Scale (TAPS 1)
   h) Sleep Duration Scale
   i) (PROMIS) Sleep Disturbance – 6a
   j) Opioid Use Questionnaire (OUQ).

   These core HEAL endpoints are collected at baseline and at week 12.
2. To assess the effect of NRD135S.E1 in comparison to placebo with respect to secondary outcome measures:
   a) UENS
   b) Norfolk QOL-DN
   c) MNSI.

   These secondary outcomes are obtained at 4 time points: baseline, and weeks 4, 8, and 12.

4.3.2 Objectives Related to Daily NRS at Different Time Points During the Study

3. Compare NRD135S.E1 and placebo in terms of:
   a) The change from baseline to all study weeks until Week 12 and to the end of SBPWOP with respect to WAP and Weekly Worst Pain (WWP).
   b) The change from the last 7 days of DB treatment to SBPWOP with respect to WAP and WWP.
   c) The occurrence of 30% and 50% reduction from baseline at all study weeks for WAP.
   d) The time from the start of DB to the:
      i) First occurrence of a week with 30% and 50% reduction for WAP from baseline
      ii) Confirmed occurrence (i.e., next week's WAP mean being smaller or equal confirms reduction)
      iii) Sustained occurrence (if confirmed and still present at Week 12).
      iv) Additionally, a cumulative display of the percent pain reduction each week will be generated for both arms.

4.3.3 Objectives Related to the Use of Rescue and Prohibited Medication

The list of prohibited medications are provided in the ISA with input from the Medical Monitor.

Starting with the screening period, the only allowable analgesic rescue medication that can be taken for pain is acetaminophen up to a maximum of 3g per day in divided doses. The amount of rescue medication taken each day will be recorded by the participant in the Medable app.

4. To compare the use of rescue medication in the NRD135S.E1 and placebo arms in terms of:
    a) Average rescue analgesic consumption by week from baseline till EoT.
    b) The change from baseline to all study weeks until Week 12 and to SBPWOP with respect to weekly consumption of rescue analgesic (i.e., total mg of acetaminophen taken per week).
    c) The change from Week 12 to SBPWOP with respect to weekly consumption of rescue analgesic (i.e., total mg of acetaminophen taken per week).
    d) Time to first intake of rescue medication and/or prohibited pain medication during Weeks 1-12 and SBPWOP separately.
    e) Frequency of any use of rescue medication and/or prohibited pain medication during Weeks 1-12 and SBPWOP separately.
5. To evaluate the natural direct and indirect effect of NRD135S.E1 on the primary endpoint while considering the use of rescue medication as a mediator.

4.3.4 Objectives Related to Additional Patient-Reported Outcome Measures

6. Compare NRD135S.E1 and placebo in terms of change from the start of DB to EoDB, start of DB to EoT, and EoDB to EoT in:
    a) 24-hour average pain measured by VAS
    b) Neuropathic Pain Symptom Inventory (NPSI)
    c) Brief Pain Inventory – Short Form (BPI-SF) scores
    d) Short form-12 (SF-12).
7. Compare NRD135S.E1 and placebo in terms of change from start of DB to EoDB and to EoT in Insomnia Severity Index (ISI©).
8. Compare NRD135S.E1 and placebo in terms of change from baseline period to last 7 days of DB and to last 7 days of SBPWOP, and from last 7 days of DB to last 7 days of SBPWOP in weekly mean of Daily Sleep Interference Score (DSIS).
9. Compare NRD135S.E1 and placebo in terms of change in CGIC at EoDB.

4.3.5 Objectives Related to Withdrawal Symptoms

10. To summarize AEs suggestive of "drug abuse, dependence and withdrawal" using a Standard MedDRA Query (SMQ) for each treatment arm.
11. To evaluate the presence of withdrawal symptoms and rebound effects after abrupt interruption of NRD135S.E1 by comparing NRD135S.E1 and placebo in terms of:
    a) Change of WAP from the baseline to SBPWOP.
    b) Change of WAP from last 7 days of DB to SBPWOP.
    c) DESS-43 score at V9.

4.3.6 Other Objectives

12. To compare NRD125S.E1 to placebo on the likability (0-10 scale). A higher likability for an active drug indicates potential abuse liability.
13. To evaluate and compare the course of symptom severity throughout the 12-week double-blind period for NRD135S.E1 and placebo in terms of longitudinal trajectories.
14. Explore sociodemographic and clinical predictors of response to NRD135S.E1

15. Characterize activity levels and sleep patterns in participants with PDPN treated with NRD135S.E1 compared to a placebo using actigraphy measures. Participants will wear the actigraphy watch the week prior to visits V1, V3, V6, V7, V8 and V9, on their non-dominant wrist for 12-20 hours per day.
16. Characterize the impact of heterogeneity based on pre-trial treatment and pre-trial responsiveness to PDPN medications on the outcome measures.
17. Subgroup analysis to examine the impact of changing in the inclusion criterion for baseline WAP on the primary efficacy WAP outcome for the subgroup of participants whose WAP is 5 or above.

5 Study Methods

5.1 General Study Design and Plan

This is a randomized, double-blind parallel design to study if NRD135S.E1 80 mg daily is superior to placebo for treating PDPN pain.

5.2 Inclusion and Exclusion Criteria and General Study Population

Specifics on the inclusion/exclusion criteria are provided in the ISA. In addition, the following criteria comes from Appendix B: Blinded inclusion criteria not listed in the ISA:

- Patient-reported daily 11-point NRS (for average pain over the last 24 hours) meets the following criteria during both the 7-day screening and 7-day baseline periods. The algorithm will be assessed centrally.
    a. Has a score of ≥ 4 points and < 10 points on the weekly mean of the 24-hour average pain NRS

5.3 Concomitant Medications

Starting with the screening period, the only allowable analgesic rescue medication that can be taken for pain is acetaminophen up to a maximum of 3g per day in divided doses. The amount of rescue medication taken each day will be recorded by the participant in the Medable app.

NRD135S.E1 may have the propensity to be affected by drug-drug interactions (DDIs), which has to be considered when concomitant medications are to be given to participants. Strong inhibitors and strong inducers of CYP3A4, as well as CYP3A4 substrates with a narrow therapeutic index, are not allowed.

The following concomitant therapies (and foods) are prohibited during the study:

- Prohibited central nervous system medications and pain-affecting medications, including over-the-counter medication and herbal medicine;
- Treatment with strong CYP3A4 inducers or inhibitors;
- Treatment with CYP3A4 substrates with a narrow therapeutic range;
- Treatment with strong modulators of Phase-II metabolism.
- Consumption of grapefruit and grapefruit juice is forbidden from randomization during the study until 24 hours after EoT.

Therapies considered necessary for the participant's wellbeing and not categorized as prohibited concomitant medications can be used in this study. Participants will be allowed to take the following medications for comorbid conditions listed below. The dose administered should not exceed the maximum dose allowed by the label, must have been stable for at least 4 weeks before screening (or more if indicated below), and remain stable throughout the study (as needed use is not allowed):

- A benzodiazepine, zolpidem, diphenhydramine, melatonin, melatonin receptor agonist, or orexin antagonist

- A Selective Serotonin Reuptake Inhibitor (SSRI)
- Oral aspirin $\leq$ 325 mg/day for cardiovascular or cerebrovascular protection

5.4 Randomization and Blinding

Randomization will be completed in a stepwise fashion, via IXRS:

- Step 1: As part of the Platform Protocol, participants will be randomized to an ISA, unblinded.
- Step 2: Once all eligibility criteria have been confirmed for both the Platform Protocol and the ISA, the site may proceed to randomization within an ISA, or randomization to treatment.

Randomization to treatment assignment will be blinded from study participants, staff from clinical sites, investigators, asset owner, IND sponsors, and/or designees. This blinding will be accomplished by providing identical investigational product and packaging for the NRD135S.E1 and its corresponding control treatment. The use of IXRS will ensure the blinding is accomplished.

127 participants were be randomized in a 1:1 ratio according to a central randomization scheme provided by the Almac centralized web-based Interactive Web Response System (IXRS) to the designated study pharmacist (or qualified designee) to the following treatment groups as follows:

1. NRD135S.E1 80 mg/day
2. Matching placebo

Randomization will be stratified by PDPN severity (moderate vs. severe, based on WAP from the baseline period).

5.4.1 Re-Randomization to an ISA

Participants that screen fail after having been randomized to an ISA, may be able to randomize to another ISA. If the participant is willing, they may be re-randomized between any other current ISAs for which they appear to be potentially eligible. Once randomized to a different ISA, the participant should be consented for that ISA and complete procedures as outlined in that ISA.

6 Sample Size

Simulations were performed in R (R Core Team, 2024) to investigate the statistical power under the selected sample size for the primary efficacy hypothesis using the ANCOVA model (1) described in Section (10.4.1), for a 2-sided significance level of $\alpha = 0.05$. Various parameter settings in the simulations were used to assess their impact on statistical power. The power of the test depends on the correlation between the baseline and week 12 daily average pain scores and therefore simulations were run for a range of correlations from $\rho = 0$ to 0.80.

Bivariate normal data (representing the baseline and week 12 WAP) was generated for the NRD135S.E1 and placebo arms for various values of correlation between baseline and week 12, assuming the NRD135S.E1 and placebo arms have the same covariance matrices. The WAP for baseline was set to 7. Data was generated using the R software. 5000 data sets were simulated for each scenario to estimate power. A sample size of $n = 61$ per arm was considered. Cohen's standardized effect size of $ES = 0.52$ was used to simulate the data which is defined to be a ratio with numerator equal to the difference (placebo minus NRD135S.E1) in the mean differences (baseline minus week 12 daily average). This difference is standardized by dividing the standard deviation of the baseline week pain in the placebo arm which was set to 1 in the simulations.

For each simulated data set, an ANCOVA model was fit to the data with the difference (baseline - week 12) as the outcome and baseline pain and a treatment indicator as covariates, see Section (10.4.1) for model details. The proportion of p-values (across all simulated data sets) for testing if the treatment indicator coefficient differs from zero that are less than 0.05 provides an estimate of the power. The solid black curve in Figure (2) shows a plot of power (versus correlation between baseline and week 12 WAP). Also shown

in Figure (2) is the power for conducting a 2-sample t-test to compare the change score of NRD135S.E1 to placebo (red dotted curve). The 2-sample t-test is considerably less powerful compared to the ANCOVA approach unless the correlation becomes large. Note that the power estimates in Figure (2) are conservative. For example, adjusting for covariates sex, age, and BMI, assuming that these two covariates explain 10% of the variability, gives higher power indicated by the blue dashed curve in Figure (2).

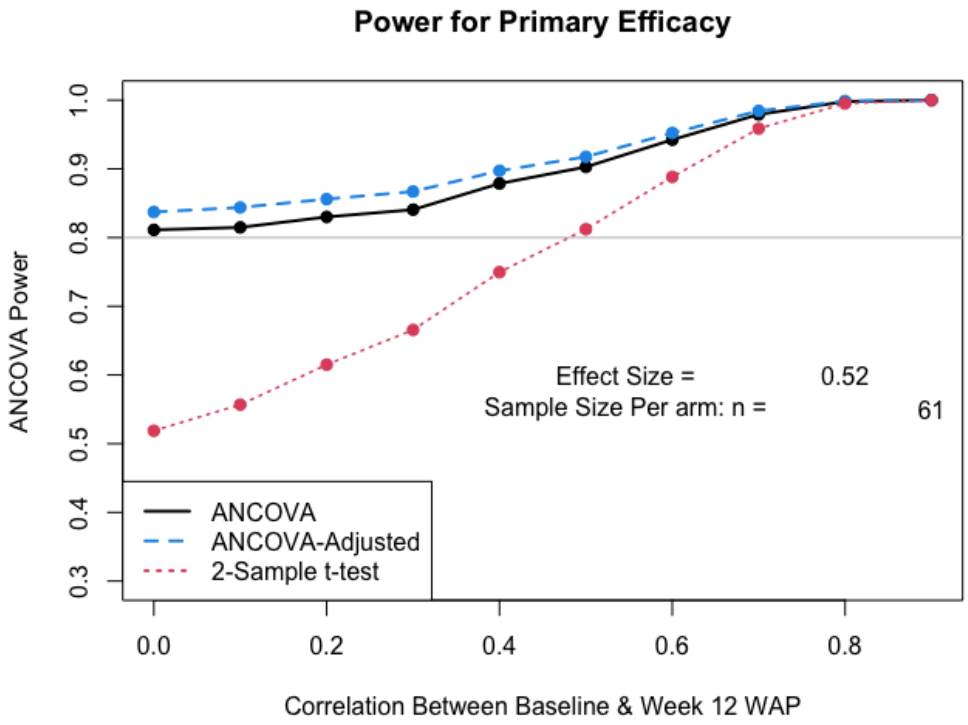

Figure 2: Power versus correlation (between baseline and last week of WAP).

For the sample size calculation, we used ANCOVA, as this approach is standard and provides a conservative basis for powering trials. However, ANCOVA was not used in the primary efficacy analysis.

6.1 Reduction of sample size

Previous versions of this SAP suggested a sample size of 130 per arm, 260 total. We proposed a reduction of sample size of 61 per arm based on the following rationale.

EPPIC Net's choice of 130 per arm was based on a SES of 0.35. The FDA expressed concerns about randomizing 130 per arm in this early Phase 2 study. FDA required the asset owner to conduct a metabolite study as a condition to proceed beyond 122 subjects. With 61 per arm, the study has 80% power to detect an ES of 0.52 (difference of 1.20 between active treatment and placebo, SD 2.35, on NRS change from baseline). The critical value for the test is 0.36 on the SES scale (matching 0.84 ± 2.35 NRS units), *i.e.*, if the estimated SES exceeds 0.36, we would reject the null at a one-sided 0.025 level. Novaremed has strong justification for delta 1.20 and SD of 2.35, and the sample size of 61 per arm was in Novaremed's original protocol submitted to FDA, and FDA approved it.

7 General Analysis Considerations

All primary hypothesis tests will be two-sided using $\alpha = 0.05$ significance level, and p-values unadjusted for multiple testing will be reported. A gatekeeping approach will be used for hypotheses that are tested only

if the testing of the hypothesis will follow based a significant finding of a test that immediately precedes the hypothesis. Point estimates, confidence intervals (at the 95% confidence level) will be computed.

The primary analyses will be performed according to the modified Intent-To-Treat (mITT) principle – all randomized subjects within ISA who received at least one dose of drug.

7.1 Timing of Analyses

There are no planned interim analyses for this trial. The final analysis will occur when the study finishes (after data lock).

7.2 Analysis Populations

The study population will consist of adult and elderly male and female individuals with moderate to severe PDPN. Vulnerable participants are not eligible since the study can be carried out in a non-vulnerable group of participants and no particular benefit has been anticipated for vulnerable participants which would be different from that in the general population. This protocol is intended for patients with moderate to severe PDPN who are willing to refrain from the use of other pain medications during the trial.

This study randomized 127 participants to either NRD135S.E1 or placebo in a 1:1 ratio.

We will consider the following analysis populations:

- *ITT*: All randomized subjects within ISA
- *mITT*: All randomized subjects within ISA who received at least one dose of DB treatment
- *Safety*: All randomized subjects within ISA who received at least one dose of DB treatment
- *PP*: All randomized subjects within ISA who received at least one dose of DB treatment and no major protocol deviations

7.3 Covariates and Subgroups

All analyses will adjust for sex, age, BMI and the WAP from the baseline week.

7.4 Interim Analyses and Data Monitoring

No interim analysis is planned for this study.

8 Summary of Study Data

All demographic and baseline variables will be summarized by means, standard deviations, medians, and interquartile ranges (IQR) for continuous measures. The frequency and percentages will be reported for all categorical measures (including binary measures).

The ISA gives tables listing prohibited medications before and during the study (these tables are also reported in the Appendix of the ISA).

9 Safety Analyses

The Safety Set (SAF) includes all subjects who received at least one dose of study medication. Subjects will be analyzed according to the treatment they received. A primary objective of this trial is to evaluate the safety of NRD135S.E1 (80mg/day) for the treatment of PDPN. The primary safety endpoint is the incidence of treatment emergent adverse events (TEAEs), reported between the administration of study drug on Day 1 and the completion of the study.

Safety Aim: Assess the safety of NRD135S.E1 80 mg daily treatment.

Safety of the NRD135S.E1 80 mg daily treatment will be assessed based on the endpoints listed in Section (4.2.1).

There will be no hypothesis testing under the safety objective. Instead, summary statistics will be presented separately for each of the two treatment groups and at different time points. The definition of TEAE will also include AEs during the SBPWOP. A separate output, tabulating frequency of AEs occurring only during SBPWOP will be recorded and assessed - this may allow assessment of a drift in AE frequency during withdrawal. We will compare the two treatment groups with respect to: (a) the proportion of patients with TEAEs; (b) the number of TEAEs; and (c) rate of TEAEs, i.e., the number of TEAEs divided by number of patient-days exposed to the study treatments. Point estimates will be accompanied by 95% confidence intervals. Safety will also be assessed using the occurrence of SAEs at the final safety follow-up visit.

10 Efficacy Analyses

10.1 General Statistical Considerations

Participants will be analyzed according to the treatment they were randomized to receive. As described in Section (4.1.1), the primary outcome is the change in the weekly average pain (WAP). The WAP is obtained by taking the (arithmetic) mean of the daily ratings of "average daily pain" on the 11-point (0-10) NRS scale during a specific week.

No interim analyses are planned for this ISA.

Noncompliance was defined as taking less than 80% or more than 120% of IP during any evaluation period (visit to visit). Discontinuation for noncompliance was at the investigator's discretion and was noted on the eCRF.

If multiple NRS within the same day for the same participant are reported, the earliest NRS will be used for the analysis.

10.2 Intercurrent Events and the Treatment of Missing Data

All available efficacy data will be used in the analysis except data during the periods when a patient was taking a prohibited pain medication or in the 3 days after last use of a prohibited pain medication (the latency period). All days on which prohibited pain medication is used plus a latency period of three days following the last day of use will be considered an intercurrent event. This policy on prohibited medication use applies for participants even if they take prohibited medications on more than two occasions when there is no safety concern on the use of such medication. Daily efficacy data obtained during the intercurrent event will be set to missing and these missing values will be imputed as discussed in the next section. Section 10.3 describes two estimands to be used as supportive analyses using multiple imputation for data set to missing for use of prohibited medication. For the first estimand, the missing values will be imputed under the missing-at-random (MAR) assumption by borrowing information from patients in the same treatment arm. The second estimand describes a sensitivity analyses that uses multiple imputation that will be conditional on a participant's previously observed outcomes and all baseline characteristics, and borrowing information from patients randomized to placebo, even for participants that were randomized to NRD135S.E1 (i.e., a missing-not-at-random, MNAR, approach).

10.2.1 Missing Data for Reasons Other Than Use of Prohibited Pain Medication

MI (assuming MAR) will be used to impute missing data for reasons other than use of prohibited pain medication, following intensive investigations to ensure reliable and valid MI.

10.2.2 Multiple Imputation (MI) details

MAR. To address missingness in the daily pain scores, we will implement multiple imputation using chained equations via the `mice` package in R. Twenty imputed datasets will be generated, each with 20 iterations, and all imputations will be initialized using a fixed random seed (1234) to ensure reproducibility.

Imputations will be conducted at the *daily observation level*, after which the *weekly average pain score* (WAP) will be computed by averaging daily pain scores within each week for each subject.

To account for potential differences in missing data mechanisms across treatment arms, imputations will be performed separately within each treatment group. Within each arm, the imputation model will include the following covariates:

- Demographics: age, sex, and BMI
- Temporal information: categorical rating period (week)
- Outcome history: lagged pain score from the previous day, included to reflect a first-order Markov assumption that current pain may depend on the most recent prior observation
- Baseline severity: average baseline pain, computed using observed values prior to randomization

The primary outcome variable will be imputed using the standard linear regression method (`norm`), while the lagged pain score at baseline will be imputed using predictive mean matching (`pmm`).

This imputation strategy preserves treatment-specific outcome distributions and avoids borrowing outcome information across arms, aligning with the assumption that the missing data process may differ by treatment assignment.

MNAR. To assess sensitivity to violations of the MAR assumption, we will implement a MNAR imputation strategy that conservatively assumes participants who used prohibited medications behave like those in the placebo group (reference-based imputation) (Carpenter *et al.*, 2013). Specifically, a modified imputation assignment variable will be defined such that any participant who used prohibited medications is imputed using the model fitted to the placebo group, regardless of their originally assigned treatment. As in the MAR scenario, imputations will be performed separately within each imputation group. The same covariates and modeling approach will be used as in the MAR imputations, including demographic factors, temporal information, baseline severity, and lagged outcome history. This approach reflects a plausible MNAR scenario in which treatment effect estimates are attenuated among participants with post-randomization protocol deviations, such as prohibited medication use.

10.3 Estimands for Supporting Primary Analyses

This section defines the estimands providing supporting information for the primary efficacy analysis and sensitivity analysis with details on handling of intercurrent events.

10.3.1 Estimand Providing Supporting Information for the Primary Efficacy Objective

The following attributes describe the estimand that provides supporting analysis for the primary efficacy analysis where multiple imputation is used instead of last observation carried forward when prohibited medication is used:

- Population: As defined by the Inclusion/Exclusion criteria.
- Endpoint: The difference in the change from baseline to week-12 between randomized groups in the WAP. The arithmetic mean of recorded (or imputed, using day-level data, if missing) NRS for average pain over the 7-days interval (i.e., the WAP) from day 78 to day 84 will be used as end-of-treatment (week 12) primary pain measure. The baseline WAP corresponds to the 7 days preceding randomization and start of DB treatment.
- Treatment of interest: NRD135S.E1 80mg daily or placebo.
- Handling of intercurrent events (IEs) prior to endpoint assessment.
  - Intercurrent or interim use of prohibited pain medication including the medication latency period (the 3 days following last use): Available data recorded during such IE will be set to missing.
  - Efficacy data collected during intake of rescue medication will be used for analysis as recorded.

  - Missing data due to any reason will be multiply imputed based on the MAR assumption.

- Policy strategy: We will be using a treatment policy strategy where individuals are analyzed following mITT above. Missing data will be imputed under the MAR assumption by borrowing information from patients in the same treatment arm.
- Summary measure: The estimate of the contrast between those randomized to NRD135S.E1 80mg daily versus placebo for the primary endpoint will be obtained from the estimated model (see Section 10.4.1) which includes only main effects for treatment, baseline WAP, sex, age, and BMI as covariates.

10.3.2 Estimand for the Sensitivity Analysis

This estimand quantifies the treatment effect of NRD135S.E1 80 mg vs placebo at 12 weeks of daily administration while accounting for the IE of prohibited pain medication use and additional IEs leading to study discontinuation prior to week-12. A treatment for chronic pain is not efficacious after discontinuation, thus strategies to handle missing data for participants who do not stay on the treatment for the study duration should not attribute any treatment benefit to participants after discontinuing the treatment. We will therefore assume MNAR and follow the steps described in section 10.2.2. The following attributes describe this sensitivity estimand:

- Population: As defined by the Inclusion/Exclusion criteria.
- Endpoint: Change from baseline to week-12 in the WAP.
- Treatment of interest: NRD135S.E1 80mg daily or placebo.
- Handling of intercurrent events (IEs) prior to endpoint assessment.
  - For use of prohibited pain medication (including the 3-day latency period) and treatment discontinuations (e.g., for poor tolerability or lack of efficacy) during week 1-12, available data recorded during the IE will be set to missing. Such missing data will be imputed under a MNAR assumption by borrowing information from the placebo arm patients (reference-based imputation).
  - Other missing data *week 1-12*, will be multiply imputed based on the MAR assumption.
  - Efficacy data collected during intake of rescue medication will be used for analysis as they are recorded.
- **Summary measure**: The estimate of the contrast between those randomized to NRD135S.E1 80mg daily versus placebo for the primary endpoint will be obtained from the estimated model (see Section 10.4.1) which includes only main effects for treatment, and baseline WAP, sex, age and BMI as covariates.

For each week, including Week 12, multiple imputation will be performed based on the model covariates and on the values at previous weeks from the patients in the same treatment arm for the first estimand described above to provide supporting information for the primary efficacy objective; for the sensitivity estimand analysis, MI will be performed under the MNAR assumption using data from patients in the placebo arm.

10.4 Primary Objectives

10.4.1 The Estimator

The endpoint for the primary analysis will be estimated using a repeated measures model with an unstructured covariance structure. The model is defined as:

$$\mathbf{Y}_i = \mathbf{X}_i\boldsymbol{\beta} + \boldsymbol{\varepsilon}_i, \quad \boldsymbol{\varepsilon}_i \sim \mathcal{N}(\mathbf{0}, \boldsymbol{\Sigma}), \tag{1}$$

where $\mathbf{Y}_i$ is the vector of change in WAP scores between Week 1-12 and baseline for subject i, $\mathbf{X}_i$ is the fixed-effects design matrix including terms for baseline covariates, week, treatment group, and the treatment-by-week interaction. The baseline covariates include sex, age, BMI and the WAP from the baseline week. The

residual vector $\boldsymbol{\varepsilon}_i$ is assumed to follow a multivariate normal distribution with mean zero and an unstructured covariance matrix. The $\boldsymbol{\varepsilon}_i$ are assumed independent of each other.

The primary contrast of interest is the marginal mean difference in change in WAP at Week 12 from baseline between treatment arms. We refer to this quantity as μ.

10.4.2 Primary Efficacy Endpoint: Change from the baseline to Week 12 in the WAP

The hypothesis for the primary efficacy endpoint is:

> NRD135S.E1 will be different than placebo for treating PDPN with respect to reducing pain as measured by the WAP during week 12 of treatment.

This hypothesis regarding the treatment effect corresponds to testing $H_0 : \mu = 0$ versus $H_a : \mu \neq 0$ obtained from the model (1) above. This will be tested using a t-test with significance level $\alpha = 0.05$.

Sensitivity analysis to investigate the effect of the assumptions underlying the treatment of missing data on the inferences will be conducted as described in Section 10.3.2.

10.4.3 Secondary Efficacy Endpoints: Occurrence of 30% and 50% reduction in WAP from baseline to Week 12

The hypothesis for the secondary efficacy endpoint is:

> NRD135S.E1 will have a higher occurrence of 30% and 50% reductions in WAP from baseline to Week 12 than placebo.

Improvement in pain is defined as follows: let X_0 denote the WAP during the baseline week and let X_{12} denote the WAP over days 78-84 from randomization. Then $100 \times (X_0 - X_{12})/X_0$ measures the percent improvement in pain. The following two estimates will be considered for testing:

- Difference between the two treatment groups with respect to proportion of patients with at least 30% improvement in WAP.
- Difference between the two treatment groups with respect to proportion of patients with at least 50% improvement in WAP.

The proportion of patients in the two treatment groups with the respective % improvement will be estimated from raw (observed) proportions including 95% CIs. Logistic regression models, conditioning on the baseline covariates, will be used for the analysis using the imputed datasets and Rubin's pooling.

A gatekeeping approach will be used where statistical inference for 30% improvement will proceed only if the test for primary efficacy of NRD135S.E1 compared to placebo is statistically significant at the 5% significance level. The odds ratio for 30% improvement in pain will be estimated with $\exp(\alpha_1)$ where α_1 is the coefficient of the treatment indicator; a 95% CI will be provided in addition to a formal hypothesis testing based on $H_0 : \alpha_1 = 0$

Statistical inference for 50% improvement will proceed only if the test for 30% improvement is statistically significant.

10.4.4 Number-Needed-to-Treat (NNT) analysis for secondary efficacy endpoints

The number-needed-to-treat (NNT) is defined as the reciprocal of the absolute risk reduction (i.e., the inverse of the difference in the proportion of participants with 30% (and 50%) percent improvements between NRD135S.E1 and placebo treated participants). Estimates of the NNT (and 95% CI) will be computed for 30% and 50% reductions (e.g., Altman, 1998).

10.5 Exploratory Objectives (EOs)

This section describes the statistical analysis approach for the exploratory objectives described in Section (4.3). Multiple testing corrections will not be applied in the exploratory analyses, as these are intended for hypothesis generation rather than confirmatory inference. Missing values for EOs will not be imputed.

A significance level of 5% will be used for all the following EOs, and we note that the study is not powered for these endpoints. Ninty-five% CIs will be computed for the treatment contrast (NRD135S.E1 versus placebo) in each of the models.

10.5.1 Objectives Related to Common EPPIC-Net General Neuropathy and Pain Outcome Measures (EO1 and EO2)

Table 1 summarizes the visit schedule, variable type, and planned analytic method for each of the Core HEAL CDEs described in Section 4.3 for EO1. For each model, we will conduct Wald tests to evaluate whether the treatment assignment coefficient differs significantly from zero. Covariates included in the models are listed in Section 7.3.

Table 2 summarizes the visit schedule, variable type, and planned analytic method for each of the Core HEAL CDEs described in Section 4.3 for the neuropathy-specific instruments (UENS, MNSI, Norfolk QOL-DN). For each model, we will conduct Wald t-tests with degrees of freedom adjusted using the Kenward-Roger approximation to test model coefficients, and a Wald t-test to test differences in estimated marginal means computed using the fixed effects from the model.

EO1	**Visits**	**Type of Measure**	**Method**
PEG	V3, V8	0–10 score	Linear regression of change (V8–V3) on treatment indicator and covariates
PCS-SF6	V3, V8	0–24 score	Linear regression of change (V8–V3) on treatment indicator and covariates
PROMIS 6b	V3, V8	6–30 score	Linear regression of change (V8–V3) on treatment indicator and covariates
PHQ-2	V3, V8	0–6 score	Linear regression of change (V8–V3) on treatment indicator and covariates
GAD-2	V3, V8	0–6 score	Linear regression of change (V8–V3) on treatment indicator and covariates
PGIC	V6, V8	1–7 ordinal scale	Ordinal regression (V8) on treatment indicator, and covariates (no PGIC at V6 because odds ratios blow up)
TAPS 1 – Alcohol	V3, V8	Yes / No	Descriptive only due to small cell sizes
TAPS 1 – Drugs	V3, V8	Yes / No	Descriptive only due to small cell sizes
TAPS 1 – Prescription	V3, V8	Yes / No	Descriptive only due to small cell sizes
TAPS 1 – Tobacco	V3, V8	Yes / No	Descriptive only due to small cell sizes
Sleep Duration	V3, V8	Continuous	Linear regression of change (V8–V3) on treatment indicator and covariates
PRIMIS 6a	V3, V8	Continuous	Linear regression of change (V8–V3) on treatment indicator and covariates
OUQ	V3, V8	Yes / No / Not sure	Descriptive only due to small cell sizes

Table 1 Summary of EO1 Measures and Analytical Methods

EO2	Visits	Type of Variable	Method
UENS	V3, V6, V7, V8	0–35 score	Change from V3 using Model (1)
Norfolk QOL-DN	V3, V6, V7, V8	-4–116 total score	Change from V3 using Model (1)
MNSI	V3, V6, V7, V8	0–8 score	Change from V3 using Model (1)

Table 2 Summary of EO2 Measures and Analytical Methods

10.5.2 Objectives Related to Daily NRS at Different Time Points During the Study (EO 3)

The primary and secondary efficacy endpoints for this study focus on WAP at 12 weeks (as described above in Section 10.4). In the analyses for this exploratory objective (EO 3), we will explore the effect of NRD135S.E1 compared to placebo on the NRS at different time points during the study and using both WAP and WWP.

EO 3a This EO will test if NRD135S.E1 is different to placebo in terms of change from baseline to all study weeks until Week 12 and until SBPWOP with respect to WAP and WWP.

We will follow the same approach of EO1 and EO2 presented in section 10.5.1, using model (1) for each week. Outcomes (WAP and WWP) will be considered as (Baseline – Week j) with $j = 1, \ldots, 12$.

A t-test will be performed to test for $\mu = 0$ versus $\mu \neq 0$ in model (1), across weeks for each of the outcomes.

EO 3b A linear regression of change WAP and WWP (last 7 days–SBPWOP) on treatment indicator and covariates will be used. A Wald test on the model indicator of treatment will be used to test for differences between NRD135S.E1 and placebo treatment.

EO 3c The hypotheses under this objective are that NRD135S.E1 will be different to placebo with respect to the proportion of occurrences of 30% and 50% reductions from baseline at all study weeks for WAP. We will estimate the difference in proportions for 30% and 50% reduction occurrence, and construct 95% confidence intervals and Chi-square tests (Fisher exact test if frequencies less than 5), assuming Bernoulli-distributed outcomes.

In addition, we will compute the number needed to treat (NNT). The NNT is defined as the reciprocal of the risk difference (RD) in the proportion of occurrences of 30% and 50% reductions from baseline. The RD and its 95% confidence interval will be estimated using a Wald-based normal approximation. The NNT point estimate will be calculated as NNT $= 1/\hat{RD}$, and its confidence limits obtained by inverting the RD confidence bounds and ordering the results. If the RD confidence interval includes zero, the corresponding NNT confidence interval will be unbounded and reported as including infinity.

EO 3d We will compare NRD135S.E1 and placebo in terms of the time from start of randomized treatment to the first occurrence of a week with 30% and 50% reduction in WAP from baseline (EO 3d(i)), confirmed occurrence (i.e., next WAP being smaller or equal confirms reduction) (EO 3d(ii)) and sustained occurrence (if confirmed each week and still present at Week 12) (EO 3d(iii)).

For EO 3d(i)-(iii), the unadjusted Kaplan-Meier estimator will be used to estimate treatment-specific cumulative incidence curves. A log-rank test will be used to test differences in cumulative incidence curves between NRD135S.E1 and placebo. Absolute frequencies and descriptive statistics of the time to each event will be reported.

A plot of percentage pain reduction, ie., $100 \times (X_0 - X_j)/X_0$ for $j = 1, \ldots, j$, will be generated across treatment groups (EO 3d(iv)).

10.5.3 Objectives Related to the Use of Rescue and Prohibited Medication (EO 4)

Based on the objectives described in section 4.3.3,

EO 4a We will follow the same approach of EO1 and EO2 presented in section 10.5.1, using model (1) for each week. The outcomes (weekly average of total rescue analgesic consumption in mg) will be computed for each week since baseline. An autoregressive, AR(1) variance-covariance matrix will be used.

EO 4b We will follow the same approach of EO1 and EO2 presented in section 10.5.1, using model (1) for each week. The outcomes (change in weekly average of total rescue analgesic consumption in mg) will be computed for each week since baseline as (Baseline – Week j) with $j = 1, \ldots, 12$. An autoregressive, AR(1) variance-covariance matrix will be used.

EO 4c A linear regression of change weekly average of total rescue analgesic consumption in mg (Week 12–SBP-WOP) on treatment indicator and covariates will be used. A Wald test on the model indicator of treatment will be used to test for differences between NRD135S.E1 and placebo treatment.

EO 4d The Kaplan-Meier estimator, as described under EO 3d in Section (10.5.2), will be used to estimate cumulative incidence curves across treatment arms and compare NRD135S.E1 to placebo in terms of time to first intake of rescue medication and/or prohibited pain medication during Weeks 1-12 and Week 13 separately. Log-rank test will be used.

EO 4e The use of rescue and prohibited medication, Y_{it} observed for subject i at week t, will be modeled using a generalized linear mixed model with Poisson distribution and log link will be used. Specifically, the model is defined as:

$$\log\left(\mathbb{E}[Y_{it} \mid \mathbf{X}_{it}, b_i]\right) = \mathbf{X}_{it}\boldsymbol{\beta} + b_i, \tag{2}$$

where $\mathbf{X}_{it}$ is the vector of fixed effects including treatment group, rating period, their interaction, and baseline covariates (sex, age, BMI, and baseline average score). The subject-specific random intercept $b_i \sim \mathcal{N}(0, \sigma_b^2)$ accounts for within-subject correlation across repeated measures. Estimation will be carried out using maximum likelihood. In this model, treatment effects are interpreted on the log scale of the mean event rate, with exponentiated coefficients corresponding to rate ratios.

Mediation analysis (EO 5)

The mediation analyses described below will not be part of the primary study report and may be performed at a later date.

Use of rescue medication as defined in section 4.3.3 is a post-randomization variable that can be considered as a mediator. Consequently, we will estimate the natural direct and indirect effect of NRD135S.E1 on change of WAP from baseline to Week 12. Figure 3 shows a directed acyclic graph describing the relationships between covariates, X, intervention NRD135S.E1, I, mediator use of rescue medication (average weekly use of rescue medication), M, and primary endpoint change of WAP from baseline to Week 12, Y. The blue and green edges show the direct and indirect effect, respectively. Assuming that standard mediation analysis assumptions hold (VanderWeele, 2015), and that the endpoint Y and the mediator M are continuous, we will define the following regression models,

$$Y_i = \theta_0 + \theta_1 I_i^{trt} + \theta_2 M_i + \theta_3 I_i^{trt} M_i + \boldsymbol{\theta}_4' \boldsymbol{X}_i + \epsilon_i, \tag{3}$$

$$M_i = \beta_0 + \beta_1 I_i^{trt} + \boldsymbol{\beta}_2' \boldsymbol{X}_i + \varepsilon_i, \tag{4}$$

where Y_i indicates change score of the WAP for participant i (baseline week WAP minus week 12 WAP), I_i^{trt} is an indicator for NRD135S.E1 treatment assignment ($I_i^{trt} = 1$ if patient i was assigned treatment NRD135S.E1 and $I_i^{trt} = 0$ if assigned to placebo), M_i indicates amount of rescue medication use, $\boldsymbol{X}_i$ is a vector of the ith patient's baseline covariates that includes sex, age, BMI and the baseline WAP. The terms ϵ_i and ε_i contain a random site effect plus random error for participant i and accounts for possible correlation of the outcomes of patients recruited in the same study site. We will then derive the natural direct and indirect effects by using the estimated parameters obtained from models (3)-(4) as shown in VanderWeele (2015). Standard errors and confidence intervals will be obtained by using the bootstrap (VanderWeele and Vansteelandt, 2009).

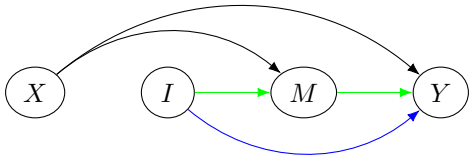

Figure 3: Directed acyclic graph showing the relationships between covariates, X, intervention NRD135S.E1, I, mediator use of rescue medication, M, and primary endpoint change of WAP from baseline to Week 12, Y.

10.6 Objectives Related to Additional Patient Reported Outcome Measures (EO 6–8)

For EO 6a–6d, we will use linear regression models to analyze change scores over three intervals: from the start of double-blind (DB) to end of DB (EoDB), from start of DB to end of treatment (EoT), and from EoDB to EoT. Each model will include the treatment indicator and baseline covariates.

Similar models will be used for EO7 considering the intervals: change from start of DB to EoDB and to EoT in Insomnia Severity Index (ISI), and for EO8, change from baseline period to last 7 days of DB and to last 7 days of SBPWOP, and from last 7 days of DB to last 7 days of SBPWOP in weekly mean of Daily Sleep Interference Score (DSIS).

10.7 Exploratory Objective Related to Clinician Global Impression of Change (EO 9)

We will compare NRD135S.E1 and placebo in terms of Clinician Global Impression of Change (CGIC). The CGIC is measured on a 7-point ordinal scale based on the following prompt "since the start of the study, the participant's overall status is", with possible responses: (1) very much improved, (2) much improved, (3) minimally improved, (4) no change, (5) minimally worse, (6) much worse, (7) very much worse. A mixed-effect ordinal logistic model (e.g., Hedeker, 2008) will be used to explore (longitudinally) differences between NRD135S.E1 and placebo at V6, V7, and V8. In particular, if Y_{ij} is the jth CGIC for participant i, then we will model

$$P(Y_{ij} \leq c|\boldsymbol{x}_i) = \frac{1}{1+\exp\{-\lambda_{ijc}\}},\ c = 1, 2, \ldots, 7,$$

where

$$\lambda_{ijc} = \gamma_c - \alpha_0 I_i^{trt} - \alpha_j \text{Time}_j - \boldsymbol{\beta}'\boldsymbol{x}_i - b_i.$$

In this model, the γ_c's are a set of strictly increasing thresholds; I_i^{trt} is a $0-1$ treatment indicator; Time_j is an indicator for the assessments V6, V7, and V8; $\boldsymbol{x}_i$ is a vector of covariates for participant i, using the same covariates as in (1); and $b_i \sim N(0, \sigma_b^2)$ is a subject-specific random effect. Estimation and testing will be performed using maximum likelihood and Wald tests respectively. Time by treatment interaction effects will be investigated to determine if any difference between NRD135S.E1 and placebo varies over the three assessment times.

10.8 Objectives Related to Withdrawal Symptoms (EO 10–11)

These analysies will not be part of the primary study report and may be performed at a later date.

10.8.1 Exploratory Objective Related to Drug Abuse, Dependence and Withdrawal (EO 10)

We will summarize AEs suggestive of "drug abuse, dependence and withdrawal" using a Standard MedDRA Query (SMQ) for each treatment arm for the double-blind treatment period and the SBPWO period separately.

10.8.2 Exploratory Objective Related to Withdrawal Symptoms (EO 11)

We will evaluate the presence of withdrawal symptoms and rebound effects after abrupt interruption of NRD135S.E1 by comparing NRD135S.E1 and placebo in terms of: (1) change from the baseline to SBPWOP in the WAP, (2) change from Week 12 to SBPWOP in the WAP and (3) DESS-43 score at V9. The DESS-43 scores for the occurrence of withdrawal symptoms obtained at the end of the SBPWOP (V9) week and this will be summarized for NRD135S.E1 and placebo treated participants. The changes in WWP and WAP between placebo and NRD135S.E1 will be estimated using 95% CIs.

10.9 Other Objectives (EO 12-17)

These analysies will not be part of the primary study report and may be performed at a later date.

10.9.1 Exploratory Objective Related Likability (EO 12)

The likability scale is measured on a 0-10 scale and will be assessed at V6, V7 and V8. NRD135S.E1 and placebo will be compared on the likability scale using the approach described for EO8.

10.9.2 Exploratory Objective Related to Course of Symptoms and Trajectory Shapes (EO 13)

We will evaluate and compare the course of symptom severity throughout the 12-week double-blind period for NRD135S.E1 and placebo in terms of longitudinal trajectories. No hypotheses are specified for this aim. Longitudinal analyses using the daily NRS pain measures over the 12-week double blind period will be performed in order to explore the pattern of symptom changes over the course of the treatment. Different trend models will be investigated (linear, quadratic and nonparametric). The nonparametric approach permits fitting flexible trajectory shapes and penalized B-splines will be used. The longitudinal models will allow the investigation as to if and when a separation between mean pain levels between NRD135S.E1 and placebo treated participants occurs and the rates of improvement between NRD135S.E1 and placebo groups over the course of treatment. In particular, by comparing and contrasting the trajectories for placebo and NRD135S.E1 treated participants, an evaluation of whether or not placebo effects are maintained or wane throughout the course of treatment can be investigated. The longitudinal approach will also be used to investigate periodic (daily, weekly, seasonable) and complex time trends.

Parametric clustering approaches (Tarpey *et al.*, 2010) will be used to identify commonalities and distinctions in pain trajectory shapes for the NRD135S.E1 and placebo treated participants as well as identify baseline characteristics that align with these groupings. The parametric clustering applies the k-means clustering algorithm (Hartigan and Wong, 1979) to a very large Monte Carlo sample from the estimated distribution of coefficients that describe the outcome trajectories for both the NRD135S.E1 and placebo study arms – this approach allows for the exploration of prototypical pain outcome trajectories that are common to both treatment groups as well as prototypical trajectories that are distinct to one or the other treatment groups.

We will also investigate if enhanced statistical power to test for differences in treatments is achievable by using the full range of longitudinal assessments (instead of just the baseline and week 12 mean NRS scores as in the ANCOVA model) by extracting an "average rate of improvement" scalar-valued measure (Tarpey *et al.*, 2021) from the outcome trajectories estimated from daily NRS ratings. The average rate of improvement scalar outcome may be a less sensitive measure of overall improvement to missing values and dropout. Additionally, enhanced statistical power for testing for a difference between the mean NRD135S.E1 and placebo trajectory over time may be possible using a weighting approach for the average rate of improvement.

10.9.3 Exploratory Objective Related to Sociodemographic Factors (EO 14)

We will explore sociodemographic and clinical predictors of response to NRD135S.E1. No formal hypothesis testing will be undertaken in this objective. For this objective, the relation of sociodemographic and clinical predictors to pain-related outcomes between treatment groups will be explored, such as differences in pain ratings between males and females, differences in outcomes associated with sociodemographic measures, the relation of changes in pain and psychological measures of distress.

10.9.4 Exploratory Objective Related to Activity Measures (EO 15)

We will characterize activity levels in participants with PDPN treated with NRD135S.E1 compared to a placebo using actigraphy measures. Actigraphy provides a rich source of data to characterize PDPN participants in terms of activity levels. There is currently very active development of statistical and machine learning methodologies for analyzing the complex data arising from actigraphy (e.g., Xiao *et al.*, 2014; Bai *et al.*, 2018). Implementing these approaches will allow the investigation of physiological and behavioral measures obtained from actigraphy that are related to pain measures due to PDPN as well as other pain-related measures (e.g., depression, anxiety). The discoveries from these analyses will undergo extensive internal cross-validation and will be reported as exploratory analyses. In particular, these explorations will allow the evaluation of periodic (daily, weekly, seasonal) and temporal trends in activity and its association with pain-related outcome measures. Functional data analysis methods for high-dimensional, correlated data along a time domain will be used to explore these associations (e.g., Ramsay and Silverman, 1997; Fan *et al.*, 2015; Reiss *et al.*, 2017; Morris, 2015).

10.9.5 Exploratory Objective Related to Heterogeneity Due to Pre-Trial Pain Treatment (EO 16)

This EO is to characterize the impact of heterogeneity due to pre-trial treatment for PDPN. To achieve this EO, this objective will be split in 2 parts: (i) explore the impact of whether or not a participant was on any PDPN treatment prior to screening and (ii) for participants on a PDPN treatment prior to screening, explore responsiveness to PDPN medications. The same models for primary (and secondary) outcomes will be used, but for (i), an additional indicator variable for any PDPN treatment prior to screening (and its interaction) will be included as a covariate. For (ii), only patients who were on a PDPN treatment prior to screening will be included in the analysis for this EO. The same models for the primary and secondary analyses will be used for (ii) except an additional covariate will be included defined as the change in pain during the 2 weeks prior to start of the IP (the interaction of this change variable with treatment will also be included in the model). This additional "change in pain" variable is defined as the following difference:

$$\text{(Pain score from day at first screen visit)} - \text{(Pain score at day of randomization)}.$$

The same analyses in (ii) will also be done for participants that were not on any PDPN pain treatment prior to screening. Further explorations will investigate heterogeneity due to pre-trial pain treatment by summarizing results based on: the type of medication, the amount of medication that was used pre-screening, and how recently pre-trial pain medication was used prior to screening.

10.9.6 Exploratory Objective Related to WAP inclusion criterion cut-off changing (EO 17)

Due to ISA protocol changes in regards to the baseline NRS WAP inclusion criterion cut-off changing from a threshold of 5 to 4, this secondary objective will examine the impact of this subgroup (baseline NRS WAP of 5 or above) on the primary efficacy analysis. This subgroup analysis will be conducted by adding a subgroup indicator variable (and its interaction with treatment group) to the ANCOVA model used for the primary efficacy analysis. Summary statistics will also be generated in order to compare subgroups on the primary outcome. This subgroup analysis will only be conducted if the sample size of the subgroup with baseline WAP score below 5 is greater than 20% of the total sample size.

10.10 PK/PD Analyses

The PK evaluation will be performed after database lock and will be described in a dedicated PK protocol. The analysis will be performed on patients from the safety set who have PK data available. Samples will be collected at the following timepoints: 0 (predose), 0.5, 1, 2, 3, 4, 6, 8, 10 and 12 hours after study drug intake. Immediately before each blood draw participants will complete the 11-point NRS for "pain right now." Plasma concentrations of NRD135S.E1 will be summarized by the usual location and scale statistics, and geometric means with Coefficient of Variation and 95% CIs. Individual participant listings will be provided. Participants will be analyzed according to the treatment they received. Derived PK parameters

will include: AUC during dosing interval (AUC_τ minimum (= predose) plasma concentration (C_{min}), C_{max}, T_{max}, $T_{1/2}$, rate constant of terminal period in plasma concentration-time curve (λ) for parent compound.

All kinetic parameters will be determined model-independently for each treatment using Phoenix WinNonlin, software. Pharmacokinetic characteristics will be determined directly from the measured concentrations; actual sampling times will be used for PK evaluation. Left-over plasma samples may be used for preliminary NRD135S.E1 metabolite assessment. Results of this preliminary metabolite assessment, if any, will be reported in a separate Research Report.

10.10.1 Pharmacodynamic (PD) Endpoints

PD endpoints will be analyzed in the mITT data FAS set from all the selected sites for PK/PD investigation. NRS "pain right now" will be summarized by the usual location and scale statistics by time point as appropriate. Individual participant listings will be provided. Participants will be analyzed according to the treatment they received.

10.10.2 Relation of Pharmacokinetic to Pharmacodynamic Endpoints

Analyses concerning the PK/PD relation will be described in the PK/PD protocol. These will include, but may not be limited to:

- The relationship between exposure and the WAP
- The relationship between exposure and the "NRS pain right now"
- The relationship between exposure and safety parameters.

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