NCT03219320

INVESTIGATIONAL PRODUCT: NYX-2925
CLINICAL PROTOCOL: NYX-2925-2001

A Randomized, Double-Blind, Parallel-Group, Placebo-Controlled, Multiple-Dose Study to Assess the Efficacy and Safety of NYX-2925 in Subjects with Neuropathic Pain Associated with Diabetic Peripheral Neuropathy

Amendment #3

Sponsor: Aptinyx Inc.
1801 Maple Avenue, Suite 4300
Evanston, IL 60201

IND# 129731

INVESTIGATOR SIGNATURE PAGE:

The signature of the investigator below constitutes his/her approval of this protocol and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol as specified in both the clinical and administrative sections, including all statements regarding confidentiality. This study will be conducted in compliance with the protocol and all applicable regulatory requirements, in accordance with Good Clinical Practices (GCPs), including International Conference on Harmonisation (ICH) Guidelines, and in general conformity with the most recent version of the Declaration of Helsinki.

Principal Investigator

_____

Printed Name

_____ _____

Signature Date

SPONSOR SIGNATURE PAGE:

This protocol will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) E6 Guideline for Good Clinical Practice, and all applicable laws and regulations including, but not limited to, those related to data privacy and clinical trial disclosure.

Aptinyx Inc.

[redacted]

[redacted]

Date

[redacted]

[redacted]

Date

[redacted]

[redacted]

Date

PROCEDURES IN CASE OF EMERGENCY

Table 1: Emergency Contact Information

24-Hour Emergency Medical Contact		
[redacted]		
Medical Monitor	[redacted]	[redacted]
Serious Adverse Event Reporting Information		
Email: [redacted]	[redacted]	

1 SYNOPSIS

IND# 129731

Name of Sponsor Company:
Aptinyx Inc.

Name of investigational product:
NYX-2925

Name of active ingredient:
(2S, 3R)-3-hydroxy-2((R)-5-isobutyryl-1-oxo-2,5-diazaspiro[3.4]octan-2-yl)butanamide

Title of Study:
A Randomized, Double-Blind, Parallel-Group, Placebo-Controlled, Multiple-Dose Study to Assess the Efficacy and Safety of NYX-2925 in Subjects with Neuropathic Pain Associated with Diabetic Peripheral Neuropathy

Principal Investigator:
To be determined

Study center(s):
Approximately 35 centers in the United States

Studied period (years):	**Phase of development:**
Estimated date first patient enrolled: July 2017 Estimated date last patient completed: November 2018	2

Objectives:

Primary objective:

- To evaluate the efficacy of multiple dose levels of NYX-2925 versus placebo in treating the neuropathic pain associated with diabetic peripheral neuropathy

Secondary objectives:

- To assess the effects of multiple dose levels of NYX-2925 versus placebo on pain characteristics, sleep interference, health status, psychological state, and global improvement
- To assess the safety and tolerability of multiple dose levels of NYX-2925

Methodology:

Study Design

The study will be a 6- to 9-week study, including a 1-to 4-week Screening Period, followed by a 4-week double-blind, randomized, placebo-controlled Treatment Period, and a 1-week Follow Up Period.

Screening Period (Week -4 to -1)

The subjects will be asked to provide informed consent for this study before any required procedures are performed. Subjects will also be required to sign an additional consent for their inclusion in a subject registry database. The database will use partially-identified subject information to review subjects' research study history within a proprietary, secure platform. Subjects who meet any of the following criteria will not be eligible for continued screening: current enrollment in another study, concurrent screening at another research site, violation of the required number of half-lives since the last research study, violation of the washout period between studies, or incorrect age for the NYX-2925-2001 study. Employees, contractors and volunteers of the study site, or relatives of any employees, contractors and volunteers of the study site are not eligible to participate.

Subjects with Type 2 diabetes who have been on stable antidiabetic medication for at least 1 month (or have stable glycemic control with diet and exercise alone) and who have been suffering from painful diabetic peripheral neuropathy in the lower extremities for at least 6 months but not more than 10 years will be screened for diabetic peripheral neuropathy using the Michigan Neuropathy Screening Instrument and the Masquerading Disorders Tool. Subjects must have a score of ≥4 and ≤9 on the 11-point Numerical Rating Scale (NRS) for average pain over the past 24 hours at Visit 1. Subjects will be assessed for widespread pain symptoms using the American College of Rheumatology Fibromyalgia Criteria. Additional procedures during Visit 1 will include the administration of the Hospital Anxiety and Depression Scale (HADS), completion of the Sheehan Suicidality Tracking Scale (S-STS), adverse event collection which will begin at the time of informed consent, demographic characteristics, medical history including concomitant medications, complete physical examination (with comprehensive neurological examination), 5-minute electrocardiogram, vital signs (sitting blood pressure and pulse), height, body weight, and collection of blood and urine samples for chemistry, Human immunodeficiency virus (HIV), hepatitis, triglycerides, hematology (including HbA1c), and urinalysis. All subjects will undergo urine drug and alcohol screen using a local urine testing kit and breathalyzer, respectively. Subjects who test positive for marijuana, opioids, benzodiazepines inconsistent with current prescriptions, or alcohol will not be allowed to continue in the study. Female subjects of childbearing potential will be tested for pregnancy using a local urine testing kit and will be counseled to begin or continue using highly effective contraception.

Eligible subjects who meet all entry criteria will enter a 1- to 4-week Screening Period, during which they will discontinue all except 1 (if applicable) of their pharmacologic analgesic treatments for neuropathic pain associated with diabetic peripheral neuropathy, and complete daily pain diaries. The duration of the Screening Period will depend upon the analgesic treatment that is being discontinued. The only allowed analgesics for painful diabetic peripheral neuropathy may not be an *N*-methyl-D-aspartate receptor ligand, must be non-opioid and non-sedative, and must not interfere with subjects' pain reporting. The allowed analgesic must have been taken for at least 1 month (30 days) prior to Visit 1, and subjects must be on a stable fixed dose that is not expected to change during the study. Subjects already taking no more than 1 allowed concomitant analgesic medication at Visit 1 may directly begin Week -1 of the Screening Period.

During Visit 1, eligible subjects will be provided a handheld device, and as a method for ensuring consistency and reliability of pain scoring, subjects will be instructed at Visits 1 through 3 on how to record their pain on the device. Subjects will also be educated at Visits 1 through 3 on appropriate expectations around their participation in a clinical study and the importance of consistently and accurately reporting their pain throughout the study. Review of these educational materials may be repeated for some or all subjects depending on findings of an ongoing blinded data review (e.g., if pain score variability is increased on a subject or site level).

Subjects will be dispensed acetaminophen to be used as rescue medication, and will be instructed to take no more than 2 g/daily (one to two 500-mg caplets every 4 to 6 hours as needed) for DPN pain.

Beginning immediately after Visit 1, pain intensity and rescue medication use will be recorded in the study-issued handheld device daily at bedtime. Subjects will enter their average pain intensity, worst pain intensity, pain on walking, and whether rescue medication was used during the past 24 hours. Pain intensity will be recorded using an 11-point (NRS), with 0 being no pain and 10 being the worst pain imaginable. Every morning upon awakening, subjects will complete the Daily Sleep Interference Scale (DSIS) via their study-issued handheld device.

Screening Period (Week -1)

The investigative site staff will contact study subjects by telephone weekly through Week -1 to reinforce the reporting instructions (i.e., diary completion) and to assess adverse events. Subjects will be asked a non-leading question to inquire about potential adverse events, “Have you experienced any new or changed symptoms since we last asked/since last week?” Subjects taking no more than 1 allowed concomitant analgesic medications at Visit 1 may directly begin Week -1 of the Screening Period.

Baseline Visit (Week 0)

At Visit 2 (Baseline Visit), study personnel will verify eligibility with the inclusion and exclusion criteria when the subjects are on site, and prior to randomization. Pain scores reported by subjects during the Screening Period will be evaluated by the interactive response technology system for raw score and for variability among scores after transmission of pain scores from the handheld devices to determine randomization eligibility The interactive response technology system will notify the site if the subjects is “Eligible” or “Not eligible.” No other information will be provided.

Subjects whose mean of the daily average pain intensity score during the preceding 7 (±1) days is within the protocol-defined algorithm and with adequate compliance with daily diary completion will be eligible for randomization. The absolute pain score and variability among scores, as well as the actual percentage required for diary compliance will be masked to investigators and subjects. Subject eligibility for randomization into the study based on these variables will be communicated to the investigator via the interactive response technology system.

Subjects will again be asked a non-leading question to assess potential adverse events. Use of concomitant medications will be documented. Vital signs (blood pressure and pulse) will be measured after sitting for 5 minutes and a 5-minute electrocardiogram will be performed. Medical history will be updated and a brief physical examination performed. Blood and urine samples will be collected for chemistry, hematology, and urinalysis testing. Subjects will undergo drug and alcohol screen using a local urine testing kit and breathalyzer, respectively. Subjects who test positive for marijuana, opioids,

benzodiazepines inconsistent with current prescriptions, or alcohol, will not be allowed to continue in the study. Female subjects of childbearing potential will be tested for pregnancy using a local urine testing kit and will be counseled to continue using highly effective contraception.

Eligible subjects will be randomized to receive either NYX-2925 or placebo for 4 weeks. Investigators and subjects will be masked to the randomization allocation.

Subjects will be dispensed a 2-week supply of investigational product and instructed to take 2 capsules by mouth once daily. Subjects will also be re-dispensed acetaminophen to use as rescue medication for DPN pain; instructions not to exceed 2 g/day will be reinforced. The appropriate use of rescue medication will be assessed by inventory of the returned caplets, as well as by subject interview.

Subjects will be instructed to continue entering their average pain intensity, worst pain intensity, pain upon walking, and whether they used rescue medication over the past 24 hours into their study-issued handheld devices every night. Every morning upon awakening, subjects will complete the DSIS via their study-issued handheld device. Pain diary compliance will be reviewed at each study visit by the study staff, and reporting instructions will be reinforced.

Quantitative sensory testing will be done using the Bedside Sensory Testing Kit. The following scales will be completed at the Baseline Visit: Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN), the Short-Form McGill Pain Questionnaire version 2 (SF-MPQ-2), the Norfolk Quality of Life Questionnaire - Diabetic Neuropathy (QOL-DN), the HADS, the Insomnia Severity Index, and the S-STS.

One week following the Week 0 visit, subjects will be contacted by telephone for an assessment of adverse events and to reinforce diary completion. Adverse events will be assessed by asking the subject a non-leading question.

Treatment Period: Week 2

Subjects will return to the clinic at the end of Week 2 for assessment of compliance with study medication, use of rescue medication, and be dispensed the final 2-week supply of investigational product and rescue medication for DPN pain. Pain diary compliance will be reviewed by the study staff, and reporting instructions will be reinforced. Adverse events will be assessed by asking the subject a non-leading question. Use of concomitant medications will be documented. Vital signs (sitting blood pressure and pulse) will be measured. Blood samples will be collected for liver function testing. Female subjects of childbearing potential will be counseled to continue using highly effective contraception. Subjects will be instructed to continue entering their average pain intensity, worst pain intensity, pain upon walking, and rescue medication use over the past 24 hours into their study-issued handheld devices every night. Every morning upon awakening, subjects will complete the DSIS via their study-issued handheld device.

Scales to be completed at the Week 2 visit include the Patient Global Impression of Change (PGI-C), the S-STS, and the Insomnia Severity Index.

One week following the Week 2 visit, subjects will be contacted by telephone for an assessment of adverse events and to reinforce diary completion.

Treatment Period: Week 4/Early Termination

During the Week 4 or Early Termination Visit, subjects will be evaluated for compliance with study medication, and return all materials and unused study medication/rescue medication to the study site. The study-issued handheld device will be returned. Pain diary compliance will be reviewed by the study staff. Adverse events will be assessed by asking the subject a non-leading question. Use of concomitant medications will be documented. Vital signs (sitting blood pressure and pulse) and body weight will be measured, brief physical examination performed, and subjects will undergo a 5-minute electrocardiogram. Blood and urine samples will be collected for chemistry, hematology, and urinalysis testing. Female subjects of childbearing potential will be tested for pregnancy using a local urine testing kit. Subjects will undergo drug and alcohol screen using a local urine testing kit and breathalyzer, respectively.

Scales to be completed at the Week 4/Early Termination Visit include BPI-DPN, PGI-C, SF-MPQ-2, HADS, QOL-DN, Insomnia Severity Index, and S-STS.

7 Days Post-Treatment Follow Up Visit (Week 5)

Subjects will return to the study site 7 days following the Week 4/Early Termination visit to assess adverse events and concomitant medication use. The adverse event assessment will include any serious adverse events that were ongoing at the time of study completion, and whether any ongoing adverse events had progressed to becoming serious. Blood samples will be collected for liver function testing.

Number of subjects (planned and analyzed):

Approximately 300 subjects enrolled by a randomized method with a pre-specified number of subjects per treatment arm.

Diagnosis and main criteria for inclusion:

Inclusion Criteria: Screening

1. An Institutional Review Board-approved written informed consent and privacy language (Health Insurance Portability and Accountability Act) authorization must be obtained from the subject prior to performing any study related procedures.
2. Subjects who consent to being included in a subject registry database.
3. Male and female subjects ≥18 and ≤75 years of age.
4. Subjects with a diagnosis of Type 2 Diabetes.
5. Subjects with a score of ≥4 and ≤9 on the 11-point NRS for average pain intensity over the past 24 hours at Visit 1.
6. Hemoglobin A1c (HbA1c) ≤11% (measured at Visit 1).
7. Stable use of diabetic medications beginning 1 month prior to Visit 1. (Adequate glycemic control with only diet and exercise is also permitted.)
8. Subjects with diabetic peripheral neuropathy, of symmetrical nature and in lower extremities for ≥6 months to ≤10 years, and diagnosed by a score of ≥3 on Michigan Neuropathy Screening Instrument.
9. Body mass index <40 kg/m^2.
10. Calculated creatinine clearance ≥60 mL/minute (Cockcroft-Gault formula).
11. Clinical laboratory values must be within normal limits or deemed not clinically significant by the investigator and sponsor-designated medical monitor.
12. Female subjects must either:
    - Be of non-childbearing potential:
        - Post-menopausal (defined as at least 1 year without any menses) prior to Visit 1; or
        - Documented as surgically sterile.
    - Or, if of childbearing potential:
        - Agree not to try to become pregnant during the study and for 28 days after the final administration of investigational product;
        - Have a negative urine pregnancy test at Visit 1; and
        - If heterosexually active, agree to consistently use 2 forms of highly effective birth control (at least 1 of which must be a barrier method) starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product.
13. Female subjects must agree not to breastfeed starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product.
14. Female subjects must not donate ova starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product.
15. Male subjects must refrain from sperm donation starting at Visit 1 and continuing throughout the study period and for 90 days after the final administration of investigational product.
16. Male subjects who are not surgically sterilized for at least 90 days, and sexually active with a female partner, must use a condom with spermicide during the study, and for 90 days after the last dose of investigational product.
17. Has not participated in an interventional study for at least 30 days. Agrees not to participate in another interventional study while on treatment. Eligibility will be reviewed during the study via sponsor participation in a research subject database.

18. Ability to understand the requirements of the study, abide by the study restrictions, as well as concomitant medications exclusions, and agree to return for the required assessments.

Inclusion Criteria: Randomization

Daily pain scores and diary compliance will be transferred into the interactive response technology system, which will be used to assess the criteria for randomization. Subjects whose mean of the daily average pain intensity score during the preceding 7 (±1) days is within the protocol-defined algorithm and with adequate compliance with daily diary completion will be eligible for randomization.

Waivers to the inclusion criteria will NOT be allowed.

Exclusion Criteria:

1. Subjects who have a current diagnosis of a major psychiatric disorder (including schizophrenia, bipolar disorder, or panic disorder), including those who have required an antipsychotic or mood stabilizer (e.g., lithium, carbamazepine, valproate) for a psychiatric condition in the past year, or subjects who have had a major depressive episode (MDE) in the past 6 months. Subjects with major depressive disorder (MDD) or generalized anxiety disorder (GAD) who have been on stable medication for the past 3 months (and are expected to remain stable for the duration of the trial) and whose condition is currently well-controlled may be included
2. Subjects who have pain that cannot be clearly differentiated from, or could interfere with the assessment of peripheral diabetic neuropathy, as measured by the Masquerading Disorders Tool at Visit 1.
3. Neurologic disorders unrelated to diabetic neuropathy (e.g., phantom limb from amputation), skin condition in the area of neuropathy that could alter sensation (e.g., plantar ulcer), or other painful conditions (e.g., arthritis) that, in the judgment of the investigator, could interfere with reporting of pain due to diabetic neuropathy.
4. History of hypoglycemia that disturbed consciousness, or ketoacidosis requiring hospitalization within past 3 months.
5. Subjects with history of severe renal impairment defined by renal dialysis or peritoneal dialysis, or who have undergone renal transplant.
6. Impaired hepatic function characterized by a previous known diagnosis of chronic liver disease, and/or the presence of abnormal serum total bilirubin, or alanine transaminase (ALT), aspartate transaminase (AST), or alkaline phosphatase $\geq$1.5 x upper limit of normal (ULN) at screening.
7. Known history of significant cardiovascular condition, such as myocardial infarction or congestive heart failure; evidence of current uncontrolled cardiac arrhythmias, angina, or electrocardiographic evidence of acute ischemia; or active conduction system abnormalities; QTcF >450 msec (males) or >470 msec (females), or uncontrolled hypertension characterized by resting systolic blood pressure values >140 mm Hg or resting diastolic >90 mm Hg.
8. Heart rate <45 bpm or >90 bpm.
9. Fasting triglycerides >250 mg/dL.
10. History of Huntington's disease, Parkinson's disease, Alzheimer's disease, Multiple Sclerosis, or a history of seizures, epilepsy, or strokes.
11. HIV infection, hepatitis, or other ongoing infectious disease that the investigator considers clinically significant.

12. Concomitant use of antiepileptic drugs, non-steroidal anti-inflammatory drugs (except cardiac preventive acetylsalicylic acid), opioids, muscle relaxants, dextromethorphan (except low dose intermittent use for cough), tramadol, topical lidocaine, topical capsaicin, and selective norepinephrine reuptake inhibitors. Subjects are allowed to enter with a maximum of 1 allowed analgesic medication for neuropathic pain that has been taken at a stable dose for at least 1 month (30 days) prior to Visit 1. Allowed analgesics may not be *N*-methyl-D-aspartate receptor ligands, must be non-opioid and non-sedative, and must not interfere with subjects' pain reporting. Tricyclic antidepressants may be continued if designated as the single analgesic medication for the treatment of pain.
13. History of or current substance abuse disorder as defined by Diagnostic and Statistical Manual of Mental Disorders - Fifth Edition.
14. Recreational and/or medicinal marijuana use within the past 6 months.
15. Positive urine drug screen for marijuana, opioids, benzodiazepines inconsistent with current prescriptions, or breathalyzer test for alcohol at Visit 1.
16. Positive urine drug screen for marijuana, opioids, benzodiazepines inconsistent with current prescriptions, or breathalyzer test for alcohol at Visit 2.
17. Sensitivity to, allergy to, or concomitant use of *N*-methyl-D-aspartate receptor ligands including ketamine, amantadine, dextromethorphan (except low dose intermittent use for cough), memantine, methadone, dextropropoxyphene, and/or ketobemidone.
18. Amputations of lower extremities (toe amputation is allowed).
19. Any condition, including serious medical conditions that could interfere with the ability of the subject to participate in the study or could confound study assessments.
20. Subjects with hypersensitivity to multiple medications, in the opinion of the investigator.
21. Subjects who meet the criteria for suicidal intent, plan and/ or behavior by scoring 3 or 4 on Questions 2 or 13, or 2 or higher on any Question 1a (only if 1b is coded YES), 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 14 based on the S-STS at Visit 1 or Visit 2.

Waivers to the exclusion criteria will NOT be allowed.

Test product, dose and mode of administration, batch number:

NYX-2925, oral capsules, 2 capsules once daily by mouth.

Reference therapy, dose and mode of administration, batch number:

Placebo, oral capsules, 2 capsules once daily by mouth

Rescue medication

Acetaminophen, up to 2 g/day, as needed for DPN pain. To be dispensed beginning at Visit 1 and re-dispensed throughout the entire study.

Duration of Study

Screening: One to 4 weeks of analgesic medication washout**.**

Maintenance: Four weeks of blinded treatment with either NYX-2925 or placebo.

Follow-Up: Assessment of LFTs, adverse events and concomitant medications 1 week following the Week 4/Early Termination Visit.

Criteria for evaluation:

Efficacy:

Primary Efficacy Endpoint:

- Change in the NRS score assessing average pain intensity in the past 24 hours from baseline (average of Days -7 to -1) to Week 4 (average of Days 22 through 28)

Secondary Efficacy Endpoint

- Change in the mean NRS score for daily worst pain intensity from baseline (average of Days -7 to -1) to Week 4 (average of Days 22 through 28)
- Change in the mean NRS score assessing average pain intensity in the past 24 hours from baseline (average of Days -7 to -1) to each week of treatment (average of Days 1 through 7, Days 8 through 14, and Days 15 through 21)
- Change in the mean NRS score assessing average pain upon walking intensity in the past 24 hours from baseline (average of Days -7 to -1) to each week of treatment (average of Days 1 through 7, Days 8 through 14, Days 15 through 21, and Days 22 through 28)
- PGI-C at Weeks 2 and 4
- Change in published subscale scores of the SF-MPQ-2 from baseline (Visit 2) to Week 4
- Change in published subscale scores of the QOL-DN from baseline (Visit 2) to Week 4
- Change in the Insomnia Severity Index from baseline (Visit 2) to Week 2 and Week 4
- Change in published subscale scores of the HADS from baseline (Visit 2) to Week 4
- Change in the mean DSIS score from baseline (average of Days -7 to -1) to each week of treatment (average of Days 1 through 7, Days 8 through 14, Days 15 through 21, and Days 22 through 28)
- Change in the BPI-DPN from baseline (Visit 2) to Week 4

Safety:

- Adverse event monitoring
- Physical examination
- Vital signs
- 5-minute electrocardiogram
- Clinical laboratory test results
- S-STS

Exploratory

- Use of rescue medication
- Percent of subjects achieving ≥30% and ≥50% pain reduction on the NRS average pain intensity from baseline (average of Days -7 to -1) to Week 4 (average of Days 22 through 28)
- Number of days to the first ≥30% reduction and first ≥50% reduction in the NRS average pain intensity
- Number of days to sustained pain reduction

Sample Size:

The planned sample size is a total of 300 randomized subjects. This sample size will provide approximately 80% power for a 2-sided t-test with Type I error of 0.048.

Statistical methods:

Safety analyses will be based on the Safety Population, which is defined as all subjects who receive at least 1 dose of investigational product. Efficacy analyses will be based on the modified Intent-to-Treat Population, which is defined as all subjects in the Safety Population with at least 1 post baseline assessment of the pain intensity NRS.

For the primary efficacy endpoint, the daily pain intensity NRS score from the handheld device will be averaged for baseline (Days -7 to -1) and Week 4 (Days 22 to 28) after randomization. Change from baseline to Week 4 will be assessed for treatment group differences with an analysis of covariance with fixed factors for study site and treatment, with baseline value as a covariate. As a sensitivity analysis, a mixed model repeated measures analysis of variance will be used to assess treatment differences. The model will include factors for study site, treatment, week, and the treatment-by-week interaction, with baseline value as a covariate.

Change in the mean NRS score assessing average pain intensity in the past 24 hours from baseline (average of Days -7 to -1) to each week of treatment will be assessed for treatment group differences with an analysis of covariance with fixed factors for study site and treatment, with baseline value as a covariate. Change from baseline for the PGI-C, SF-MPQ-2, QOL-DN, Insomnia Severity Index, HADS, DSIS, BPI-DPN will be analyzed similarly.

The use of rescue medication and percent of subjects meeting responder criteria will be summarized descriptively.

The number of days to the first ≥30% reduction and first ≥50% reduction in the NRS average pain intensity will be assessed for treatment group differences with the log-rank test.

Adverse events will be categorized by system organ class and preferred term with the Medical Dictionary for Regulatory Activities. Summary tables for treatment-emergent adverse events (TEAEs) will include number and percent of subjects experiencing TEAEs by system organ class and preferred term.

Mean change in clinical laboratory and vital signs from baseline (Visit 2) to Week 4 (Visit 4) will be summarized descriptively. Clinical laboratory results considered clinically important by the investigator will be identified. Subjects with suicidal ideation or behavior will be identified with the S-STS.

2 TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

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3 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

The following abbreviations and special terms are used in this study protocol.

Table 2: Abbreviations and Special Terms

Abbreviation or specialist term	Explanation
AUC	Area Under the Plasma Concentration versus Time Curve
BPI-DPN	Brief Pain Inventory for Diabetic Peripheral Neuropathy
CFR	Code of Federal Regulations
C_{max}	Plasma Concentration
CSF	Cerebrospinal fluid
DSIS	Daily Sleep Interference Scale
eCRF	Electronic case report form
FDA	Food and Drug Administration
GAD	Generalized Anxiety Disorder
GCP	Good Clinical Practice
HADS	Hospital Anxiety and Depression Scale
HbA1c	Hemoglobin A1c; glycated hemoglobin
HCV RNA	Hepatitis C Virus-Ribonucleic Acid
HIV	Human Immunodeficiency Virus
ICH	International Conference on Harmonisation
IRB	Institutional Review Board
MDD	Major Depressive Disorder
MDE	Major Depressive Episode
NF	National Formulary
NMDA	N-methyl-D-aspartate
NMDAR	N-methyl-D-aspartate receptor
NRS	Numerical Rating Scale
PGI-C	Patient Global Impression of Change
QOL-DN	Norfolk Quality of Life Questionnaire - Diabetic Neuropathy
SF-MPQ-2	Short-Form McGill Pain Questionnaire version 2
S-STS	Sheehan Suicidality Tracking Scale
TEAE	Treatment-emergent adverse events
USP	United States Pharmacopeia

Note: Abbreviations that appear only in tables or figures are defined in the appropriate tables or figures.

4 INTRODUCTION

NYX-2925 is a novel small molecule being developed for the treatment of neuropathic pain and fibromyalgia. NYX-2925 is an *N*-methyl-D-aspartate receptor (NMDAR) functional glycine-site partial agonist, and at low concentrations of endogenous agonist (glycine or D-serine), NYX-2925 partially activates NMDAR. NYX-2925 appears to act at a binding site that is distinct from NMDAR agonists or antagonists studied to date, such as D-cycloserine, ketamine, MK-801, or kynurenic acid. The NYX-2925 mode of action is distinct from that of all of existing and emerging drugs that are indicated for the treatment of neuropathic pain and fibromyalgia. While current medications target individual elements of pain signal transmission or modulation, NYX-2925 modulates multiple synaptic relays within pain circuits.

Glutamate is the major excitatory neurotransmitter in the central nervous system and acts through activation of glutamate receptors. A portion of the receptors bind preferentially to *N*-methyl-D-aspartate (NMDA), and are therefore, termed NMDARs. Unlike other glutamate receptors found in the brain, such as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid or kainic acid receptors, the NMDARs are unique in that they have distinct binding sites for both glutamate and glycine, and binding by both ligands is required for receptor activation. The NMDARs are implicated in a number of physiological and pathological processes, including anxiety, cognition, learning, stroke, schizophrenia, Parkinson's disease, and neuropathic pain (Traynelis 2010, Mony 2009, Tai 2001).

The central nervous system modulates the experience of pain in people with neuropathic pain, with the rostroventralmedial medulla (Silva 2016), the dorsal anterior cingulate cortex (Russo 2015), the insula and other brain regions (Ossipov 2010) all thought to be involved. Neuropathic pain is caused by disease or injury of the somatosensory system as opposed to nociceptive pain where the sensory system is physiologically normal (Jensen 2014). While neuropathic pain can initially arise within the central or the peripheral nervous system through a wide range of etiologies, central nervous system modulation of the experience of pain is common, regardless of the specific precipitating factors or initial location of the pain. The prevalence of neuropathic pain is approximately 8% in the population in the United States (Gilron 2015, Toth 2013, Bouhassira 2008). Individuals suffering from neuropathic pain, irrespective of the underlying disorder, currently have limited treatment options available. Current treatment options predominantly include antidepressants and antiepileptics. These therapies have shown some efficacy in treating neuropathic pain symptoms, although for a large proportion of patients, treatment is insufficient.

Preclinical pharmacology studies support the study of NYX-2925 in both fibromyalgia and neuropathic pain, both of which are chronic pain syndromes involving central nervous system modulation of pain signals. NYX-2925 is effective in several models of neuropathic pain, and has been shown to be effective in the chronic unpredictable stress model of fibromyalgia. Animal models of neuropathic pain in which NYX-2925 was tested include the rat Bennett model of neuropathic pain, the rat streptozotocin model of diabetic peripheral neuropathy, and the rat Taxol® model of chemotherapy-induced neuropathic pain. Unlike gabapentin (150 mg/kg orally), NYX-2925 (1 to 30 mg/kg orally) produced a rapid and long-lasting analgesia in the rat Bennett model of neuropathic pain without changes in locomotor activity after a single dose. Further, in

the chronic unpredictable stress model of fibromyalgia, NYX-2925 (10 mg/kg) has been shown to produce mechanical analgesia. In summary, these data support testing NYX-2925 in both fibromyalgia and neuropathic pain.

Pharmacokinetic studies have been performed in rats and dogs, and suggest that NYX-2925 is rapidly absorbed into the bloodstream, and time to peak plasma concentration is reached within 1 hour. NYX-2925 is rapidly eliminated from plasma with an apparent terminal elimination half-life in the range of 1 to 2 hours. Brain exposure is approximately 5% to 10% relative to plasma levels of NYX-2925.

Both single-dose and daily repeat-dose toxicology studies have been performed in rats and dogs. The maximum tolerated dose following a single oral exposure is at least 1000 mg/kg in both rats and dogs. After 6 weeks of daily oral doses, the no-observed-adverse-effect levels were the highest doses tested (180 mg/kg in dogs and 480 mg/kg in rats). There were no adverse findings in either species. The plasma levels (exposure) of NYX-2925 at the highest doses in these studies are considered to be at least 50- to 100-fold the projected human therapeutic dose.

NYX-2925 has been administered to humans. The first-in-human study (NYX-2925-1001), for which results are available, was a combined single and multiple ascending dose, placebo-controlled study in healthy volunteers that also includes food-effect, cerebrospinal fluid (CSF), and elderly cohorts.

In the single dose portion of the study, single doses of NYX-2925 were administered orally. Five ascending dose groups (50 mg, 150 mg, 400 mg, 800 mg, and 1200 mg) and an elderly cohort (300 mg) with 8 subjects each (6 on active drug, 2 on placebo in each group) were dosed. Seven days after the initial dose, the 400 mg dose group received a second repeated dose (6 active drug, 1 placebo) to examine the effect of food on plasma drug concentrations. A CSF cohort (50 mg) was also completed with 6 subjects receiving NYX-2925. In 7 dose groups or cohorts, 29 males and 25 females were enrolled. A total of 52 of the 54 single dose subjects completed the study. Two subjects randomized to placebo discontinued study participation. One subject was lost to follow-up and 1 subject discontinued due to physician decision (related to subject noncompliance). One subject in the elderly cohort receiving 300 mg experienced a treatment-emergent adverse event (TEAE) which was not considered related to NYX-2925. Five subjects in the CSF cohort receiving 50 mg experienced at least one TEAE, one of which (procedural headache) was deemed related to NYX-2925. One subject receiving placebo experienced a TEAE. There were no TEAEs reported for clinical laboratories, safety electrocardiograms, or vital signs.

In the multiple ascending dose phase, there were three ascending dose groups (150, 600 and 900 mg) with 8 subjects in each dose group; 6 received active drug and 2 received placebo for up to 7 days. There were also 6 subjects in the CSF cohort who were dosed with NYX-2925 300 mg (no placebo control) and 5 completed the study. A total of 15 males and 15 females were enrolled. Study medication was given once daily for up to 7 days. Two subjects in the CSF cohort discontinued study participation, 1 due to other (related to subject noncompliance) and 1 due to protocol violation. All subjects in 150 mg, 600 mg, and 900 mg dose groups received all 7 doses of study medication. Twelve subjects (1 NYX-2925 150 mg, 3 NYX-2925 600 mg, 1 NYX-2925 900 mg, 2 placebo, and 5 NYX-2925 300 mg CSF cohort) reported at least 1 TEAE. There was one subject in the 150 mg dose group with urinary retention assessed as unrelated by the investigator, and possibly related by the sponsor. There was 1 subject with at least 1 study drug

related TEAE (flatulence and bloating) in the 600 mg dose group, 1 placebo subject with at least 1 study drug related TEAE (fatigue and lethargy), and 1 CSF cohort subject with at least 1 study drug related TEAE (headache). No grade 3 or higher TEAEs, serious TEAEs, or TEAEs leading to study discontinuation were reported. No individual preferred adverse event term was reported for more than 1 subject in the 150 mg, 600 mg, or 900 mg dose groups. In the CSF cohort, 3 subjects reported procedural headache, 5 subjects reported procedural pain, and 2 subjects reported vomiting. There were no TEAEs reported for clinical laboratories, safety electrocardiograms, or vital signs.

Concerning cardiovascular safety, a thorough analysis of ECGs demonstrated that NYX-2925 did not increase QTc at any dose up to 1200 mg. The maximum geometric mean peak plasma concentration (C_{max}) (20.1 μg/ml) was well above the expected exposure in clinical care. There were no clinically significant changes in HR, PR and QRS or in ECG diagnostic statements.

The pharmacokinetics of NYX-2925 in humans are similar to the pharmacokinetics in rats and dogs. Pharmacokinetic analysis revealed that absorption of NYX-2925 was relatively rapid and peak concentrations were achieved within 2 hours after dosing and declined in a roughly monophasic manner over 24 hours. The pharmacokinetics were dose proportional over the dose range tested (within the variability) and showed minimal accumulation following once per day dosing for 7 days. A high-fat, high-caloric meal decreased the rate of absorption (delayed T_{max}), lowered C_{max} by 10%, but had no impact on the extent of exposure (area under the plasma concentration versus time curve [AUC] values were comparable), suggesting that the compound may be administered with or without food. The majority, approximately 60% to 70%, of the administered dose was eliminated as unchanged NYX-2925 in the urine. The central nervous system exposure was confirmed from the CSF concentration time profile in both CSF dose groups. The CSF concentrations increased proportionally as the dose increased.

In summary, NYX-2925 has been sufficiently tested in preclinical studies for safety and tolerability over a wide dose range, with exposures greater than the proposed clinical doses. General toxicology studies support clinical studies up to and including 6 weeks of daily doses. Results from the first-in-human study indicate that NYX-2925 is safe and well-tolerated in the male and female healthy volunteers dosed to date at single doses up to 1200 mg, and multiple doses up to 900 mg given daily for 7 days. The preclinical and phase 1 clinical data support further testing in humans.

In addition to the current trial, there are 2 other ongoing clinical studies with NYX-2925. NYX-2925-1003 is a phase 1, double-blind, placebo-controlled, pilot, crossover study to assess the effects of NYX-2925 on sleep architecture as assessed by polysomnography in healthy male subjects aged 40-55 when their bedtime is advanced by 3 hours in a sleep laboratory. Also ongoing is study NYX-2925-2002, in subjects with fibromyalgia. The primary objective of this study is to determine whether NYX-2925 modulates central nervous system activity in key pain neural circuitry, consistent with the potential to provide analgesia in subjects with fibromyalgia, as assessed by neuroimaging methods. Secondarily, this study will provide a preliminary characterization of the safety of NYX-2925 in subjects with fibromyalgia.

Further, there is planned study; a phase 1, double-blind, randomized, sponsor-open, parallel, placebo-controlled exploratory study to assess the effect of multiple dose levels of NYX-2925 on

the brain function of healthy volunteers as measured by electroencephalogram and event related potentials.

To date, one serious adverse event, cholelithiasis requiring inpatient hospitalization, has been reported in the clinical development program for NYX-2925, that being in the current study, NYX-2925-2001. The event is considered not related to NYX-2925.

This study (NYX-2925-2001) is a randomized, double-blind, placebo-controlled, multiple-dose study to assess the efficacy and safety of NYX-2925 in subjects with neuropathic pain associated with diabetic peripheral neuropathy. NYX-2925 will be provided as capsules for oral administration.

5 STUDY OBJECTIVES AND ENDPOINTS

5.1 Primary Objective

The primary objective of this study is to evaluate the efficacy of multiple dose levels of NYX-2925 versus placebo in treating the neuropathic pain associated with diabetic peripheral neuropathy.

5.2 Secondary Objectives

Secondary objectives of the study are to assess the:

- Effects of multiple dose levels of NYX-2925 versus placebo on pain characteristics, sleep interference, health status, psychological state, and global improvement.
- Safety and tolerability of multiple dose levels of NYX-2925.

5.3 Study Endpoints

5.3.1 Efficacy Endpoints

The primary endpoint is the change in the numerical rating scale (NRS) score assessing average pain intensity in the past 24 hours from baseline (average of Days -7 to -1) to Week 4 (average of Days 22 through 28).

Secondary efficacy endpoints include:

- Change in the mean NRS score for daily worst pain intensity from baseline (average of Days -7 to -1) to Week 4 (average of Days 22 through 28)
- Change in the mean NRS score assessing average pain intensity in the past 24 hours from baseline (average of Days -7 to -1) to each week of treatment (average of Days 1 through 7, Days 8 through 14, and Days 15 through 21)
- Change in the mean NRS score assessing average pain upon walking intensity in the past 24 hours from baseline (average of Days -7 to -1) to each week of treatment (average of Days 1 through 7, Days 8 through 14, Days 15 through 21, and Days 22 through 28)
- Patient Global Impression of Change (PGI-C) at Weeks 2 and 4
- Change in published subscale scores of the Short-Form McGill Pain Questionnaire version 2 (SF-MPQ-2) from baseline (Visit 2) to Week 4
- Change in published subscale scores of the Norfolk Quality of Life Questionnaire - Diabetic Neuropathy (QOL-DN) from baseline (Visit 2) to Week 4
- Change in the Insomnia Severity Index from baseline (Visit 2) to Week 2 and Week 4
- Change in published subscale scores of the Hospital Anxiety and Depression Scale (HADS) from baseline (Visit 2) to Week 4

- Change in the mean Daily Sleep Interference Scale (DSIS) score from baseline (average of Days -7 to -1) to each week of treatment (average of Days 1 through 7, Days 8 through 14, Days 15 through 21, and Days 22 through 28)
- Change in the Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN) from baseline (Visit 2) to Week 4

5.3.2 Safety Endpoints

The safety endpoints will include adverse events, vital signs, physical examination findings, electrocardiograms, clinical laboratory results, and the Sheehan Suicidality Tracking Scale (S-STS).

5.3.3 Exploratory Efficacy Endpoints

Exploratory efficacy endpoints include:

- Use of rescue medication
- Percent of subjects achieving ≥30% and ≥50% pain reduction on the NRS average pain intensity from baseline (average of Days -7 to -1) to Week 4 (average of Days 22 through 28)
- Number of days to the first ≥30% reduction and first ≥50% reduction in the NRS average pain intensity
- Number of days to sustained pain reduction

6 INVESTIGATIONAL PLAN

6.1 Overall Study Design and Plan: Description

The study will be a 6- to 9-week study, including a 1- to 4-week Screening Period, followed by a 4-week double-blind, randomized, placebo-controlled Treatment Period, and a 1-week Follow-Up Period (Figure 1). The schedule of assessments is provided in Table 3. The study will include approximately 35 study sites in the United States.

Screening Period (Week -4 to -1)

Upon Institutional Review Board (IRB) approval of the protocol, subjects will be asked to provide informed consent for this study before any required procedures are performed. Subjects will also be required to sign an additional consent for their inclusion in a subject registry database. The database will use partially-identified subject information to review subjects' research study history within a proprietary, secure platform. Subjects who meet any of the following criteria will not be eligible for continued screening: current enrollment in another study, concurrent screening at another research site, violation of the required number of half-lives since the last research study, violation of the washout period between studies, or incorrect age for the NYX-2925-2001 study. Employees, contractors and volunteers of the study site, or relatives of any employees, contractors and volunteers of the study site are not eligible for study participation.

Subjects with Type 2 diabetes who have been on stable antidiabetic medication for at least 1 month (or have stable glycemic control with diet and exercise alone) and who have been suffering from painful diabetic peripheral neuropathy in the lower extremities for at least 6 months but not more than 10 years will be screened for diabetic peripheral neuropathy using the Michigan Neuropathy Screening Instrument and the Masquerading Disorders Tool. Subjects must have a score of ≥4 and ≤9 on the 11-point NRS for average pain over the past 24 hours at Visit 1. Subjects will be assessed for widespread pain symptoms using the American College of Rheumatology Fibromyalgia Criteria. Additional procedures during Visit 1 will include the administration of the HADS, completion of the S-STS, adverse event collection which will begin at the time of informed consent, demographic characteristics, medical history including concomitant medications, complete physical examination (with comprehensive neurological examination), 5-minute electrocardiogram, vital signs (sitting blood pressure and pulse after 5 minutes), height, body weight, and collection of blood and urine samples for chemistry, HIV, hepatitis, and triglycerides, hematology (including HbA1c), and urinalysis. All subjects will undergo urine drug and alcohol screen using a local urine testing kit and breathalyzer, respectively. Subjects who test positive for marijuana, opioids, benzodiazepines inconsistent with current prescriptions, or alcohol will not be allowed to continue in the study. Female subjects of childbearing potential will be tested for pregnancy using a local urine testing kit and will be counseled to begin or continue using highly effective contraception.

Eligible subjects who meet all entry criteria will enter a 1- to 4-week Screening Period, during which they will discontinue all except 1(if applicable) of their pharmacologic analgesic treatments for neuropathic pain associated with diabetic peripheral neuropathy, and complete daily pain diaries. The duration of the Screening Period will depend upon the analgesic treatment

that is being discontinued. The only allowed analgesic for painful diabetic peripheral neuropathy may not be an *N*-methyl-D-aspartate receptor ligands, must be non-opioid and non-sedative, and must not interfere with subjects' pain reporting (Section 8.7). The allowed analgesics must have been taken for at least 1 month (30 days) prior to Visit 1, and subjects must be on a stable fixed dose that is not expected to change during the study.

The discontinuation of all (but 1 allowed, as applicable) analgesic medications will be managed by the principal investigator at the investigative site. Subjects already taking no more than 1 allowed concomitant analgesic medication at Visit 1 may directly begin Week -1 of the Screening Period.

During Visit 1, eligible subjects will be provided a handheld device, and as a method for ensuring consistency and reliability of pain scoring, and subjects will be instructed at Visits 1 through 3 on how to record their pain on the device [redacted] Subjects will also be educated at Visits 1 through 3 on appropriate expectations around their participation in a clinical study [redacted], and the importance of consistently and accurately reporting their pain throughout the study. Review of these educational materials may be repeated for some or all subjects depending on findings of an ongoing blinded data review (e.g., if pain score variability is increased on a subject or site level).

Subjects will be dispensed acetaminophen to be used as rescue medication, and will be instructed to take no more than 2 g/daily (one to two 500-mg caplets every 4 to 6 hours as needed) for DPN pain.

Beginning immediately after Visit 1, pain intensity and rescue medication use will be recorded in the study-issued handheld device daily at bedtime. Subjects will enter their average pain intensity, worst pain intensity, pain on walking, and whether rescue medication was used during the past 24 hours. Pain intensity will be recorded using an 11-point NRS, with 0 being no pain and 10 being the worst pain imaginable. Every morning upon awakening, subjects will complete the DSIS via their study-issued handheld device.

Screening Period (Week -1)

The investigative site staff will contact study subjects by telephone weekly through Week -1 to reinforce the reporting instructions (i.e., diary completion) and to assess adverse events. Subjects will be asked a non-leading question to inquire about potential adverse events, "Have you experienced any new or changed symptoms since we last asked/since last week?" Subjects taking no more than 1 allowed concomitant analgesic medication at Visit 1 may directly begin Week -1 of the Screening Period.

Baseline (Week 0, Visit 2)

At Visit 2 (Baseline Visit), study personnel will verify eligibility with the inclusion and exclusion criteria when the subjects are on site, and prior to randomization. Pain scores reported by subjects during the Screening Period will be evaluated by the interactive response technology system for raw score and for variability among scores after transmission of pain scores from the handheld devices to determine eligibility. The interactive response technology system will notify the site if the subject is "Eligible" or "Not eligible". No other information will be provided.

Subjects whose mean of the daily average pain intensity score during the preceding 7 (±1) days is within the protocol-defined algorithm (Section 12.2) and with adequate compliance with daily diary completion will be eligible for randomization. The absolute pain score and variability among scores, as well as the actual percentage required for diary compliance, will be masked to investigators and subjects. The appropriate use of rescue medication will be assessed by inventory of the returned caplets, as well as by subject interview. Subject eligibility for randomization into the study based on these variables will be communicated to the investigator via the interactive response technology system.

Subjects will again be asked a non-leading question to assess potential adverse events, for example, "Have you experienced any new or changes symptoms since we last asked/since your last visit?" Use of concomitant medications will be documented. Vital signs (blood pressure and pulse) will be measured after sitting for 5 minutes and a 5-minute electrocardiogram will be performed. Medical history will be updated and a brief physical examination performed. Blood and urine samples will be collected for chemistry, hematology, and urinalysis testing. Subjects will undergo drug and alcohol screen using a local urine testing kit and breathalyzer, respectively. Subjects who test positive for marijuana, opioids, benzodiazepines inconsistent with current prescriptions, or alcohol, will not be allowed to continue in the study. Female subjects of childbearing potential will be tested for pregnancy using a local urine testing kit and will be counseled to continue using highly effective contraception.

Eligible subjects will be randomized to receive either NYX-2925 or placebo for 4 weeks. Investigators and subjects will be masked to the randomization allocation.

Subjects will be dispensed a 2-week supply of investigational product and instructed to take 2 capsules by mouth once daily. Subjects will also be re-dispensed acetaminophen to use as rescue medication for DPN pain; instructions not to exceed 2 g/day will be reinforced.

Subjects will be instructed to continue entering their average pain intensity, worst pain intensity, pain upon walking, and whether they used rescue medication over the past 24 hours into their study-issued handheld devices every night. Every morning upon awakening, subjects will complete the DSIS via their study-issued handheld device. Pain diary compliance will be reviewed at each study visit by the study staff, and reporting instructions will be reinforced.

Quantitative sensory testing will be done using the Bedside Sensory Testing Kit. The following scales will be completed at the Baseline Visit: BPI-DPN, the SF-MPQ-2, the QOL-DN, the HADS, the Insomnia Severity Index, and the S-STS.

One week following the Week 0 visit, subjects will be contacted by telephone for an assessment of adverse events and to reinforce diary completion. Adverse events will be assessed by asking the subject a non-leading question.

Treatment Period (Week 2, Visit 3)

Subjects will return to the clinic at the end of Week 2 for assessment of compliance with study medication, use of rescue medication, and be dispensed the final 2-week supply of investigational product and rescue medication for DPN pain. Pain diary compliance will be reviewed by the study staff, and reporting instructions will be reinforced. Adverse events will be assessed by asking the subject a non-leading question; for example, "Have you experienced any new or changed symptoms since we last asked/since your last visit?" Use of concomitant medications will be documented. Vital signs (sitting blood pressure and pulse after 5 minutes) will be measured. Blood samples will be collected for liver function testing. Female subjects of childbearing potential will be counseled to continue using highly effective contraception.

Subjects will be instructed to continue entering their average pain intensity, worst pain intensity, pain upon walking, and rescue medication use over the past 24 hours into their study-issued handheld devices every night. Every morning upon awakening, subjects will complete the DSIS via their study-issued handheld device.

Scales to be completed at the Week 2 visit include the PGI-C, the S-STS, and the Insomnia Severity Index.

One week following the Week 2 visit, subjects will be contacted by telephone for an assessment of adverse events and to reinforce diary completion.

Treatment Period (Week 4/Early Termination, Visit 4)

During the Week 4 or Early Termination Visit, subjects will be evaluated for compliance with study medication, and return all materials and unused study medication/rescue medication to the study site. The study-issued handheld device will be returned. Pain diary compliance will be reviewed by the study staff. Adverse events will be assessed by asking the subject a non-leading question; for example, "Have you experienced any new or changed symptoms since we last asked/since your last visit?" Use of concomitant medications will be documented. Vital signs (sitting blood pressure and pulse after 5 minutes) and body weight will be measured, brief physical examination performed, and subjects will undergo a 5-minute electrocardiogram. Blood and urine samples will be collected for chemistry, hematology, and urinalysis testing. Female subjects of childbearing potential will be tested for pregnancy using a local urine testing kit. Subjects will undergo drug and alcohol screen using a local urine testing kit and breathalyzer, respectively.

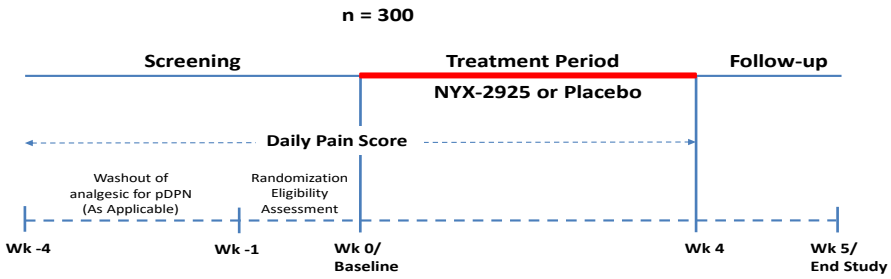
Scales to be completed at the Week 4/Early Termination Visit include BPI-DPN, PGI-C, SF-MPQ-2, HADS, QOL-DN, Insomnia Severity Index, and S-STS.

Follow-Up Visit (Week 5/7 days Post-Treatment, Visit 5)

Subjects will return to the study site 7 days following the Week 4/Early Termination visit to assess adverse events and concomitant medication use. The adverse event assessment will include any serious adverse events that were ongoing at the time of study completion, and

whether any ongoing adverse events had progressed to becoming serious. Blood samples will be collected for liver function testing.

Figure 1: Study Design



pDPN = painful diabetic peripheral neuropathy; Wk = week

Table 3: Schedule of Procedures

Study Period	Screening	Baseline	Treatment		Follow-Up
Study Week (Day)	1 to 4 weeks[a] (-28 to -1)	Week 0 (1)[b]	Week 2 (14 ± 2)	Week 4[c] (28 ± 2)	Week 5 (35 ± 2)
Study Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Assessment					
Informed consent prior to any study procedure	X				
Assess inclusion/exclusion criteria	X	X			
Instructions on reporting pain	X	X	X		
Education on appropriate expectations around participation in a clinical study[d]	X	X	X		
Michigan Neuropathy Screening Instrument	X				
Masquerading Disorders Tool	X				
Bedside Sensory Testing Kit		X			
American College of Rheumatology Fibromyalgia Criteria	X				
Demographic characteristics (age, sex, race, and ethnicity)	X				
Medical history	X	update			
Height	X				
Body weight	X			X	
Vital sign measurements (sitting)[e]	X	X	X	X	
Physical examination[f]	X	X		X	
5-minute electrocardiogram after 5 minutes in supine position	X	X		X	
Urine pregnancy test for females of childbearing potential	X	X		X	
HIV and hepatitis tests[g]	X				
Drug and alcohol screen[h]	X	X		X	
Obtain samples for serum chemistry, hematology, and urinalysis[i]	X	X	X[i]	X	X[i]
Discontinue all but 1 concomitant analgesic medication	X[j]				
Record average pain intensity over past 24 hours[k]					
Dispense handheld electronic diary	X				
Return handheld electronic diary				X	
Dispense rescue medication (acetaminophen)[l]	X	X	X		
Return rescue medication and perform accountability[m]		X	X	X	

Study Period	Screening	Baseline	Treatment		Follow-Up
Study Week (Day)	1 to 4 weeks[a] (-28 to -1)	Week 0 (1)[b]	Week 2 (14 ± 2)	Week 4[c] (28 ± 2)	Week 5 (35 ± 2)
Study Visit	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Record average pain intensity, worst pain intensity, pain upon walking,[k] and rescue medication use over past 24 hours at bedtime for each day	X	X	X		
Record Daily Sleep Interference Scale (DSIS) each morning	X	X	X		
Phone call to reinforce diary instructions[n]	X	X	X	X	
Assess pain and diary compliance		X	X	X	
Randomize eligible subjects		X			
Dispense investigational product		X	X		
Return investigational product and assess compliance[m]			X	X	
Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN)		X		X	
Short-Form McGill Pain Questionnaire (SF-MPQ-2)		X		X	
Norfolk Quality of Life Questionnaire – Diabetic Neuropathy (QOL-DN)		X		X	
Insomnia Severity Index		X	X	X	
Hospital Anxiety and Depression Scale (HADS)	X	X		X	
Patient Global Impression of Change (PGI-C) scale			X	X	
Sheehan Suicidality Tracking Scale (S-STS)	X	X	X	X	
Concomitant medications	X	X	X	X	X
Adverse events[o]	X	X	X	X	X

a) Subjects will discontinue all but 1 allowed concomitant pharmacologic analgesic medication. Duration of the period will depend upon the analgesic treatment(s) being discontinued (not to exceed 4 weeks). Subjects taking no more than 1 allowed concomitant analgesic medication at Visit 1 may directly begin Week -1 of the Screening Period.

b) Visit window for Baseline Visit is 6 to 10 days after start of Week -1

c) Subjects who prematurely discontinue randomized treatment will have Week 4 procedures performed.

d) Review of these educational materials may be repeated for some or all subjects or depending on findings of an ongoing blinded data review (e.g., if pain score variability is increased on a subject or site level).

e) Vital signs will include blood pressure and pulse after at least 5 minutes of being seated, and prior to blood sample collection.

f) A complete physical examination will be performed at Visit 1 and a brief physical examination will be performed at Visits 2 and 4. The physical examination at Visit 1 will include a comprehensive neurological examination.

g) If the screening hepatitis C antibody test result is reactive, a nucleic acid test for hepatitis C virus-ribonucleic acid (HCV RNA) should be performed for confirmation of chronic infection. If the screening HIV test is positive, a confirmatory Western Blot test should be performed to confirm HIV status.

h) Urine drug screen and alcohol breathalyzer.

i) Samples will be obtained following an 8-hour fast. The Week 2 and Week 5 samples will only be analyzed for liver function tests and no fasting will be required. HbA1c will be collected at Screening (Visit 1) only.
j) Analgesic medications discontinued during the Screening Period cannot be restarted until after the Week 4 visit.
k) Numerical Rating Scale of pain intensity (0 = no pain to 10 = worst pain imaginable) will be completed once daily at bedtime. At Visit 1, average pain intensity over the past 24 hours will be recorded onto device by site personnel.
l) Subjects will be instructed to take no more than 2 g/daily (one to two 500-mg caplets every 4 to 6 hours as needed) for pain.
m) Instruct study participants to bring all used and unused investigational product and rescue medication to each visit for compliance monitoring.
n) Phone call will be made weekly during weeks with no study visits, to reinforce diary completion.
o) Weekly phone calls will be made during weeks with no study visits, to assess adverse events.

6.2 Scientific Rationale for Study Design

Double-blind, randomized, placebo-controlled study designs provide unbiased estimates of efficacy and safety. Withdrawal of all but 1 concomitant analgesic medication during the Screening Period is expected to reduce inter-subject variability in treatment response (Katz 2005, Katz 2015). The 1-month duration of treatment is considered sufficient to identify clinically important pain relief.

Only masked versions of the informed consent and protocol will be provided to study sites. The masked versions will obscure the number of dose groups (i.e., the probability of receiving placebo) and the randomization criteria (Section 7.1). Masking is expected to reduce variability in treatment response and to reduce the response to placebo.

6.3 Justification of Dose

Multiple dose levels of NYX-2925 are planned. No dose is more than 1/6th of the maximum single dose (1200 mg) given in phase 1, which was safe and well-tolerated. Further, no dose is more than 2/9th of the maximum multiple dose (900 mg) given once daily for 7 days in phase 1, which was safe and well-tolerated. Both the 1200 mg single dose and the 900 mg multiple dose were associated with pharmacokinetic exposures (maximum concentration and area under the concentration curve) below the no-observed-adverse-effect levels for both the dog and rat in the Good Laboratory Practice toxicology studies. NYX-2925 has a terminal elimination half-life of 4 to 5 hours and demonstrated no accumulation with once daily dosing over 7 days in phase 1. This is consistent with pharmacokinetic data in rats and dogs, following much higher doses over six weeks. Approximately 65% of the NYX-2925 dose is eliminated as unchanged drug in the urine. Thus, no drug accumulation is expected in this study. Both male and female subjects were included in the phase 1 studies and provide adequate support for studying both female and male subjects with neuropathic pain, when using appropriate contraception precautions. Thus, the selected dose strengths chosen for this study are well supported by the available preclinical and clinical safety and pharmacokinetic data. The 28-day duration of exposure to NYX-2925 in this study is supported by the general toxicology studies in rats and dogs, comprising 42 and 55 daily doses, respectively.

The dose strengths of NYX-2925 planned for this study are expected to produce maximum plasma concentrations ranging from approximately 170 to 3400 ng/mL. These levels approximate the plasma maximum concentration associated with efficacy in the rat Bennett, Taxol®, and streptozotocin models. In those pharmacology models, analgesia was observed at doses between 1 and 30 mg/kg, corresponding to maximum plasma concentrations levels of 210 to 4500 ng/mL, respectively. Brain exposure in humans, as measured in CSF, is anticipated to approximate the CSF levels measured in rats at analgesic doses. Additionally, the anticipated CSF levels in humans should be 3- to 6-orders of magnitude greater than the NYX-2925 concentration associated with half-maximal response at the NMDAR target.

Preclinical data support daily dosing as sufficient to result in efficacy in pain syndromes. First, when given to rats, NYX-2925 enhances hippocampal long-term potentiation at 24 hours and as much as 1 week following a single-oral dose. This enhancement of synaptic plasticity is a probable mechanism for the long duration of effects in the pain models. Following a single oral dose in the rat Bennett model of neuropathic pain, analgesic activity was consistently seen at

24 hours post-dose, and in some experiments at 1 week post-dose. In a rat Bennett model experiment that included gabapentin as a positive control, gabapentin was active only at the 1-hour time point. In the rat Taxol® model of chemotherapy-induced neuropathy, NYX-2925 was active at both 24 hours and 1 week after administration of a single dose, whereas the positive control, bupivacaine, was active only at the 1-hour testing interval. Gabapentin (150 mg/kg orally) was inactive in this model. Finally, in the rat model of streptozotocin-induced diabetic neuropathy, NYX-2925 analgesic activity was also evident at 24 hours and 1 week following a single dose. Given that the mean elimination half-life of NYX-2925 in the rat is approximately 2 hours, these data indicate that the analgesic activity of NYX-2925, which is consistently present for 24 hours after a single dose and often present for a week, persists beyond the presence of drug in the rat. Along with the corresponding 1-week persistence of effects on the enhancement of synaptic plasticity, the proposed underlying mechanism of drug action, the rat pain model data support the idea that NYX-2925 effects will persist for at least 24 hours following a single dose in humans. Taken together, these preclinical data support daily dosing in humans.

7 SELECTION AND WITHDRAWAL OF SUBJECTS

7.1 Subject Inclusion Criteria

Inclusion Criteria: Screening

1. An IRB-approved written informed consent and privacy language (Health Insurance Portability and Accountability Act) authorization must be obtained from the subject prior to performing any study-related procedures.
2. Subjects who consent to being included in a subject registry database.
3. Male and female subjects ≥18 and ≤75 years of age.
4. Subjects with a diagnosis of Type 2 diabetes.
5. Subjects with a score of ≥4 and ≤9 on the 11-point NRS for average pain intensity over the past 24 hours at Visit 1.
6. Hemoglobin A1c (HbA1c) ≤11% (measured at Visit 1).
7. Stable use of diabetic medications beginning 1 month prior to Visit 1. (Adequate glycemic control with only diet and exercise is also permitted).
8. Subjects with diabetic peripheral neuropathy, of symmetrical nature and in lower extremities for ≥6 months to ≤10 years, and diagnosed by a score of ≥3 on the Michigan Neuropathy Screening Instrument.
9. Body mass index <40 kg/m^2.
10. Calculated creatinine clearance ≥60 mL/minute (Cockcroft-Gault formula).
11. Clinical laboratory values must be within normal limits or deemed not clinically significant by the investigator and sponsor-designated medical monitor.
12. Female subjects must either:

- Be of non-childbearing potential:
    - Post-menopausal (defined as at least 1 year without any menses) prior to Visit 1; or
    - Documented as surgically sterile.
- Or, if of childbearing potential:
    - Agree not to try to become pregnant during the study and for 28 days after the final administration of investigational product;
    - Have a negative urine pregnancy test at Visit 1; and
    - If heterosexually active, agree to consistently use 2 forms of highly effective birth control (at least 1 of which must be a barrier method) starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product.

13. Female subjects must agree not to breastfeed starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product.
14. Female subjects must not donate ova starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product.
15. Male subjects must refrain from sperm donation starting at Visit 1 and continuing throughout the study period and for 90 days after the final administration of investigational product.
16. Male subjects who are not surgically sterilized for at least 90 days, and sexually active with a female partner, must use a condom with spermicide during the study, and for 90 days after the last dose of investigational product.
17. Has not participated in an interventional study for at least 30 days. Agrees not to participate in another interventional study while on treatment. Eligibility will be reviewed during the study via sponsor participation in a research subject database.
18. Ability to understand the requirements of the study, abide by the study restrictions, as well as concomitant medications exclusions, and agree to return for the required assessments.

Inclusion Criteria: Randomization

Daily pain scores and diary compliance will be transferred into the interactive response technology system, which will assess the criteria for randomization. Subjects whose mean of the daily average pain intensity score during the preceding 7 (±1) days is within the protocol-defined algorithm and with adequate compliance with daily diary completion will be eligible for randomization.

Waivers to the inclusion criteria will NOT be allowed.

7.2 Subject Exclusion Criteria

1. Subjects who have a current diagnosis of major psychiatric disorder (including schizophrenia, bipolar disorder, or panic disorder), including those who have required an antipsychotic or mood stabilizer (e.g., lithium, carbamazepine, valproate) for a psychiatric condition in the past year, or subjects who have had a major depressive episode (MDE) in the past 6 months. Subjects with major depressive disorder (MDD) or generalized anxiety disorder (GAD) who have been on stable medications for the past 3 months (and are expected to remain stable for the duration of the trial) and whose condition is currently well-controlled may be included.
2. Subjects who have pain that cannot be clearly differentiated from, or could interfere with the assessment of peripheral diabetic neuropathy, as measured by the Masquerading Disorders Tool at Visit 1.
3. Neurologic disorders unrelated to diabetic neuropathy (e.g., phantom limb from amputation), skin condition in the area of neuropathy that could alter sensation (e.g., plantar ulcer), or other painful conditions (e.g., arthritis) that, in the judgment of the investigator, could interfere with reporting of pain due to diabetic neuropathy.

4. History of hypoglycemia that disturbed consciousness, or ketoacidosis requiring hospitalization within past 3 months.
5. Subjects with history of severe renal impairment defined by renal dialysis or peritoneal dialysis, or who have undergone renal transplant.
6. Impaired hepatic function characterized by a previous known diagnosis of chronic liver disease, and/or the presence of abnormal serum total bilirubin, or alanine transaminase (ALT), aspartate transaminase (AST), or alkaline phosphatase ≥1.5 x upper limit of normal (ULN) at screening.
7. Known history of significant cardiovascular condition, such as myocardial infarction or congestive heart failure; evidence of current uncontrolled cardiac arrhythmias, angina, or electrocardiographic evidence of acute ischemia; or active conduction system abnormalities; QTcF >450 msec (males) or >470 msec (females), or uncontrolled hypertension characterized by resting systolic blood pressure values >140 mm Hg or resting diastolic >90 mm Hg.
8. Heart rate <45 bpm or >90 bpm.
9. Fasting triglycerides >250 mg/dL.
10. History of Huntington's disease, Parkinson's disease, Alzheimer's disease, Multiple Sclerosis, or a history of seizures, epilepsy, or strokes.
11. Human immunodeficiency virus (HIV) infection, hepatitis, or other ongoing infectious disease that the investigator considers clinically significant.
12. Concomitant use of antiepileptic drugs, non-steroidal anti-inflammatory drugs (except cardiac preventive acetylsalicylic acid), opioids, muscle relaxants, dextromethorphan (except low dose intermittent use for cough), tramadol, topical lidocaine, topical capsaicin, and selective norepinephrine reuptake inhibitors. Subjects are allowed to enter with a maximum of 1 allowed analgesic medication for neuropathic pain that has been taken at a stable dose for at least 1 month (30 days) prior to Visit 1. Allowed analgesics may not be *N*-methyl-D-aspartate receptor ligands, must be non-opioid and non-sedative and must not interfere with subjects' pain reporting (Section 8.7). Tricyclic antidepressants may be continued if designated as the single analgesic medication for the treatment of pain.
13. History of or current substance abuse disorder as defined by Diagnostic and Statistical Manual of Mental Disorders - Fifth Edition.
14. Recreational and/or medicinal marijuana use within the past 6 months.
15. Positive urine drug screen for marijuana, opioids, benzodiazepines inconsistent with current prescriptions, or breathalyzer test for alcohol at Visit 1.
16. Positive urine drug screen for marijuana, opioids, any benzodiazepines inconsistent with current prescriptions, or breathalyzer test for alcohol at Visit 2.
17. Sensitivity to, allergy to, or concomitant use of *N*-methyl-D-aspartate receptor ligands including ketamine, amantadine, dextromethorphan (except low dose intermittent use for cough), memantine, methadone, dextropropoxyphene, and/or ketobemidone.
18. Amputations of lower extremities (toe amputation is allowed).

19. Any condition, including serious medical conditions that could interfere with the ability of the subject to participate in the study or could confound study assessments.
20. Subjects with hypersensitivity to multiple medications, in the opinion of the investigator.
21. Subjects who meet the criteria for suicidal intent, plan, and/or behavior by scoring 3 or 4 on Questions 2 or 13, or 2 or higher on any Question 1a (only if 1b is coded YES), 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 14 based on the S-STS at Visit 1 or Visit 2.

Waivers to the exclusion criteria will NOT be allowed.

7.3 Screen Failures

Subjects who sign and date the informed consent form, but who fail to meet the inclusion and exclusion criteria, are considered screen failures. Reason(s) for screen failure must be documented by the investigator and provided to the sponsor in a timely fashion. Screen failures who had failed due to not meeting criteria under Protocol Amendment 2 may be rescreened with Sponsor approval. No other subjects may be rescreened.

7.4 Subject Withdrawal Criteria

Subjects who withdraw from the Treatment Period will not be replaced. Any subject may withdraw consent at any point during the study. The investigator may discontinue a subject at any time if it is deemed medically appropriate, or for subject noncompliance with study requirements. Subjects who terminate early should return to the clinic for the Early Termination visit. Subjects may be withdrawn from the study if any of the following criteria are met:

- Occurrence of an adverse event, intercurrent illness, or laboratory abnormality that, in the opinion of the investigator, warrants the subject's permanent withdrawal from the study for subject safety
- Lack of efficacy
- Subject withdraws consent
- Subject noncompliance
- Lost to follow-up
- Pregnancy
- Sponsor termination of study
- An individual stopping criteria is met

7.5 Methods of Birth Control

If heterosexually active, female subjects must agree to consistently use 2 forms of highly effective birth control (at least 1of which must be a barrier method) starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product. Methods of highly effective birth control include:

- Oral or parenteral contraceptives

- Intrauterine device (either hormonal or non-hormonal type acceptable)
- Barrier

Female subjects must agree not to breastfeed starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product. Female subjects must not donate ova starting at Visit 1 and continuing throughout the study period and for 28 days after the final administration of investigational product.

Male subjects must refrain from sperm donation starting at Visit 1 and continuing throughout the study period and for 90 days after the final administration of investigational product. Male subjects who are not surgically sterilized for at least 90 days, and sexually active with a female partner, must use a condom with spermicide during the study, and for 90 days after the last dose of investigational product.

8 STUDY TREATMENT

8.1 Dosing and Administration

This is a double-blind study. Subjects, study personnel, and the sponsor will be blinded to study treatment.

Subjects will be dispensed blinded investigational product according to the randomization schedule. Investigational product will be dispensed at the Week 0 (Baseline) and Week 2 visits; at each of the 2 visits, subjects will receive a 2-week supply of investigational product. Subjects will be instructed to take 2 capsules of investigational product once daily by mouth.

Randomized treatment will be:

NYX-2925 by mouth once daily for 4 weeks, or

Placebo by mouth once daily for 4 weeks

Subjects will also be dispensed 1 bottle of acetaminophen to be used as rescue medication at the visits identified in Table 3. Rescue medication will consist of 500 mg caplets of acetaminophen. Subjects will be instructed that up to 2 g/day of rescue medication are allowed and should be reported daily via their study-issued handheld device.

8.2 Investigational Product Description, Appearance, Packaging, and Labeling

NYX-2925 is a small molecule ((2S, 3R)-3-hydroxy-2-((R)-5-isobutyryl-1-oxo-2,5-diazaspiro[3.4]octan-2-yl)butanamide), that will be provided as capsules for oral administration. Matching placebo capsules will also be provided.

The oral formulation of the investigational product comprises inert United States Pharmacopeia (USP)-grade excipients in a capsule made of hydroxyl-propyl cellulose. The content of the capsule is a dry blend of NYX-2925, microcrystalline cellulose, National Formulary (NF), pregelatinized starch, NF, and magnesium stearate USP.

Matching placebo capsules will contain only the inactive ingredients listed previously.

The investigational product will be provided in a blister-pack kit. The labels will include “NYX-2925 Oral Capsules or Placebo to Match”, capsule count, kit number, storage conditions, retest date, sponsor name, and investigational use statement. Each kit of investigational product will include 18 capsules (nine 2-capsule rows) of either matching placebo capsules or NYX-2925 capsules according to the randomization schedule. The investigational product will be packaged in a way that each dose will be 2 capsules/row.

The sponsor will provide investigative sites with sufficient amounts of investigational product to conduct the study.

8.3 Preparation and Handling

The investigative site will receive blister pack kits of blinded investigational product, and there will be no investigational product preparation done at the investigative site. The investigator may

delegate investigational product handling to site staff who will dispense kits of investigational product for subject administration according to the interactive response technology system. The interactive response technology system is being utilized to ensure the correct distribution of blinded investigational product according to the randomization schedule. The maintenance of treatment randomization codes will be maintained within the interactive response technology system.

8.4 Storage

Investigational product should be stored in a locked, limited access location according to the label. The capsules should be stored at 20ºC to 25ºC with excursions permitted between 15ºC to 30ºC. The investigative site should contact the sponsor for directions regarding non-permitted excursions.

8.5 Accountability

The investigator or designee at the investigative site will conduct investigational product accountability. The investigative site personnel will receive, inspect, and acknowledge condition of investigational product; document the amount received, dispensed and returned, and will dispense, and maintain the investigational product accountability records. A sponsor representative will inspect investigational product and accountability records. Site personnel will conduct subject level accountability, account for and document used/unused investigational product, and retain kits of returned investigational product to the investigative site's limited access storage facility.

Upon completion or termination of the study, and after sponsor accountability is completed, all used and unused supplies will be returned or destroyed as instructed by sponsor representative.

8.6 Treatment Compliance

Site personnel will assess subject compliance with investigational product dosing. In addition, incorrect or partially administered doses will be documented.

8.7 Concomitant and Excluded Medications

Subjects will discontinue all except 1 of their analgesic treatments for neuropathic pain associated with diabetic peripheral neuropathy. The allowed analgesic medication for neuropathic pain may not be an *N*-methyl-D-aspartate receptor ligand, must be non-opioid and non-sedative, and must not interfere with the subject's pain reporting. The allowed analgesic must have been taken for at least 1 month (30 days) prior to Visit 1, and subjects must be on a stable fixed dose that is not expected to change during the study. Subjects may continue their antidiabetic medications.

Concomitant use of antiepileptic drugs, non-steroidal anti-inflammatory drugs (except cardiac preventive acetylsalicylic acid), opioids, muscle relaxants, dextromethorphan (except low dose intermittent use for cough), tramadol, topical lidocaine, topical capsaicin, and selective norepinephrine reuptake inhibitors is prohibited. Tricyclic antidepressants may be continued if designated as the single analgesic medication for the treatment of pain. Concomitant use of the following *N*-methyl-D-aspartate receptor ligands; ketamine, amantadine, dextromethorphan

(except low dose intermittent use for cough), memantine, methadone, dextropropoxyphene, and/or ketobemidone is prohibited. Recreational or medicinal use of marijuana is prohibited.

Subjects will be dispensed acetaminophen to be used as rescue medication, and will be instructed to take no more than 2 g/daily (one to two 500-mg caplets every 4 to 6 hours as needed) for DPN pain. Rescue medication will be provided by the sponsor.

If it is questionable whether a medication(s) falls into the category of excluded medications named above, the investigator should review the medication with the sponsor prior to the enrollment of the subject. Washout from excluded medication is allowed only if the investigator deems it is medically appropriate. The washout period must cover 5 half-lives of the drug and must be completed within the duration of the Screening Period.

Concomitant medications used within 30 days prior to screening and through the follow-up visit must be documented in the source documents and on the corresponding electronic case report form (eCRF).

9 STUDY ASSESSMENTS AND PROCEDURES

9.1 Screening Assessments

9.1.1 Masquerading Disorders Tool

The Masquerading Disorders Tool (Analgesic Solutions, Wayland, MA) is a screening instrument to help identify subjects with disorders that masquerade as painful diabetic peripheral neuropathy, such as Morton's neuroma, peripheral vascular disease, Achilles tendonitis, arthritis, and tarsal tunnel syndrome. The screening instrument consists of symptom screening questions, and an in-clinic history and examination to target the nature and etiology of the subject's peripheral pain. The results of this assessment tool are provisional, with the final decision based on the clinical judgment of the investigator.

9.1.2 Michigan Neuropathy Screening Instrument

The Michigan Neuropathy Screening Instrument is a 15-item, self-administered questionnaire and a lower extremity examination that includes inspection of vibratory sensation and ankle reflexes. It can be used to assess distal symmetrical peripheral neuropathy in diabetes (Herman 2012).

9.1.3 Bedside Sensory Testing Kit

The Bedside Sensory Testing Kit (Analgesic Solutions, Wayland, MA) is used to assess a subject's sensory profile to allow phenotypic identification of subgroups of subjects with painful diabetic peripheral neuropathy who might respond differently to a given treatment. The kit consists of multiple sensory instruments that are applied to different peripheral anatomic sites to evaluate the following phenomena: extent of damage of low threshold mechanoreceptive small fibers in the feet, dynamic mechanical allodynia, punctate hyperalgesia, high threshold mechanoreceptive function, and cold allodynia.

9.1.4 American College of Rheumatology Fibromyalgia Criteria

The 2010 American College of Rheumatology Fibromyalgia Criteria is a clinician-administered survey that is used as a diagnostic tool for fibromyalgia. The diagnostic criteria for fibromyalgia are met by the following 3 conditions:

- Widespread Pain Index ≥7 and Symptom Severity Score ≥5, or the Widespread Pain Index is 3 to 6 and the Symptom Severity Score is ≥9;
- Symptoms have been present at a similar level for at least 3 months; and
- Subject does not have a disorder that would otherwise explain the pain (Wolfe 2010).

9.2 Efficacy Assessments

9.2.1 Numeric Rating Scale of Pain Intensity (NRS)

The NRS of pain intensity is a unidimensional, segmented numeric version of the visual analog scale. A subject selects a whole number (0 to 10) that best indicates the intensity of his/her pain.

The format consists of a horizontal line which is anchored by terms defining pain levels where 0 represents no pain and 10 represents worst pain imaginable.

Subjects will report at bedtime their average pain intensity, worst pain intensity, and pain upon walking during the past 24 hours, using the 11-point NRS.

9.2.2 Patient Global Impression of Change (PGI-C)

The PGI-C is a 7-point scale that allows subjects to rate the change in the disease state from study initiation to specific time points during the study or at the end of the study. It provides the subject's impression of overall change since beginning the study.

9.2.3 Short-Form McGill Pain Questionnaire, version 2 (SF-MPQ-2)

The SF-MPQ-2 measures the sensory, affective, and evaluative qualities of pain, including symptoms relevant to neuropathic pain. The tool includes visual analogue as well as verbal rating scales of pain intensity on a total of 22 neuropathic and non-neuropathic pain descriptors that are each evaluated on a 10-point numerical rating scale (Dworkin 2009).

9.2.4 Norfolk Quality of Life Questionnaire – Diabetic Neuropathy (QOL-DN)

The Norfolk QOL-DN is a 47-item, subject-reported questionnaire designed to measure the relationship between symptomatic diabetic neuropathy and quality of life from the perspective of the patient. It is composed of 2 parts: questions related to symptoms experienced by the patient and questions related to the impact of the patient's neuropathy on activities of daily life. A factor analysis performed on the Norfolk QOL-DN separated the questions into 5 domains: activities of daily living, symptoms, small fiber neuropathy, large fiber neuropathy, and autonomic neuropathy. The QOL-DN is sensitive to the different features of diabetic neuropathy including small fiber, large fiber, and autonomic nerve function (Vinik 2005).

9.2.5 Hospital Anxiety and Depression Scale (HADS)

The HADS is a self-reported screening tool for anxiety and depression in non-psychiatric clinical populations. The scale consists of 14 items (7 each for anxiety and depression), each rated on a 4-point scale ranging from 0 (not at all) to 3 (very often). Responses are based on the relative frequency of symptoms over the preceding week. Possible scores range from 0 to 21 for each subscale. An analysis of scores on the two subscales supported the differentiation of each mood state into four ranges: 'non cases' (scores 0-7), 'mild cases' (scores 8-10), 'moderate cases' (scores 11-15), and 'severe cases' (scores 16 or higher) (Zigmond 1983).

9.2.6 Insomnia Severity Index

The ISI is a 7-item self-report questionnaire assessing the nature, severity, and impact of insomnia. The usual recall period is the "last month" and the dimensions evaluated are: severity of sleep onset, sleep maintenance and early morning awakening problems, sleep dissatisfaction, interference of sleep difficulties with daytime functioning, noticeability of sleep problems by others, and distress caused by the sleep difficulties. A 5-point Likert scale is used to rate each item (e.g., 0 = no problem; 4 = very severe problem), yielding a total score ranging from 0 to 28. The total score is interpreted as follows: absence of insomnia (0–7); sub-threshold insomnia (8-14); moderate insomnia (15–21); and severe insomnia (22–28) (Bastien 2001).

9.2.7 Daily Sleep Interference Scale (DSIS)

The DSIS was developed to quantify sleep interference due to pain. The DSIS is a single-item measure that is completed by patients once a day (upon awakening) to accurately capture variability in sleep interference due to pain on a daily basis, thus minimizing recall bias. The DSIS has an 11-point response scale that asks patients to ‘‘Select the number that best describes how much your pain has interfered with your sleep during the past 24 hours.’’ Response options range from 0 (Did not interfere with sleep) to 10 (Completely interfered with sleep / unable to sleep due to pain). The DSIS is designed to be used in a patient daily diary that patients fill out upon awakening each morning (Vernon 2008).

9.2.8 Brief Pain Inventory for Diabetic Peripheral Neuropathy (BPI-DPN)

The BPI is a patient-completed numeric rating scale that assesses the severity of pain (Severity scale), its impact on daily functioning (Interference scale), and other aspects of pain (e.g., location of pain, relief from medications). A modified version of the BPI, including the 4-item pain Severity scale (Worst Pain, Least Pain, Average Pain, and Pain Now) and the 7-item pain Interference scale (General Activity, Mood, Walking Ability, Normal Work, Relations With Others, Sleep, Enjoyment of Life) has been validated in patients with painful diabetic peripheral neuropathy), which distinguishes between pain due to DPN and pain due to other causes. Each BPI item uses a 0-10 numeric rating scale anchored at zero for “no pain” and 10 for “pain as bad as you can imagine” for Severity, and “does not interfere” to “completely interferes” for Interference. The 4 Severity items and the 7 Interference items can also each be averaged to form 2 composite scores, the Pain Severity Index and the Pain Interference Index (Zelman 2005).

9.3 Safety Assessments

9.3.1 Safety Parameters

Refer to the Table 3 (Schedule of Procedures) or Section 10 (Schedule of Procedures) for time points.

9.3.2 Vital Sign Measurements

Each assessment includes blood pressure and pulse after at least 5 minutes of being seated, and prior to blood sample collection. Vital signs may be repeated after 20 minutes for eligibility assessments.

9.3.3 Physical Examination

A complete or brief physical examination will be performed as specified in the schedule of procedures table. A complete physical examination will include assessment of the following body systems: head, eyes, ears, nose, and throat; lymph nodes; neurologic; respiratory; cardiovascular; gastrointestinal; musculoskeletal; dermatologic; and extremities.

The physical examination at Visit 1 will include a comprehensive neurological examination. The brief physical examination will be completed per investigator discretion.

Body weight and height will be measured at selected visits (Table 3).

9.3.4 Electrocardiogram

Electrocardiograms (5-minute duration) will be collected after 5 minutes of supine rest and prior to blood sample collection, if possible. The device will be a Spaulding electrocardiogram device that acquires a 12-lead electrocardiogram continuously at each time point for up to 5 minutes. The 5 minutes of electrocardiogram data will be converted into a summary electrocardiogram (simulated 10-second electrocardiogram) for each electrocardiogram time point and submitted to the designated electrocardiogram laboratory cardiologists for measurements and diagnoses as though it were a standard 10-second electrocardiogram. Electrocardiograms may be repeated for eligibility assessments.

The investigator or qualified sub-investigator will review all electrocardiogram interpretations and interval duration measurements for clinical significance. Standard electrocardiogram parameters will be measured. Clinically significant, abnormal 12-lead electrocardiograms should be repeated. Clinically significant electrocardiogram changes should be recorded as an adverse event.

The Spaulding electrocardiogram device should be used for all assessments during the study unless it is non-operational, in which case another electrocardiogram may be performed locally and transmitted to Spaulding for central reading via printout.

9.3.5 Clinical Laboratory Assessments

Blood samples for serum chemistry and hematology will be obtained after an 8-hour fast, except for the Week 2 and Week 5 liver function tests. The study investigator should monitor subjects during the study for signs of hepatic abnormality potentially meeting the stopping criteria (Section 11).

The investigator will review clinical laboratory test results as they become available, for determination and documentation of clinical significance (if there are out of range values). The investigative site will contact the medical monitor for any liver function test results that potentially meet the stopping criteria. Possibly drug related or clinically relevant abnormal values of uncertain causality must be repeated. Persistently abnormal laboratory values should be followed at the investigator's discretion. Clinical labs may be repeated for eligibility assessments. Abnormal liver function tests (specified in section 11) must be repeated within 48-72 hours.

Hematology assessments will include total white blood cell count, differential (neutrophils, lymphocytes, monocytes, eosinophils, and basophils), hemoglobin, hematocrit, red blood cell count, platelet count and HbA1c (at Visit 1).

Serum chemistry assessments will include electrolytes (bicarbonate, calcium, chloride, phosphorous, potassium, sodium, and magnesium), enzymes (alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, and gamma-glutamyl transferase), and other (albumin, total bilirubin, direct bilirubin, indirect bilirubin, creatinine, glucose, triglycerides (at screening), total protein, blood urea nitrogen, and uric acid).

Qualitative urinalysis will include pH, specific gravity, glucose, ketones, protein, blood, leukocyte esterase, urobilinogen, nitrites, and reflex microscopy.

All women of childbearing potential will receive urine pregnancy tests (ß-human chorionic gonadotropin).

The urine drug panel will include cannabinoids, opioids, and benzodiazepines; an alcohol screen will also be performed with a breathalyzer.

Hepatitis and HIV screen includes: Hepatitis B surface antigen, hepatitis C antibody, and HIV. If the screening hepatitis C antibody test result is reactive, a nucleic acid test for HCV RNA should be performed for confirmation of chronic infection. If the screening HIV test is positive, a confirmatory Western Blot test should be performed to confirm HIV status.

9.3.6 Sheehan Suicidality Tracking Scale (S-STS)

The standard version of the S-STS (Sheehan 2014[2]) is a 16-item scale that assesses the seriousness of suicidality phenomena on a Likert-type scale (0 to 4) ranging from 0 = "not at all" (0) to 4 = "extremely". It also assesses the frequency of key phenomena and the overall time spent in suicidality. The standard version is available in identical clinician- and subject-rated formats. The S-STS accommodates a wide range of time frames. In clinical trials, the frequently used variants are "in the past week", "in the past month", "since the last visit", "lifetime look back", and "in the past day".

The S-STS maps to both the C-CASA, and to all of the categories in the FDA Draft Guidance documents of both 2010 and 2012, and in addition provides coverage for all suicidal phenomena presented in the draft FDA guidance. The S-STS has been studied and validated against other instruments used for the assessment of suicidality in a study, and been shown provide at least equal sensitivity to detecting suicidal ideation and behavior (Sheehan 2014 [1]).

Suicidality identified after baseline will be recorded as an adverse event.

9.4 Safety and Pharmacovigilance

9.4.1 Adverse Events – Relationship to Investigational Product

An adverse event is any untoward, undesired, unplanned clinical event in the form of signs, symptoms, disease, or laboratory or physiological observations occurring in a human being participating in a clinical study with an Aptinyx investigational product, regardless of causal relationship. A pre-existing condition is one that is present before investigational product dosing and is reported as part of the subject's medical history. Pre-existing conditions should be reported as an adverse event only if the frequency, intensity, or character of the pre-existing condition worsens during the course of the study.

Laboratory abnormalities are not considered adverse events unless they are associated with clinical signs or symptoms, or require medical intervention. However, a laboratory abnormality (e.g., a clinically significant change detected on clinical chemistry, coagulation, hematology, urinalysis) that is independent from the underlying medical condition and that requires medical or surgical intervention, or leads to investigational product interruption or discontinuation, must be considered an adverse event.

For each reported adverse event, an investigator must assess the relationship to investigational product using the following scale:

Unrelated

- Does not follow a known response pattern to the suspect investigational product (if response pattern is previously known).
- Can clearly be explained by the known characteristics of the subject's clinical state or other modes of therapy administered to the subject.

Unlikely Related

- The temporal sequence from dosing of the investigational product suggests that a relationship is unlikely.
- Follows a response pattern that is unlike that of the suspect investigational product (if response pattern is previously known).
- Could be reasonably explained by the subject's clinical state or other modes of therapy administered to the subject.

Possibly Related

- Follows a reasonable temporal sequence from dosing of the investigational product.
- May follow a known response pattern to the suspect investigational product (if response pattern is previously known).
- Could also be reasonably explained by the subject's clinical state or other modes of therapy administered to the subject.

Probably Related

- Follows a reasonable temporal sequence from dosing of the investigational product.
- Could not be reasonably explained by the known characteristics of the subject's clinical state or any other modes of therapy administered to the subject.
- Is confirmed by improvement on stopping or slowing dosing of the investigational product, if applicable.

When an assessment is not provided, the event will be treated as possibly related for purposes of regulatory reporting.

9.4.2 Recording Adverse Events

A "pre-existing" condition is one that is present before investigational product dosing and is reported as part of the subject's medical history. Pre-existing conditions should be reported as an adverse event only if the frequency, intensity, or character of the pre-existing condition worsens during the course of the study. Adverse events will be collected from the time the informed consent is signed to the end of the subject's participation in the study.

Subjects should be instructed to report all potential adverse events to the investigator, and, queried in a non-leading manner, without specific prompting (e.g., "How are you feeling?"). The site study staff should assess emerging symptoms of dissociative reaction similar to those caused

by NMDA antagonists including memory impairment, disturbance in time, body or environmental perception, stilted speech, emotional withdrawal, impaired coordination, motor retardation, bizarre reasoning or illusory experiences in any sensory perception, or confused state; such symptoms may be captured as adverse events.

To avoid vague, ambiguous, or colloquial expressions, all adverse events should be recorded in standard medical terminology rather than in the subject's own words. Each adverse event will also be described in terms of duration (start and stop date), severity, relationship to investigational product, action(s) taken, and outcome. Diagnoses (rather than symptoms) should be recorded wherever possible.

9.4.3 Reporting Adverse Events

All adverse events must be documented, evaluated, and reported in the source documents and eCRF. Adverse event collection begins after the subject has completed the informed consent and continues until the subject's participation in the study ends. Ongoing adverse events should be followed to a satisfactory resolution in the investigator's opinion. Subjects should be instructed to report all adverse events to the investigator. In addition, the investigator should seek to elicit any clinical or objective reactions by specific questioning (e.g., "How have you been feeling?") and as appropriate by examination. Information on all adverse events should be recorded on the eCRF. All clearly related signs, symptoms, and results of diagnostic procedures performed in relation to an adverse event should be grouped together and recorded as a single diagnosis.

9.4.3.1 Severity of Adverse Events

All adverse events will be assessed for severity, using the following general grading scale:

Mild: Transient or mild discomfort (<48 hours); no medical intervention/therapy required.

Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required.

Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy, required hospitalization possible.

Life threatening: Extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization, or hospice care probable.

When changes in the severity of an adverse event occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates).

9.4.3.2 Action Taken for Adverse Events

For each reported adverse event, an investigator must document the action taken according to the following criteria:

- No action taken
- Concomitant medication taken
- Hospitalization or prolongation of hospitalization

- Discontinued study
- Nondrug therapy
- Other (specify)

The investigator must also document the action taken in regards to investigational product (as a result of a given adverse event) according to the following criteria:

- Dose not changed
- Investigational product interrupted
- Investigational product withdrawn
- Not applicable
- Unknown

9.4.3.3 Outcome for Adverse Events

Adverse events should be followed until resolution. For each reported adverse event, the investigator must document the outcome according to the following criteria:

- Fatal
- Not recovered/not resolved
- Recovered/resolved
- Recovered/resolved with sequelae
- Recovering/resolving
- Unknown

9.5 Serious Adverse Events

Serious adverse events are reported to the sponsor-designated medical monitor using the Aptinyx provided form by email or fax at:

- [redacted]
- [redacted]

A serious adverse event is any adverse event occurring at any dose that results in any of the following outcomes:

- Death: “Death” is an outcome and is NOT the adverse event. In the event of death, the cause of death should be recorded as the adverse event. The only exception is “sudden death” when the cause is unknown.
- Is a life-threatening experience: Life-threatening adverse events include any adverse drug experience, which, in the view of the investigator, places the subject at immediate risk of death from the reaction as it occurs. It does not include a reaction that, had it occurred in a more serious form, might have caused death.
- Requires inpatient hospitalization or prolongation of existing hospitalization

- Results in a persistent or significant disability/incapacity: Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.
- Results in a congenital anomaly/birth defect
- Additionally, important medical events that may not result in death, be life threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed previously. Example: allergic bronchospasm requiring intensive treatment in an emergency room or at home.

All serious adverse events that result in death or are life threatening, regardless of causal relationship, must be reported to the sponsor-designated medical monitor within 24 hours of the site's knowledge of the event. A copy of the initial serious adverse event report must be received within 1 business day.

All other serious adverse events or other events reportable to the United States Food and Drug Administration (FDA) and/or IRB will be forwarded to the sponsor-designated medical monitor within 1 business day.

The serious adverse event report should provide as much of the required information as is available at the time. The following minimum information is required for reporting a serious adverse event: subject identification, reporting source, and an event outcome. Supplemental information may be transmitted using a follow-up report and should not delay the initial report. Aptinyx may contact the investigational site to solicit additional information or to follow-up on the event.

If there is any doubt whether the information constitutes a serious adverse event, the information will be treated as a serious adverse event for the purposes of this protocol. Serious adverse event reporting will begin at the time of consent and will end 30 days after the last dose.

All relevant documentation pertaining to a serious adverse event (additional laboratory tests, consultation reports, discharge summaries, postmortem reports, etc.) will be provided to the sponsor-designated medical monitor in a timely manner. Serious adverse events will be followed until resolution or return to baseline (when worsening of a pre-existing condition is reported). If a serious adverse event does not return to baseline but reaches a stable situation that is not expected to change, this may be documented on the serious adverse event form.

The Sponsor may break the treatment code for subjects who experience a serious adverse event in order to determine if the individual case or group of cases required expedited regulatory reporting. Individual treatment codes will be available to limited staff who are responsible to break codes for reporting purposes.

All serious adverse events will be reported to the FDA.

9.6 Other Reportable Events

Reports of overdose (with or without an adverse event), abuse, dependency, inadvertent or accidental exposure, pregnancy, and unexpected therapeutic benefit should be forwarded in the same timeframe as a serious adverse event. Overdose occurs when a subject is dosed or has taken

a dose greater than the intended or scheduled dose specified by the protocol. All pregnancies occurring during the study must be followed for information regarding the course of pregnancy, delivery, and condition of the newborn. Follow-up should be provided by the investigator to the sponsor-designated medical monitor in a timely manner. When the newborn is healthy, further follow-up is not necessary. Male subjects must inform the investigator if their partner becomes pregnant during the study. Female subjects of childbearing potential and female partners of male subjects must use an acceptable method of birth control during the participation in this study.

10 SCHEDULE OF PROCEDURES

The schedule of procedures is summarized in Table 3 .

10.1 Screening Period (Week -4 to -1)

The subject will be asked to provide informed consent for this study before any required procedures are performed. Subjects will also be asked to sign an additional consent for their inclusion in a subject registry database. Adverse event collection will begin at the time of informed consent.

After the informed consents are signed, the following procedures and assessments should be completed at Visit 1:

- Confirmation of diabetic peripheral neuropathy by the following assessments:
  - Michigan Neuropathy Screening Instrument
  - Masquerading Disorders Tool
  - American College of Rheumatology Fibromyalgia Criteria
- NRS for average pain intensity over the past 24 hours
- Demographic characteristics (age, sex, race, and ethnicity)
- Medical history
- Prior (within last 30 days) and concomitant medications
- Height and body weight
- Sitting vital signs (blood pressure and pulse after 5 minutes)
- Complete physical examination, including comprehensive neurological examination
- Electrocardiogram after 5 minutes in supine position
- Urine pregnancy test for females of childbearing potential
- Urine drug test
- Breathalyzer test for alcohol
- Blood samples for fasting (8 hours) serum chemistry HIV, hepatitis B surface antigen, hepatitis C antibody, triglycerides, and hematology (including HbA1c)
- Urine sample for urinalysis
- HADS
- S-STS
- Adverse events

Eligible subjects who meet all inclusion and exclusion criteria will enter the 1- to 4-week Screening Period. The following procedures and assessments will be performed:

- Subjects who are taking more than 1 analgesic medication will discontinue all but 1 of the medications as allowed by the protocol before the start of daily pain and sleep evaluations. The duration of their Screening Period will depend on the time required to discontinue analgesic medications, but the total duration of the Screening Period cannot exceed 4 weeks. Subjects who are taking no more than 1 allowed analgesic medication will begin daily pain and sleep evaluations at bedtime immediately after Visit 1 (i.e., will begin Week -1).
- Subjects will be provided a handheld device, and as a method for ensuring consistency and reliability of pain scoring, instructed on how to record their pain on the device [redacted] Subjects will also be educated on appropriate expectations around their participation in a clinical study [redacted] On each of the days preceding Visit 2 (Baseline Visit), subjects will record the following on the handheld device:
  - At bedtime: average pain intensity, worst pain intensity, pain on walking, and use of rescue medication during the past 24 hours
  - Upon waking: DSIS
- Subjects will be dispensed acetaminophen to be used as rescue medication, and will be instructed to take no more than 2 g/daily (one to two 500-mg caplets every 4 to 6 hours as needed) for DPN pain.
- The investigative site staff will contact study subjects by telephone weekly until and including Week -1 to reinforce the reporting instructions (7±2 days prior to Visit 2) and assess adverse events.

10.2 Baseline (Week 0)

At Visit 2 (Baseline Visit), the following procedures and assessments will be performed:

- Medical history update
- Concomitant medication use assessed
- Rescue medication supplies returned, appropriate use assessed, and compliance/accountability performed
- Adverse events assessed
- Sitting vital signs (blood pressure and pulse after 5 minutes)
- Brief physical examination
- Electrocardiogram after 5 minutes in supine position
- Urine pregnancy test for females of childbearing potential
- Urine drug test

- Breathalyzer test for alcohol
- Urine sample for urinalysis
- Bedside Sensory Testing Kit
- Pain scores reported by subjects during the Screening Period and diary compliance evaluated by the interactive response technology system for raw score and for variability among scores by automated transmission of pain scores from the handheld devices

Subjects who continue to meet all entry criteria and are randomized by the interactive response technology system will undergo the following procedures and assessments:

- Blood samples for fasting (8 hours) serum chemistry and hematology
- HADS
- S-STS
- BPI-DPN
- SF-MPQ-2
- QOL-DN
- Insomnia Severity Index
- Investigational product dispensed
- Rescue medication dispensed

Subjects will be reminded on how to record their pain on the device [redacted] Subjects will also be reminded about appropriate expectations around their participation in a clinical study [redacted] Review of these educational materials may be repeated for some or all subjects depending on findings of an ongoing blinded data review (e.g., if pain score variability is increased on a subject or site level. On each day until returning to the study site at Week 2, subjects will record the following on the handheld device:

- At bedtime: average pain intensity, worst pain intensity, pain on walking, and use of rescue medication during the past 24 hours.
- Upon waking: DSIS

The investigative site staff will contact study subjects by telephone one week following Week 0 to reinforce the diary completion and assess adverse events.

Subjects will be instructed to return to the study site at Week 2.

10.3 Treatment Period (Week 2 and Week 4)

10.3.1 Week 2 (Visit 3)

At Visit 3, the following procedures and assessments will be performed:

- Concomitant medication use assessed
- Adverse events assessed
- Sitting vital signs (blood pressure and pulse after 5 minutes)
- Serum chemistry for liver function testing (only)
- PGI-C
- Insomnia Severity Index
- Rescue medication supplies returned, appropriate use assessed, and compliance/accountability performed
- Investigational product and supplies returned and compliance/accountability performed
- Compliance with entry of pain intensity and rescue medication into daily diary
- Investigational product dispensed
- Rescue medication dispensed

Subjects will be reminded on how to record their pain on the device [redacted] Subjects will also be reminded about appropriate expectations around their participation in a clinical study [redacted] Review of these educational materials may be repeated for some or all subjects depending on findings of an ongoing blinded data review (e.g., if pain score variability is increased on a subject or site level. On each of day until returning to the study site at Week 4, subjects will record the following on the handheld device:

- At bedtime: average pain intensity, worst pain intensity, pain on walking, and use of rescue medication during the past 24 hours.
- Upon waking: DSIS

The investigative site staff will contact study subjects by telephone one week following Week 0 to reinforce the diary completion and assess adverse events.

Subjects will be instructed to return to the study site at Week 4.

10.3.2 Week 4 (Visit 4)/Early Termination

At Visit 4, or at the time of early termination from the study, the following procedures and assessments will be performed:

- Concomitant medication use assessed
- Adverse events assessed
- Body weight
- Sitting vital signs (blood pressure and pulse after 5 minutes)
- Brief physical examination
- Electrocardiogram after 5 minutes in supine position

- Urine pregnancy test for females of childbearing potential
- Urine drug test
- Breathalyzer test for alcohol
- Blood samples for fasting (8 hours) serum chemistry and hematology
- Urine sample for urinalysis
- BPI-DPN
- SF-MPQ-2
- QOL-DN
- Insomnia Severity Index
- HADS
- PGI-C
- S-STS
- Rescue medication supplies returned, appropriate use assessed, and compliance/accountability performed
- Investigational product and supplies returned and compliance/accountability performed
- Study-issued handheld device returned
- Compliance with entry of pain intensity and rescue medication into daily diary

Subjects will be instructed to return to the study site after 7 days.

10.4 Follow-Up Period (Week 5/7 Days Post-Treatment)

Subjects will return to the study site 7 days following the Week 4/Early Termination visit to assess adverse events and concomitant medication use. The adverse event assessment will include any serious adverse events that were ongoing at the time of study completion, and whether any ongoing adverse events had progressed to becoming serious. Blood samples will be collected for liver function testing.

11 STOPPING CRITERIA

Subjects will be withdrawn from the treatment period of the study if any of the following criteria are met. Repeat testing must be completed within 48-72 hours:

Repeated results (i.e. values confirmed by repeat testing):

- Serum total bilirubin ≥ 2.5 mg/dL, or
- Subject meets one of the following criteria
  - ALT or AST >3 x ULN, or
  - ALT or AST ≥ 3 x ULN, and total bilirubin ≥ 2 x ULN, or
  - ALT or AST ≥ 3 x ULN, with the appearance of fatigue, nausea, vomiting, right upper quadrant pain, or tenderness, fever, rash, or eosinophilia (>5%), or
  - Alkaline phosphatase ≥ 2x ULN.

12 STATISTICS

12.1 Sample Size Determination

The planned sample size is 300 randomized subjects. This sample size will provide approximately 80% power for a 2-sided t-test with Type I error of 0.048.

12.2 Randomization

Daily pain scores and diary compliance will be transferred into the interactive response technology system, which will be used to assess the criteria for randomization. Subjects whose mean of the daily average pain intensity score during the preceding 7 (±1) days is within the protocol-defined algorithm and with adequate compliance with daily diary completion will be eligible for randomization.

Eligible subjects will be randomized to receive either NYX-2925 or placebo for 4 weeks in a pre-specified allocation. The randomization will be stratified by study site.

12.3 Statistical Analysis Plan

The planned statistical analysis methods, including procedures for accounting for missing, unused, and spurious data, will be described in more detail in the Statistical Analysis Plan, which will be finalized prior to study completion. Deviations from the original Statistical Analysis Plan will be described in the final clinical study report.

Descriptive statistics for categorical variables will include the number and percent of subjects with each characteristic. Percentages will be based on the number of subjects with non-missing values. Descriptive statistics for ordinal and continuous variables will include the number of subjects with non-missing values, mean, median, standard deviation, minimum value, and maximum value.

All relevant data collected in the eCRF will be shown for each subject in the individual subject data listings.

12.4 Analysis Populations

Safety analyses will be based on the Safety Population, which is defined as all subjects who receive at least 1 dose of investigational product.

Efficacy analyses will be based on the modified Intent-to-Treat Population, which is defined as all subjects in the Safety Population with at least 1 post baseline assessment of the pain intensity NRS.

12.5 Analyses of Efficacy

12.5.1 Primary Efficacy Endpoint

12.5.1.1 Primary Efficacy Analysis

For the primary efficacy endpoint, the daily pain intensity NRS score from the handheld device will be averaged for baseline (Days -7 to -1) and Week 4 (Days 22 to 28) after randomization. Change from baseline to Week 4 will be assessed for treatment group differences with an analysis of covariance with fixed factors for study site and treatment, with baseline value as a covariate.

12.5.1.2 Sensitivity Analysis

For the primary efficacy endpoint, the daily pain intensity NRS score from the handheld device will be averaged for baseline (Days -7 to -1) and each week (Days 1 to 7, Days 8 to 14, Days 15 to 21, and Days 22 to 28) after randomization. A mixed model repeated measures analysis of variance will be used to assess treatment differences. The model will include factors for study site, treatment, week, and the treatment-by-week interaction, with baseline value as a covariate.

12.5.2 Other Efficacy Endpoints

Change in the mean NRS score assessing average pain intensity in the past 24 hours from baseline (average of Days -7 to -1) to each week of treatment (average of Days 1 through 7, Days 8 through 14, and Days 15 through 21) will be assessed for treatment group differences with an analysis of covariance with fixed factors for study site and treatment, with baseline value as a covariate. Change from baseline for the PGI-C, SF-MPQ-2, QOL-DN, Insomnia Severity Index, HADS, DSIS, BPI-DPN will be analyzed similarly.

The use of rescue medication and percent of subjects meeting responder criteria (≥30% reduction and first ≥50% reduction in the NRS average pain intensity) will be summarized descriptively.

The number of days to the first ≥30% reduction and first ≥50% reduction in the NRS average pain intensity and the number of days to sustained pain reduction will be assessed for treatment group differences with the log-rank test. Subjects who fail to achieve the target reduction will be censored at the end of their final week with NRS pain intensity measurements.

12.5.3 Multiple Comparisons

The primary efficacy endpoint and primary statistical analysis are protocol-specified. A Type I error of 0.005 will be allocated to the interim analysis (if applicable) and a Type I error of 0.048 will be allocated to the final analysis (Fleming 1984). There will be no adjustment for multiple treatment group comparisons in this phase 2 study.

12.6 Analyses of Safety

All safety summaries will be descriptive; no statistical testing will be performed.

12.6.1 Adverse Events

A TEAE is defined as an adverse event with an onset that occurs after receiving investigational product, or a continuing adverse event diagnosed prior to the date of first dose of investigational product, which increases in severity after the start of dosing. Adverse events will be categorized by system organ class and preferred term with the Medical Dictionary for Regulatory Activities.

Summary tables for TEAEs will include number and percent of subjects experiencing TEAEs by system organ class and preferred term. If a subject has more than 1 TEAE that codes to the same preferred term, the subject will be counted only once for that preferred term. Similarly, if a subject has more than 1 TEAE within a system organ class category, the subject will be counted only once in that system organ class category.

The following TEAE summaries will be provided for the Screening Period and Treatment Period (including Follow-Up):

- Overall summary of TEAEs
- TEAEs by system organ class and preferred term
- Drug-related TEAEs by system organ class and preferred term
- TEAEs by system organ class, preferred term, and severity

Serious TEAEs and TEAEs leading to investigational product discontinuation will be identified.

12.6.2 Clinical Laboratory Tests

Mean change from baseline (Visit 2) to Week 4 (Visit 4) will be summarized descriptively for each analyte.

Clinical laboratory results considered clinically important by the investigator will be identified. Individual results for clinical laboratory tests (serum chemistry, hematology, and urinalysis) outside the normal range will be flagged in the data listings.

Clinical laboratory assessments will be conducted by the following central laboratory:

[redacted]

12.6.3 Electrocardiogram

The interpretation of electrocardiogram and interval duration measurements by the designated electrocardiogram laboratory cardiologists will be reviewed by the investigator. Clinically significant deteriorations from baseline will be reported and summarized as TEAEs.

12.6.4 Vital Sign Measurements

Change from baseline to each scheduled measurement will be summarized descriptively. Baseline will be defined as the last evaluation prior to dosing with investigational product on Day 1.

12.6.5 Suicidal Ideation

Subjects with suicidal ideation or behavior will be identified with the S-STS.

12.7 Interim Analysis

One interim analysis may be conducted after approximately 50% of subjects (i.e., approximately 150 subjects) have completed Visit 4. Based on the interim analysis, 1 or more dose groups of NYX-2925 could be discontinued and the total sample size re-estimated. A Type I error of 0.005 would be allocated to the interim analysis. Complete details of the interim analysis would be provided in a separate analysis plan. It is not anticipated that the interim analysis would lead the Sponsor to decide to terminate the trial, although such a decision, made for any reason, would be made after consultation with FDA.

13 ADMINISTRATIVE

13.1 Source Documents

Source documents are defined as the result of original observations and activities of a clinical investigation. Source documents may include, but are not limited to, study progress notes, e-mail correspondences, informed consent forms, clinical laboratory reports, medical histories, hospital records, and drug accountability records. All source documents will be maintained by the investigator(s) and made available for inspection by sponsor representatives, the FDA, and other applicable regulatory authorities.

13.2 Study Monitoring

Site visits will be conducted by an authorized sponsor representative (site monitor) to inspect study data, source documents, and eCRFs in accordance with International Conference on Harmonisation (ICH) guidelines, good clinical practices (GCPs), and local regulations or guidelines. The monitor will inspect the study data at regular intervals throughout the study to verify adherence to the protocol, as well as completeness, consistency, and accuracy of study data.

The investigator will permit sponsor representatives, its third party vendors, the FDA, and/or respective health authorities to inspect facilities and records relevant to this study.

13.3 Case Report Forms

An eCRF will be used to record all subject data required in this protocol. The eCRF must be completed by designated and trained study personnel. The eCRF will be signed by the principal investigator or a subinvestigator listed on the FDA Form 1572. It is the responsibility of the principal investigator to ensure the eCRFs are completed and submitted to Aptinyx (or designee) in an accurate and timely manner. The processing of eCRFs will include an audit trail (to include changes made, reason for change, date of change and person making the change).

13.4 Protocol Amendment(s)

If a protocol has been filed with regulatory agencies or submitted to an IRB and requires changes, a protocol amendment must be written. The sponsor will make changes to the protocol. All amendments will be sent to a central IRB on the study sites' behalf, if applicable. Sites using a local IRB are responsible for submitting the amendment for review and approval.

13.5 Audits and Inspections

During the course of the study, or after completion of the study, each study site may be subject to an audit by an Aptinyx Quality Assurance Auditor (or an auditor appointed by Aptinyx or its authorized representative) and/or an inspector from the FDA and/or other regulatory authority. Every attempt will be made to notify the investigator in writing in advance of the audit.

13.6 Institutional Review Board

This protocol, the informed consent document, and all relevant supporting data must be submitted to the IRB for approval. Institutional Review Board approval of the protocol, informed consent document, any subject-facing materials, and any advertisement used to recruit study subjects must be obtained before the study may be initiated. The IRB must comply with requirements set forth in the Code of Federal Regulations (CFR) part 56.

The investigator is responsible for keeping the IRB advised of the progress of the study, changes to research activity, unanticipated problems involving risk to human subjects or others, and any changes made to the protocol as deemed appropriate, but in any case, at least once a year. The investigator is also responsible for notifying the IRB of any significant adverse events or protocol deviations that occur during the study, and meet IRB reporting requirements.

The investigator agrees that he or she will not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects, as referenced in 21 CFR 312.66.

13.7 Compliance with Regulatory Requirements

This protocol will be conducted in compliance with the protocol and all regulatory requirements, in accordance with GCP, including ICH Guidelines, and in general conformity with the most recent version of the Declaration of Helsinki.

13.8 Informed Consent

Written informed consent must be obtained from potential study participants prior to the initiation of non-routine study-related tests. The original signed informed consent form for each participating subject shall be filed with records kept by the investigators(s). A copy of the signed informed consent document must be provided to the subject. If applicable, written consent will be obtained using a certified translation. If the informed consent form is revised, subjects must be re-consented in a timely manner.

13.9 Study File Management

The investigator is responsible for ensuring that the study files are maintained. The study file will include, but is not limited to, source documents, correspondence, regulatory documents (IRB approvals/correspondence, study logs, FDA 1572 forms, financial disclosures, clinical study material records, investigational product accountability records, and medical records).

13.10 Study Completion

Aptinyx requires the following data and materials be completed before a study can be considered terminated or completed: source documents are completed, investigational product reconciliation activities are completed, study procedures and assessments are source verified and completed.

13.11 Confidentiality

Personal study subject data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precautions to ensure the

confidentiality of personal health information, and in accordance with applicable national and/or local laws and regulations on personal health information protection.

Monitors, auditors, and other authorized agents of Aptinyx, the IRB approving this research and applicable regulatory authorities will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects, to the extent permitted by the law and regulations. In any presentation of the results of this study at meetings or in publications, the subjects' identity will remain confidential.

13.12 Compensation, Insurance, and Indemnity

Information regarding compensation, insurance, and indemnity is addressed in the Clinical Trial Research Agreement.

13.13 Financial Disclosure

The investigator(s) are responsible for providing financial disclosure(s) in covered clinical studies. Principal investigators and subinvestigators are required to disclose applicable financial information, and to promptly update Aptinyx with any relevant changes throughout the study and for 1 year after study completion.

13.14 Records Retention

According to United States Investigational New Drug regulations (21 CFR 312.62), records and documents pertaining to the conduct of this study and the distribution of investigational products including but not limited to source documents, eCRFs, informed consent forms, clinical laboratory test results, and drug inventory records will be retained. These records will be kept on file by the principal investigator for 2 years after a marketing application is approved for the drug for the indication for which it is being studied. If no application is filed or approved, these records must be kept for 2 years after the investigation has been discontinued and the FDA has been notified. Per ICH guidelines, documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region, or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. Aptinyx will notify the investigator when records and documents no longer need to be retained. No study records should be destroyed without prior authorization.

13.15 Publication Policy

The publication policy is outlined in the Clinical Trial Agreement.

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