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NYX-783-2004

A Study to Evaluate the Safety and Efficacy of NYX-783 in  
Subjects with Post-Traumatic Stress Disorder

Protocol

01 May 2020

**INVESTIGATIONAL DRUG: NYX-783**  
**CLINICAL PROTOCOL: NYX-783-2004**

**A Study to Evaluate the Safety and Efficacy of NYX-783 in  
Subjects with Post-Traumatic Stress Disorder**

Sponsor: Aptinyx Inc.



IND Number: 135011

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**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 Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guidelines, and in general conformity with the most recent version of the Declaration of Helsinki.

Principal Investigator

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Printed Name

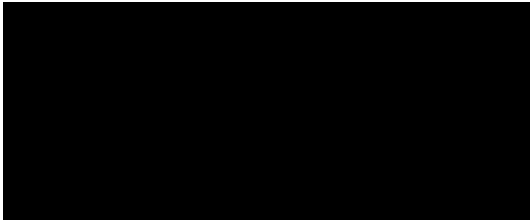
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Signature

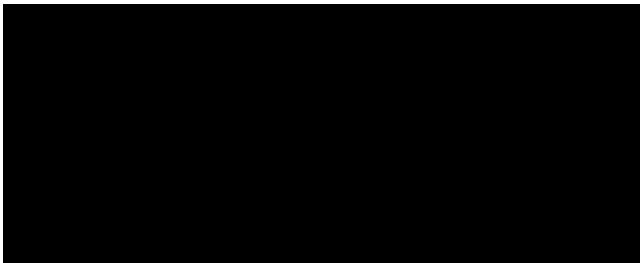
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Date

**APPROVAL PAGE:**



**Aptinyx Inc.**



## PROCEDURES IN CASE OF EMERGENCY

**Table 1: Emergency Contact Information**

Role in Study	Name	Contact Information
[REDACTED]	[REDACTED]	[REDACTED]
<b>Serious Adverse Event Reporting Information</b>		
Email: [REDACTED]	[REDACTED]	

## 2. SYNOPSIS

<b>Name of sponsor/company:</b> Aptinyx Inc.	
<b>Name of investigational product:</b> NYX-783	
<b>Name of active ingredient:</b> [REDACTED]	
<b>Title of study:</b> A Study to Evaluate the Safety and Efficacy of NYX-783 in Subjects with Post-Traumatic Stress Disorder	
<b>Study center:</b> Multicenter (up to approximately 30 enrolling sites)	
<b>Studied period (years):</b> Estimated date first subject enrolled (screened): January 2019 Estimated date last subject enrolled: June 2020	<b>Phase of development:</b> 2
<p><b>Study Objective:</b> The overarching goal of this exploratory study is to investigate the safety and tolerability of NYX-783 in a PTSD population, and to characterize the response profile of PTSD symptoms to NYX-783 treatment, as assessed by the intrusion, avoidance, and negative alterations in cognition and mood, and arousal &amp; reactivity subscores as well as the total score on the Clinician-Administered PTSD Scale for the Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup> edition (CAPS-5)</p> <p><b>Primary Objective:</b></p> <ul style="list-style-type: none"> <li>To assess the effects of NYX-783 compared to placebo on reducing the severity of Post-Traumatic Stress Disorder (PTSD) symptoms as measured by the Clinician-Administered PTSD Scale for the Diagnostic and Statistical Manual of Mental Disorders, 5<sup>th</sup> edition (CAPS-5).</li> </ul> <p><b>Secondary Objectives:</b></p> <ul style="list-style-type: none"> <li>To assess the effect of NYX-783 compared to placebo in the following outcomes: a) change in symptoms of PTSD as measured by the PTSD-Checklist for DSM-5 (PCL-5), b) change in sleep quality as measured by the Pittsburgh Sleep Quality Index (PSQI) Global Score and the PSQI Addendum for PTSD (PSQI-A) Global Score and the PSQI Addendum for PTSD (PSQI-A) Global Score, and c) change in cognitive function as measured by the Symbol Coding from the Brief Assessment of Cognition (BAC Symbol Coding).</li> <li>To assess the effect of NYX-783 compared to placebo in the change in global clinical severity of PTSD symptoms as measured by the Clinical Global Impressions-Severity (CGI-S) scale.</li> <li>To compare NYX-783 vs. placebo in the change in levels of anxiety and depression as measured by the Hospital Anxiety and Depression Scale (HADS).</li> <li>To assess the safety and tolerability of NYX-783 compared to placebo.</li> </ul> <p><b>Exploratory Objectives</b></p> <ul style="list-style-type: none"> <li>[REDACTED]</li> </ul>	

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]

**Methodology:**

Study Design

This is a Phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group, outpatient study using a sequential parallel comparison design (SCPD). The study will consist of 3 periods: Screening, Treatment, and Follow-up. The Treatment period will be conducted in 2 stages to examine the efficacy and safety of NYX-783 compared to placebo in the treatment of PTSD symptoms. Randomization in Stage 1 (Weeks 1-4) will be 4:1:1 (placebo: NYX-783 50 mg QD: NYX-783 10 mg QD, respectively *treatment arms [1], [2] and [3]*). In Stage 2, both placebo responders and non-responders (CAPS-5 total score reduction from baseline [Day 1]  $\leq 35\%$  and CAPS-5 total score  $\geq 26$  at Week 4) from Stage 1 will be re-randomized (in a stratified manner) in a blinded fashion in a 3:2:2 ratio (placebo: NYX-783 50 mg QD: NYX-783 10 mg QD) (*treatment arms [4],[5],[6] and [7],[8],[9]*, respectively). All subjects who received active NYX-783 in Stage 1 will receive placebo in Stage 2 (*treatment arms [10] and [11]*), which will provide information about the duration of effects of NYX-783 as taken during Stage 1, while taking placebo during Stage 2.

**Screening period:**

Written informed consent must be provided by potential study participants prior to the initiation of any protocol-specified procedures. Subject eligibility will be confirmed in a 2-step process: 1) Screening Visit (Days -28 to -14): Subjects will be screened for PTSD using the PCL-5, with the Life Events Checklist for DSM-5 (LEC-5) being used to assess the nature of the primary traumatic event. The primary eligibility criteria will be confirmed by the investigative site using the Structured Interview for DSM-5® (SCID-5-CT), and the within the past month version of the CAPS-5. Additional eligibility assessments will include demographics, medical history, vital signs, physical examination (PE), height, weight, electrocardiogram (ECG), laboratory samples, suicidality, and concomitant medication information. Other clinical measures will be used to assess sleep quality, cognitive effects, as well as mood and anxiety symptoms. 2) SAFER interview (Day -14  $\pm 3$  days): Eligible subjects with a primary diagnosis of PTSD, a PCL-5  $\geq 38$ , and a CAPS-5 total score  $\geq 30$  as assessed by the investigative site, will participate in a SAFER remote phone interview to confirm the

diagnosis of PTSD and evaluate related primary exclusion criteria. Subjects who meet the eligibility criteria during the SAFER interview will be qualified to proceed to the Day 1 randomization visit.

**Treatment Period:**

On Day 1, severity of PTSD symptoms will be assessed by the investigative site using the CAPS-5 and PCL-5. Other clinical measures will be used to assess sleep quality, cognitive effects, as well as mood and anxiety symptoms. Assessments will also include vital signs, ECG, weight, laboratory samples, and concomitant medication information. Eligible subjects will be randomized to receive NYX-783 50 mg QD, NYX-783 10 mg QD, or matching placebo. Study drug will be administered at the investigative site on Day 1 and dispensed for subsequent outpatient administration.

Subjects will return to the clinic for study visits at Weeks 2, 4, 6, and 8/early termination (or conduct remote assessments at Week 2 and Week 6). On these days, PTSD severity will be assessed by CAPS-5 and PCL-5 at the investigative site. Other clinical measures will be used to assess sleep quality, cognitive effects, as well as mood and anxiety symptoms. Additional safety assessments will also be completed. Blood samples for plasma concentrations of NYX-783 will be collected at Week 4 and Week 8/early termination. Blood samples for optional pharmacogenomic testing will be collected on Day 1. Optional blood samples for biomarker (RNA) testing will be collected on Days 1 and Weeks 4 and 8/early termination.

**Follow-up Period:**

A safety follow-up visit will be completed approximately 7 days after the last dose of study drug is administered.

Safety throughout the entire study will be assessed by adverse events, vital signs, weight, physical examination, ECGs, clinical laboratory values, and the Sheehan Suicidality Tracking Scale (S-STs).

Rater qualification, training, and surveillance will be centrally monitored during the study.

A Data and Safety Monitoring Board will periodically review available safety data to determine if it is safe to proceed.

**Number of subjects (planned):** Approximately 156 subjects

**Main criteria for inclusion:**

Inclusion Criteria

1. Male and female subjects between the ages of 18 to 65 years (inclusive) at the time of signing the informed consent.
2. A primary diagnosis of PTSD (DSM-5 criteria, APA, 2013) according to the CAPS-5 and SCID-5-CT, with the primary traumatic event occurring  $\geq 12$  months prior to screening. NOTE: In cases of “delayed-onset PTSD”, clinically manifest PTSD symptoms must be present for at least 6 months prior to screening.
3. PCL-5  $\geq 38$  at screening.
4. CAPS-5 score total score  $\geq 30$  at screening.
5. Qualified to participate according to the SAFER interview.
6. Female subjects of childbearing potential must have a negative serum pregnancy test at screening and be practicing an adequate method of birth control (e.g., oral or parenteral contraceptives, intrauterine device, barrier, abstinence). Subjects may not be breastfeeding or plan to become pregnant or donate ova during the study and for 30 days after the last dose of study drug.
7. Male subjects who are sexually active with female partner(s) must agree to the following during the study and for 30 days after the last dose of study drug: a) use an acceptable method of birth control (condom with spermicide or surgical sterilization) and b) refrain from sexual activity



with female partners who do not use an acceptable method of birth control. Barrier contraception (condom with spermicide) **must** be used by all male subjects who are not surgically sterilized at least 90 days prior to screening. Male subjects must also agree to refrain from sperm donation during the study and until 30 days after the last dose of study drug.

8. Ability to understand the requirements of the study, provide written informed consent, abide by the study restrictions, and agree to return for the required assessments.
9. Willing to use a smartphone-based video recording technology for monitoring compliance with study drug administration.
10. The subject has a stable social situation in the investigator's opinion, e.g. during the course of the study no changes are expected in work, marital, or accommodation status.

#### Exclusion Criteria

1. Complex PTSD, defined as a condition that may develop following exposure to an event or series of events of an extreme and prolonged or repetitive nature of which the subject experienced as extremely threatening or horrific and from which escape was difficult or impossible (e.g., torture, slavery, genocide campaigns, prolonged domestic violence, repeated childhood sexual or physical abuse). If affect dysregulation and interpersonal dysfunction are primary over other core PTSD symptoms, in the investigator's opinion, they should be considered as exclusionary criteria. NOTE: Non-interpersonal trauma (e.g. motor vehicle accident, mass transportation accident, domestic fire, mass shooting survivor) prior to 18 years of age are not exclusionary, and, the trauma can be considered the primary traumatic event (index trauma).
2. Trauma focused psychotherapies including, prolonged exposure therapy, cognitive behavioral therapy, eye movement desensitization and reprocessing (EMDR) within 30 days prior to screening or during the study.
3. Primary traumatic event occurred prior to 2001.
4. Primary traumatic event was followed by further major traumatic life episodes in the 6 months prior to screening.
5. Primary traumatic event was associated with traumatic brain injury (TBI) categorized as Moderate-to-Severe TBI, according to the Mayo TBI Severity Classification System. ([Malec 2007](#)) (see Appendix A).
6. Substance use disorder within the 6 months prior to screening. Use of any illicit drugs of abuse during the study period is prohibited.
7. History of alcohol abuse within the 6 months prior to screening. NOTE: Up to an average of 1 drink/day is allowed during the study.
8. Positive screen for alcohol or drugs of abuse, including phencyclidine, barbiturates, benzodiazepines, opiates, methadone, cocaine, cannabinoids, and amphetamines at screening or Day 1. Subjects who have a positive screen for cannabinoids or other medically prescribed drugs of abuse at screening, must have a negative screen at Day 1 and agree not to use during the study.
9. Any history of schizophrenia or other psychotic disorder, bipolar disorder, psychosis, or other Axis 1 disorder (except for major depressive disorder or anxiety disorders that followed exposure to the trauma, or an anxiety disorder showed a worsening after trauma). NOTE: Anxiety disorders (such as selective mutism, specific phobia, social anxiety disorder, panic disorder, agoraphobia, and generalized anxiety disorder) are exclusionary only if the diagnosis preceded the primary traumatic event, and, the subject was seeking and received treatment for the symptoms prior to the primary traumatic event. A current secondary diagnosis of major depressive disorder without full remission is not exclusionary; if treated, the dose of a permitted

- antidepressant must be stable for at least 30 days prior to screening and expected to continue during the study.
10. Clozapine use or electroconvulsive therapy within 12 months prior to screening or during the study.
  11. Current use of medications with primarily central nervous system (CNS) activity (e.g., antipsychotics, anticonvulsant drugs [including gabapentinoids], psychostimulants, benzodiazepines, methylphenidate, doxazosin, prazosin, clonidine, first-generation sedating H<sub>1</sub> antihistamines eszopiclone, zolpidem extended-release or any other sedative-hypnotic medications within 3 days prior to screening or during the study. **NOTE:** Use of certain non-benzodiazepine sleep inducers (e.g., zolpidem immediate-release [up to 10 mg at bedtime], zaleplon [up to 20 mg at bedtime]), melatonin, or ramelteon for sleep are allowed.
  12. Use of nefazodone, selective serotonin reuptake inhibitors (SSRIs, such as fluoxetine, sertraline, escitalopram, citalopram, paroxetine), serotonin and norepinephrine reuptake inhibitors (SNRI-s, such as desvenlafaxine, duloxetine, venlafaxine), reversible and non-reversible monoamine oxidase inhibitors (such as selegiline, phenelzine, tranylcypromine), tricyclic antidepressants (such as amitriptyline, imipramine, nortriptyline), noradrenergic and specific serotonergic antidepressant (NaSSA) mirtazapine, serotonin modulator and stimulator antidepressants such as vilazodone or vortioxetine, or the norepinephrine, serotonin and dopamine reuptake inhibitor bupropion within 30 days (90 days for fluoxetine) prior to screening or during the study. **NOTE:** Subjects on stable treatment ( $\geq 30$  days prior to screening) with St. John's Wort may continue this therapy during the study with no expected change in dose.
  13. Treatment with an injectable depot neuroleptic within 90 days prior to screening or during the study.
  14. Subjects who meet the criteria for suicidal intent, plan and/or behavior on the S-STS at screening or Day 1. These criteria are a score of 3 or 4 on questions 2 or 13, or 2 or higher on any of the following questions: 1a (only if 1b is coded YES), 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 14. (**NOTE:** calculation of S-STS total score is not required)
  15. In the process of litigating for compensation for a psychiatric disorder. Subjects who are in the process of applying for medical or VA benefits and/or those who have a settled claim prior to their enrollment in the trial are eligible.
  16. History of Huntington's, Parkinson's, and/or Alzheimer's disease, multiple sclerosis, seizures (history of childhood febrile seizures are not exclusionary), epilepsy, or stroke.
  17. History of allergy, sensitivity, or intolerance to *N*-methyl-D-aspartate receptor (NMDAR) ligands, including ketamine, dextromethorphan, memantine, methadone, dextropropoxyphene, or ketobemidone, as well as current use of such agents.
  18. Received an investigational drug product or device within 30 days (or 5 half-lives, whichever is longer for a drug product) of dosing.
  19. Previously received NYX-783.
  20. Screening QT interval corrected for heart rate (HR) by Fridericia's formula (QTcF)  $>450$  (males) or  $470$  (females) milliseconds (msec) or an ECG that is not suitable for QT measurements (e.g., poorly defined termination of T-wave in the investigator's opinion).
  21. A known familial history or known presence of long QT syndrome, or a known history of past or current clinically significant arrhythmias or ischemic heart disease.
  22. Body mass index  $>35$  kg/m<sup>2</sup> at screening.
  23. HR  $\leq 45$  or  $>95$  beats per minute at screening.
  24. Uncontrolled Type I or Type II diabetes mellitus or uncontrolled hypertension.

<ol style="list-style-type: none"><li>25. Estimated creatinine clearance &lt;60 mL/minute (calculated by the Cockcroft-Gault equation) at screening or history of renal disease, as assessed by the investigator or the sponsor-designated medical monitor.</li><li>26. Known diagnosis of a current infectious disease, including human immunodeficiency virus infection, hepatitis, or other ongoing infectious disease that the investigator considers clinically significant.</li><li>27. Current evidence of dysplasia or history of malignancy (including lymphoma and leukemia) in the last 5 years, except for successfully treated non-metastatic basal cell or squamous cell carcinoma of the skin or localized carcinoma in situ of the cervix.</li><li>28. History of gastrointestinal disease or surgery (except simple appendectomy or hernia repair), leading to impaired drug absorption.</li><li>29. Known abnormal laboratory results, ECG results, medical history or concurrent conditions which, in the opinion of the investigator or sponsor, would preclude safe study participation or interfere with study procedures/assessments.</li><li>30. Uncorrected hypothyroidism or hyperthyroidism. NOTE: Subjects with compensated hypothyroidism with normal thyroid-stimulating hormone levels may be enrolled.</li><li>31. Impaired hepatic function characterized by a previous known diagnosis of chronic liver disease and/or the presence of direct bilirubin &gt;1.5x, or, alanine transaminase, aspartate transaminase, alkaline phosphatase, or gamma-glutamyl transferase &gt;2x the upper limit of normal at screening.</li><li>32. Current borderline personality disorder, antisocial personality disorder, or other personality disorder (e.g. subject showed pervasive pattern of disregard for and violation of the rights of others, or subject has had previous repetitive suicidal behavior [including aborted, interrupted or ineffective suicide attempts, gestures], self-mutilating behavior, or has ever been homicidal) of sufficient severity, in the investigator's opinion, to interfere with study participation.</li><li>33. Use of NMDAR-binding drugs (e.g., ketamine, dextromethorphan, memantine, methadone, lamotrigine, esketamine) within 60 days prior to dosing or during the study.</li><li>34. 3,4-Methylenedioxymetamphetamine (MDMA)-assisted psychotherapy within 90 days prior to screening or during the study.</li><li>35. Expected to initiate any psychotherapy during the study.</li></ol>
<p><b>Investigational product, dosage and mode of administration:</b> NYX-783 50-mg oral capsule(s) or matching placebo There are 3 treatment arms:</p> <ul style="list-style-type: none"><li>• NYX-783 50 mg QD</li><li>• NYX-783 10 mg QD</li><li>• Placebo QD</li></ul>
<p><b>Participant Duration: (approximately 12 weeks total duration)</b></p> <ul style="list-style-type: none"><li>• Screening: Up to 21 days before randomization</li><li>• 8-week treatment period: 4 weeks of NYX-783 50 mg QD or 10 mg QD and 4 weeks of placebo or 8 weeks of placebo followed by a one-week post-therapy safety follow up period<ul style="list-style-type: none"><li>○ Stage 1: 4 weeks</li><li>○ Stage 2: 4 weeks</li></ul></li></ul>
<p><b>Criteria for evaluation:</b></p>

**Efficacy:**

- CAPS-5
- HADS
- PCL-5
- PSQI
- PSQI-A
- CGI-S
- BAC Symbol Coding
- [REDACTED]
- [REDACTED]
- [REDACTED]

**Safety:**

- PE findings
- Weight
- Vital sign measurements
- ECG results
- Clinical laboratory test results
- AE monitoring
- S-STs

[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]

**Statistical methods:**

The primary efficacy analysis population is Intention-to-treat (ITT) population, defined as all randomized subjects. Unless otherwise specified, all other efficacy analyses also will be conducted using the ITT population.

The primary efficacy endpoint (change in CAPS-5 score following 4 weeks of treatment in each respective stage (Stage 1 [baseline to Week 4] and Stage 2 [Week 4 to Week 8]) will be analyzed with a stage-specific mixed model for repeated measures (MMRM).

Estimated treatment effects will be combined across Stage 1 and Stage 2, using prespecified 0.6/0.4 weighting for Stage 1/Stage 2. The models will include variables for treatment group, visit, treatment-by-visit interaction term, baseline CAPS-5 score and baseline-by-visit interaction.

Treatment arms 1, 2, 3 and 7, 8, 9 will be analyzed for the primary efficacy variable. Secondary efficacy variables will be analyzed using the same populations and similar MMRM models as those used for the primary efficacy analysis. Treatment arms 4, 5, 6 and 10, 11 will be utilized for exploratory efficacy analyses to keep integrity of the trial.

Treatment differences in subject characteristics at baseline will be assessed using Fisher's exact test for categorical variables and Type III sums of squares analysis of variance (ANOVA) for continuous variables.

Unless otherwise specified, all tests of treatment effect will be conducted at one-sided  $\alpha = 0.10$ .

**Sample Size:** Response to treatment will be measured using the CAPS-5 score change from baseline to Week 4 (Stage 1) and from Week 4 to Week 8 (Stage 2). If the effect size of NYX-783 is in line with the effect sizes observed in similar historical studies, this study is powered at approximately 80% to reject the null hypothesis at a one-sided  $\alpha=0.10$  level for any single specified efficacy comparison. The sample size estimate assumes a 15% dropout during stage 1 and 11% dropout during stage 2. The sample size may be adjusted if the actual dropout rate exceeds this assumption.

**Interim Analysis:** No interim analysis is planned.

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#### 4. LIST OF ABBREVIATIONS

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

**Table 2: Abbreviations and Special Terms**

Abbreviation or specialist term	Explanation
AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
AST	Aspartate aminotransferase
AUC	Area under the plasma-concentration versus time curve
AUC <sub>0-24</sub>	AUC from pre-dose to 24 hours after drug administration
β-HCG	Beta-human chorionic gonadotropin
BAC Symbol Coding	Symbol Coding from the Brief Assessment of Cognition
BPM	Beats per minute
CAPS-5	Clinician-Administered PTSD Scale for DSM-5
██████████	██
CFR	Code of Federal Regulations
CGI-S	Clinical Global Impressions - Severity
C <sub>max</sub>	Maximum plasma concentration
CNS	Central nervous system
CSF	Cerebrospinal fluid
DCS	D-cycloserine
DES-II	Dissociation Experiences Scale-II (2 <sup>nd</sup> edition)
dL	Deciliter
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
ECG	Electrocardiogram
eCRF	Electronic case report form
FDA	Food and Drug Administration
GABA	Gamma-aminobutyric acid
GCP	Good Clinical Practice

<b>Abbreviation or specialist term</b>	<b>Explanation</b>
HADS	Hospital Anxiety and Depression Scale
hERG	Human ether-à-go-go-related gene
HIV	Human immunodeficiency virus
HR	Heart rate
ICF	Informed consent form
ICH	International Conference for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IRB	Institutional Review Board
IRT	Interactive response technology
LEC-5	Life Events Checklist for DSM-5
LS	Least-squares
MAD	Multiple ascending dose
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
Mg	Milligram
miRNA	MicroRNA
mL	Milliliter
MPV	Mean platelet volume
mRNA	Messenger RNA
Msec	Millisecond(s)
NaSSA	Noradrenergic and specific serotonergic antidepressant
MTD	Maximum tolerated dose
NMDA	<i>N</i> -methyl-D-aspartate
NMDAR	<i>N</i> -methyl-D-aspartate receptor
NOAEL	No-observed-adverse-effect level
PCL-5	PTSD checklist for DSM-5
PE	Physical examination
█	█
PHI	Personal health information
PK	Pharmacokinetic
PO	By mouth
PSQI	Pittsburgh Sleep Quality Index

<b>Abbreviation or specialist term</b>	<b>Explanation</b>
PSQI-A	PSQI Addendum for PTSD
PTSD	Post-traumatic stress disorder
QD	Once daily
QTc	Corrected QT interval
QTcF	QT interval corrected for heart rate by Fridericia's formula
RDW	Red cell distribution width
RNA	Ribonucleic acid
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SCID-5-CT	Structured Clinical Interview for DSM-5 (Clinical Trials Version)
█	█
SNRI	Serotonin and norepinephrine reuptake inhibitor
SOC	System organ class
SPCD	Sequential parallel comparison design
SSRI	Selective serotonin reuptake inhibitor
S-STS	Sheehan Suicidality Tracking Scale
$t_{1/2}$	Apparent terminal elimination half-life
TBI	Traumatic Brain Injury
TEAE	Treatment-emergent adverse event
$T_{max}$	Time to maximum plasma concentration
ULN	Upper limit of normal

## 5. INTRODUCTION

NYX-783 [REDACTED] is an orally bioavailable, small molecule [REDACTED] being developed for the treatment of post-traumatic stress disorder (PTSD). This new molecular entity is a modulator of the *N*-methyl-D-aspartate receptor (NMDAR), and acts as a co-agonist to glutamate at the NMDAR. The NMDARs are a large family of ionotropic glutamate receptors found predominantly in the central nervous system (CNS). (Hollmann 1994, Paoletti 2007)

Glutamate, the physiologic agonist at NMDAR, is the major excitatory neurotransmitter in the CNS. NMDARs are tetrameric, mixed ion channels (i.e.,  $\text{Ca}^{+2}$ ,  $\text{Na}^{+}$ , and  $\text{K}^{+}$ ) containing two glycine-binding GluN1 subunits and two glutamate-binding GluN2 subunits. There are a number of variants of the glycine binding subunits and four gene products of the glutamate subunits referred to as GluN2A, GluN2B, GluN2C, and GluN2D. Full activation of NMDA receptors requires the simultaneous binding of both co-agonists, glycine and glutamate. (Hansen 2017)

Unlike NMDAR antagonists, which block or inhibit the NMDAR, and unlike D-cycloserine (DCS, an NMDAR partial agonist), which mediates its effects through binding at the GluN1 glycine co-agonist site, NYX-783 modulates the NMDAR by binding to a unique modulatory site. NYX-783 binding to this novel site triggers altered receptor conformation, resulting in activity at all four GluN2 subtypes (GluN2A, GluN2B, GluN2C, and GluN2D). NMDAR-mediated synaptic plasticity is thought to be a primary mechanism in the formation of new memories and NMDA receptor subunits have differential roles in fear acquisition and extinction.

PTSD is a common and often severe condition that is evoked by a terrifying event, either personally experienced or witnessed by the individual. (APA 2013) Most people exposed to severe traumatic events may have temporary difficulty adjusting and coping, but with time symptoms get better. In PTSD, symptoms such as re-experiencing traumatic event, avoidance, numbing and elevated arousal show no spontaneous improvement, with symptoms lasting for months or even decades, and interfering with daily functioning and interpersonal relationships. (Almli 2014) The lifetime prevalence of PTSD is about 8% in the general population, but occurs in much higher proportions in special populations at higher risk for exposure to trauma. (Kilpatrick 2013, Almli 2014) A meta-analysis of 33 studies found that the prevalence of PTSD in service members returning from deployment in Afghanistan and Iraq was 23%. (Fulton 2015) PTSD is associated with significant psychological and physical burden on the individual level, and economic burdens from societal aspects. (Ellis 2018)

PTSD, according to the DSM-5 diagnostic criteria, is characterized by 4 major symptom clusters following a traumatic event. The symptom categories cover: 1) **intrusive symptoms**: traumatic event persistently re-experienced through unwanted upsetting memories, nightmares, flashbacks, emotional distress after exposure to traumatic reminders, physical activity after exposure to traumatic reminders; 2) **avoidance symptoms**, including trauma-related thoughts or feelings and trauma-related external reminders; 3) **negative alterations in cognition and mood** (examples include inability to recall key features of the trauma, overly negative thoughts and assumptions about oneself or the world, exaggerated blame of self or others for causing the trauma, negative affect, decreased interest in activities, feeling isolated, or difficulty experiencing positive affect); and 4) **alterations of arousal and reactivity symptoms** incorporating irritability or aggression, risky or destructive behavior, hypervigilance, heightened startle reaction, difficulty

concentrating, or difficulty sleeping. In addition, DSM-5 distinguishes a subtype of PTSD, “with dissociative symptoms” in which the individual recurrently experiences depersonalization and/or derealization. (APA 2013)

Beyond the core mental symptom categories of PTSD, impairments have been identified in a number of domains of **cognitive functioning**, including working memory, executive functions, sustained attention, and processing speed. (Twamley 2009, Kanagaratnam 2007, Samuelson 2006)

In a meta-analysis of data from 21 placebo-controlled trials, selective serotonin re-uptake inhibitors (SSRIs) were found to be superior to placebo in the treatment of PTSD, but the effect size was small (standardized mean difference = -0.23). (Hoskins 2015) Currently, two SSRIs, sertraline and paroxetine, have been approved by FDA for treatment of PTSD. Fluoxetine and venlafaxine also have shown efficacy in reducing PTSD symptoms in placebo -controlled clinical trials. (Hoskins 2015) Overall, however, response rates with SSRIs have rarely exceeded 60%, and less than 20-30% of subjects have been shown to achieve full remission. (Berger 2009)

The premise of this trial stems from the role of NMDARs in a number of physiological and pathological processes, including PTSD (Steckler 2012, Sherin 2011, Ravindran 2009) and affected cognitive domains. (Gonzalez 2015, Tsai 2016) The key element of PTSD is a pervasive sense of impending danger, fearfulness, and heightened arousal in situations where no actual threat or danger is present. (Pole 2007) Persistent fear memory may be the result of enhanced fear acquisition, impaired fear extinction, and/or deficiencies in contextual processing. (Garfinkel 2014)

NMDARs have been shown to regulate fear acquisition and extinction due to their role in synaptic development and plasticity. (Radulovic 2018, Luchkina 2018) Further, there appears to be differential roles for the NMDAR receptor subtypes. For example, GluN2A receptor subunit activation may have a role in the formation and/or stabilization of new learned fear responses, whereas GluN2B receptor activation may be involved with the suppression/extinction of learned fear responses. (Dalton 2012)

NYX-783 has exhibited efficacy in several pre-clinical models of PTSD symptoms. Specifically, in a model of intrusive fear memory, a single oral dose of NYX-783 (1 mg/kg) facilitated extinction of conditioned fear similarly to a single dose of the positive control DCS (15 mg/kg given subcutaneously). When contextual fear memory was measured 14 days after the original conditioning session, spontaneous recovery of freezing behavior was observed in rats previously treated with DCS. Importantly, no spontaneous recovery of freezing behavior was observed in rats previously treated with NYX-783. Thus, a single treatment with NYX-783 reduced freezing in a rat model of intrusive fear memory when measured either proximal or remote to fear conditioning. Additionally, in an assay that measures affective state (heterospecific rough-and-tumble play), NYX-783 increased the number of rat hedonic ultrasonic vocalizations across a broad dose range. NYX-783 also decreased rat immobility in the Porsolt forced swim assay, which exhibits high predictive validity for antidepressant activity. These data may be significant given the widespread use of antidepressants to treat PTSD symptoms.

Safety pharmacology studies indicate that NYX-783 has low potential for off-target pharmacology. NYX-783 had no significant activity in a panel of 80 receptor-binding assays that included gamma-aminobutyric acid (GABA), opiate, serotonin, and tachykinin receptors, ion

channels, and transporters. NYX-783 was inactive in modulating human ether-à-go-go-related gene (hERG) activity and had no effect on hemodynamic or ECG parameters in male isolated guinea pig hearts (Langendorff method) up to  $\leq 500 \mu\text{M}$ .

In general, NYX-783 was well-tolerated after single oral doses up to and including 600 mg/kg in rats and 1000 mg/kg in dogs. In a 91-day repeat-dose study, NYX-783 was well tolerated at all dose levels, and the no-observed-adverse-effect-level (NOAEL) in rats was the highest dose tested, 500 mg/kg. In the daily repeat-dose, 91-day dog toxicology study, NYX-783 was evaluated at doses of 2, 6, and 20 mg/kg. There were notable changes in liver parameters at 6 and 20 mg/kg; the liver-associated findings at 6 mg/kg were considered non-adverse and recovered after a 4-week drug holiday. Also, at the 20 mg/kg dose, elevations in serum levels of liver enzymes, primarily ALT, were observed. The liver-associated findings at 20 mg/kg were considered adverse and did not recover. Therefore, the NOAEL in dog was 6 mg/kg/day. All adverse observations occurred at doses in excess of the planned clinical doses.

Results are available from all 70 subjects in the Phase 1, first-in-human study of NYX-783 (NYX-783-1002) in which healthy male and female subjects were enrolled. In this study, safety and pharmacokinetics of single doses (10, 50, 200, and 600 mg) and multiple doses (once daily for 7 days of 2, 50, and 200 mg) of NYX-783 were evaluated. Seventy subjects were included in the safety population and 54 active-treated subjects were included in the PK population. NYX-783 was safe and well-tolerated at the studied doses. No grade 3, grade 4, or serious adverse events were reported. Further, there were no laboratory results or ECG findings reported as adverse events. There were 2 unrelated adverse events (emesis and viral gastroenteritis) that lead to withdrawal from the study. There were 2 mild adverse events (headache and fatigue) that the investigator considered possibly related to NYX-783. A maximum tolerated dose was not reached in the study.

The PK data from study NYX-783-1002 have shown that following oral administration of 2 to 600 mg, NYX-783 was rapidly absorbed and  $C_{\text{max}}$  was reached within 1 to 2 hours post dose. Concentrations declined in a roughly monophasic manner over 24 hours, and a roughly biphasic manner over 48 hours after 7 days of dosing. NYX-783 had a  $t_{1/2}$  of approximately 4 hours and demonstrated dose-proportional PK with minimal accumulation following QD dosing for 7 days. Data suggests that NYX-783 can be administered with or without food, and, plasma exposures to NYX-783 in the elderly cohort were comparable. The majority (50-60%) of the administered dose of NYX-783 was excreted unchanged in the urine. CSF samples were obtained in subjects taking a single 50 mg dose of NYX-783. NYX-783 crossed the blood brain barrier, with CSF concentrations at 4 and 8 hours approximately 12% to 14% of plasma  $C_{\text{max}}$  at the same dose level.



## 6. STUDY OBJECTIVES AND ENDPOINTS

**Table 3: Study Objectives and Endpoints**

Objectives	Endpoints
Primary	
To assess the effects of NYX-783 compared to placebo in reducing the severity of PTSD symptoms as measured by the CAPS-5	Change in CAPS-5 total score and subscores following 4 weeks of treatment in each respective stage (Stage 1 [baseline to Week 4] and Stage 2 [Week 4 to Week 8])
Secondary	
<p>To assess the effect of NYX-783 compared to placebo in the following outcomes:</p> <ul style="list-style-type: none"> <li>a) change in symptoms of PTSD as measured by the PTSD-Checklist for DSM-5 (PCL-5)</li> <li>b) change in sleep quality as measured by the Pittsburgh Sleep Quality Index (PSQI) Global Score and the PSQI Addendum for PTSD (PSQI-A) Global Score</li> <li>c) change in cognitive function as measured by the Symbol Coding from the Brief Assessment of Cognition (BAC Symbol Coding)</li> </ul>	Change in each of the respective outcomes following 4 weeks of treatment in each respective stage (Stage 1 [baseline to Week 4] and Stage 2 [Week 4 to Week 8])
To assess the effect of NYX-783 compared to placebo in the change in global clinical severity of PTSD symptoms as measured by the CGI-S scale	Change in CGI-S following 4 weeks of treatment in each respective stage
To compare NYX-783 vs. placebo in the changes in levels of anxiety and depression as measured by the Hospital Anxiety and Depression Scale (HADS)	Change in HADS-A and HADS-D subscores following 4 weeks of treatment in each respective stage (Stage 1 [baseline to Week 4] and Stage 2 [Week 4 to Week 8])
To assess the safety and tolerability of NYX-783 compared to placebo.	Incidence of adverse events and any changes in vital signs, physical examination findings (including weight), 12-lead ECG, clinical laboratory values, suicidality (Sheehan Suicidality Tracking Scale)
Exploratory	
<p>[REDACTED]</p> <p>[REDACTED]</p>	<p>[REDACTED]</p> <p>[REDACTED]</p>

Objectives	Endpoints
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]

**6.1. Criteria for Evaluation**

**6.1.1. Efficacy Criteria**

The following criteria will be used to assess efficacy: CAPS-5, HADS, PCL-5, CGI-S, PSQI, PSQI-A, and BAC Symbol Coding.

**6.1.2. Safety Criteria**

The following criteria will be used to assess safety: physical examination (PE) findings, weight, vital sign measurements (blood pressure, heart rate [HR], and respiratory rate), ECG results, clinical laboratory results, adverse events (AEs), and suicidality using the Sheehan Suicidality Tracking Scale (Sheehan 2014 [a], Sheehan 2014 [b]) instrument (S-STs).

**6.1.3. Exploratory Criteria**

[REDACTED]

## 7. INVESTIGATIONAL PLAN

### 7.1. Overall Study Design and Plan: Description

This is a Phase 2, multicenter, randomized, double-blind, placebo-controlled, parallel-group, outpatient study of approximately 12 weeks duration (Screening: 3 weeks; Treatment: 8 weeks; Follow-up: 1 week). In this study, a sequential parallel comparison design (SPCD) will be used to examine the efficacy and safety of oral NYX-783 compared to placebo in the treatment of PTSD symptoms. On Day 1, approximately 156 eligible subjects will be randomized to receive placebo, NYX-783 50 mg QD, or NYX-783 10 mg QD in a 4:1:1 ratio, respectively (Figure 1). Placebo non-responders (CAPS-5 total score reduction from baseline [Day 1]  $\leq 35\%$  and CAPS-5 total score  $\geq 26$  at Week 4) and placebo responders identified during Stage 1 (Week 1-4) will then be then re-randomized in a blinded fashion to placebo, NYX-783 50 mg QD or NYX-783 10 mg QD in Stage 2 (Weeks 4-8) in a 3:2:2 ratio. Subjects who received active treatment during Stage 1 will receive placebo in Stage 2, thus providing information about the maintenance effect of NYX-783 treatment after re-randomization to placebo. Subjects who complete the study will have received either 4 weeks of NYX-783 50 mg QD or 10 mg QD (and 4 weeks of placebo) or 8 weeks of placebo. The sample size estimate is based on an assumption of 15% dropout during period 1, and 11 % dropout rate in period 2.

All potential study participants must provide written informed consent before initiating any protocol-specified procedures. A 2-step approach will be used to identify the study population during the Screening period:

1) **Screening visit (Days -28 to -14)**. Potential subjects will be screened for the presence of PTSD using the self-report PCL-5, with the Life Events Checklist for DSM-5 (LEC-5) being used to assess the nature of the primary traumatic event. Subjects with a PCL-5 severity score  $\geq 38$  will be interviewed by an investigative site clinician or trained mental health professional using the Structured Interview for DSM-5 (SCID-5-CT) to exclude comorbidities. The CAPS-5 will be used to confirm the diagnosis of PTSD and to identify the primary traumatic event. The severity of PTSD symptoms within the past month will be assessed based on the CAPS-5; a CAPS-5 total score  $\geq 30$  is required for study inclusion. Other screening procedures will include demographics, medical history, vital sign measurements, PE, height, weight, ECG, laboratory samples (chemistry, hematology, urinalysis, drug screen), suicidality, and concomitant medication information. Other clinical measures will be used to assess sleep quality, cognitive effects, and mood and anxiety symptoms.

2) **SAFER interview (Day -14  $\pm$  3 days)**. Subjects who meet the diagnostic criteria at the Screening visit will then participate in telephone-based interview (**SAFER interview**) to confirm the diagnosis of PTSD and review potential comorbidities and study exclusion criteria. Those subjects meeting the diagnostic and selection criteria will be eligible for proceeding to the randomization visit.

On Day 1, eligible subjects will be randomized to receive NYX-783 50 mg QD, NYX-783 10 mg QD, or matching placebo. Assessments on Day 1 will include vital signs, ECG, weight, laboratory samples (pharmacogenomics [optional], plasma concentration, RNA [optional], chemistry, hematology, urinalysis and drug screen), suicidality, and concomitant medication review. Clinical measures will be used to assess severity of PTSD symptoms, sleep quality,

cognitive effects, and mood and anxiety symptoms. Study drug will be administered at the investigative site on Day 1 and dispensed for subsequent outpatient administration.

At the follow-up visits on Weeks 2, 4, 6, and 8/early termination, PTSD severity in the past week will be assessed by the investigative site using the CAPS-5 and PCL-5. Other clinical measures will be used to assess sleep quality, cognitive effects, mood, and anxiety symptoms. Safety assessments will also be performed at these visits and at the safety follow-up visit, which occurs approximately 7 days after the last dose of study drug.

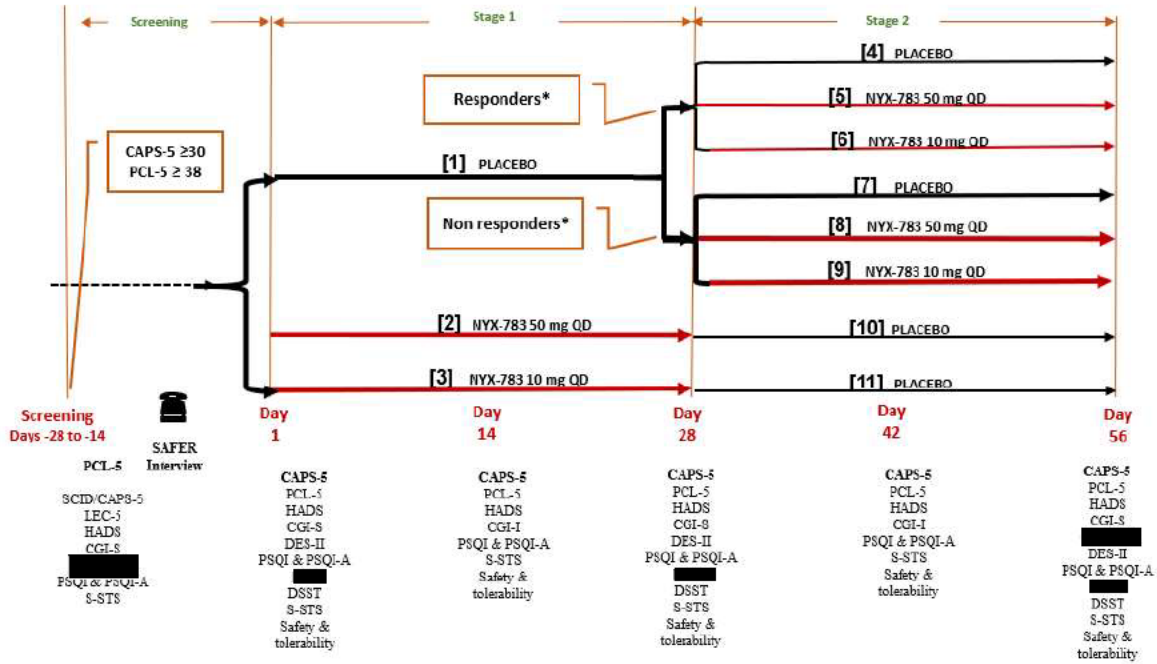
Blood samples for evaluating plasma concentrations will be collected at Week 4 and Week 8/early termination. Blood samples for pharmacogenomic (PGX) testing will be collected on Day 1 for those subjects who have signed consent to participate in this exploratory analysis. Optional blood samples for biomarker (RNA) testing will be collected on Day 1, Week 4, and Week 8/early termination.

Safety throughout the entire study will be assessed by adverse events, vital sign measurements, weight, PE, ECG testing, clinical laboratory testing, and the Sheehan Suicidality Tracking Scale (S-STS) instrument.

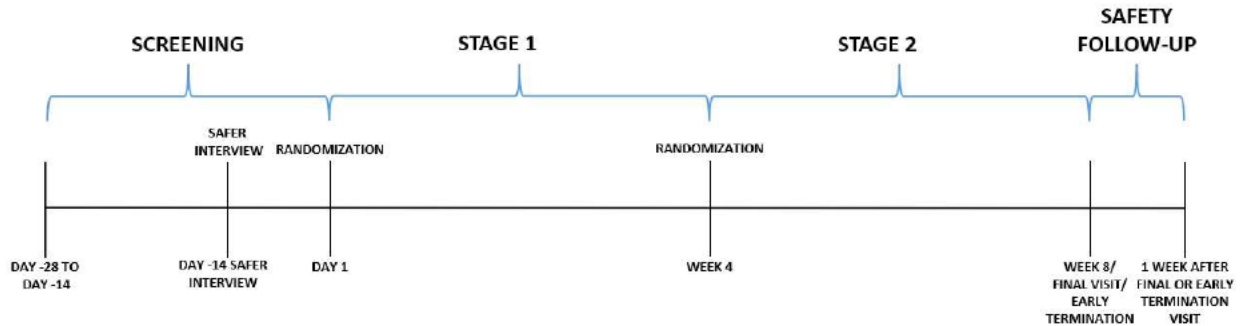
Rater qualification, training, and surveillance will be centrally monitored during the study.

A Data and Safety Monitoring Board (DSMB) will perform periodic reviews of available safety data to ensure it is safe to proceed.

**Figure 1: Study Design**



Placebo non-responder: CAPS-5 reduction from baseline ≤ 35% and CAPS-5 total score ≤ 26 at Week 4.  
 BAC-symbol coding from the Brief Assessment of Cognition. CAPS-5: Clinicians Administered PTSD Scale for DSM-5, █ CGI-S; CGI-I: Clinical Global Impression-Improvement. Clinical Global Impression-Severity. █ DES: Dissociative Experiences Scale. DSST: Digit Symbol Substitution Test. HADS: Hospital Anxiety and Depression Scale. PCL-5: PTSD Checklist for DSM-5, PSQI: Pittsburgh Sleep Quality Inventory, PSQI-A: PSQI-Addendum for PTSD, SCID-5: Structured Interview for DSM-5; █ S-STs: Sheehan Suicidality Tracking Scale ☎ Phone Interview



**Table 4: Schedule of Assessments**

Phases/Visits  Tasks / Events	Screening		Double-blind Treatment Phase					Early Termination	Safety Follow-Up
	Visit 1 Screening	SAFER	Day 1	Week 2	Week 4	Week 6	Week 8		
			Stage 1						
			Stage 2						
Days relative to start dosing of study drug	-28 to -14	-14 <sup>a</sup>	1	14 <sup>x</sup>	28 <sup>a</sup>	42 <sup>x</sup>	56 <sup>a</sup>	NA	63 <sup>ap</sup>
<b>ENTRY / SAFETY / ADMINISTRATIVE</b>									
Informed consent	X								
Inclusion/exclusion criteria	X	X <sup>j</sup>	X						
Demographics / habits <sup>b</sup>	X								
Medical/psychiatric history	X		X						
PTSD diagnosis (CAPS-5)	X								
SAFER interview (Confirmation of PTSD diagnosis and overview of inclusion/exclusion criteria)		X <sup>j</sup>							
LEC-5 (Part of PCL-5 with LEC-5 and Criterion A)	X								
S-STSW	X <sup>s</sup>		X <sup>u</sup>	X <sup>uw</sup>	X <sup>u</sup>	X <sup>uw</sup>	X <sup>u</sup>	X <sup>u</sup>	
Vital signs <sup>c</sup>	X		X	X	X	X	X	X	X <sup>r</sup>
Height	X <sup>e</sup>								
Weight	X <sup>e</sup>		X	X	X	X	X	X	X <sup>r</sup>
Physical examination	X				X		X	X	X <sup>r</sup>
12-lead ECG <sup>d</sup>	X		X		X		X	X	X <sup>r</sup>
Randomization			X		X				
Prior and concomitant medications <sup>w</sup>	X		X	X <sup>w</sup>	X	X <sup>w</sup>	X	X	X
Study drug dispensing <sup>o</sup>			X	X	X	X			
Study drug administration			X <sup>k</sup>	X	X <sup>l</sup>	X	X		
Study drug compliance monitoring <sup>f</sup>			X	X	X	X	X	X	
Adverse events <sup>ew</sup>	X	X	X	X <sup>w</sup>	X	X <sup>w</sup>	X	X	X
<b>LABORATORY TESTS</b>									
Clinical chemistry <sup>q</sup>	X		X	X	X	X	X	X	X <sup>r</sup>
Hematology	X		X		X		X	X	X <sup>r</sup>
HIV and hepatitis tests	X								

Pregnancy test <sup>v</sup>	X <sup>h</sup>		X <sup>i</sup>		X <sup>i</sup>		X <sup>i</sup>	X <sup>i</sup>
Drug and alcohol screen	X <sup>h</sup>		X <sup>i</sup>		X <sup>i</sup>		X <sup>h</sup>	X <sup>h</sup>
Urinalysis	X		X		X		X	X
<b>Clinical Measures</b>								
CAPS-5 <sup>w</sup>	X <sup>s</sup>		X <sup>t</sup>	X <sup>tw</sup>	X <sup>t</sup>	X <sup>tw</sup>	X <sup>t</sup>	X <sup>t</sup>
PCL-5	X <sup>s</sup>		X <sup>t</sup>	X <sup>t</sup>	X <sup>t</sup>	X <sup>t</sup>	X <sup>t</sup>	X <sup>t</sup>
HADS <sup>t</sup>	X		X	X	X	X	X	X
CGI-S	X		X	X	X	X	X	X
PSQI	X		X	X	X	X	X	X
PSQI-A	X		X	X	X	X	X	X
DES-II			X		X		X	X
BAC-Symbol Coding			X		X		X	X

Abbreviations: BAC-Symbol Coding = Symbol Coding from the Brief Assessment of Cognition; CAPS-5 = Clinician Administered PTSD Scale for DSM-5; [REDACTED] CGI-S = Clinical Global Impression – Severity; DES-II = Dissociative Experiences Scale-II; DNA = Deoxyribonucleic acid; ECG = electrocardiogram; HADS = Hospital Anxiety and Depression Scale; HIV = human immunodeficiency virus; LEC-5 = Life Events Checklist for DSM-5; [REDACTED] PCL-5 = PTSD Checklist for DSM-5; [REDACTED] PSQI = Pittsburgh Sleep Quality Index; PSQI-A = Pittsburgh Sleep Quality Index Addendum; PTSD = Post-Traumatic Stress Disorder; SCID-5-CT = Structured Clinical Interview for DSM-5 (Clinical Trials Version); [REDACTED]; S-STIS = Sheehan Suicidality Tracking Scale.

- a) Visit window is ± 3 days.
- b) Includes alcohol or illicit drug use history, employment status, smoking history, and index traumatic event.
- c) Collect vital signs after at least 5 minutes of rest, seated or supine.
- d) ECG after at least 5 minutes supine rest.
- e) Include body mass index calculation.
- f) Subjects use smartphone video application monitoring. Investigative site personnel calculate compliance and document accountability via capsule count/blister package inspection.
- g) Serious Adverse Event (SAE) reporting begins at time of informed consent and continues until 30 days after the last dose of study drug. Non-serious AE collection commences with the time of first dose of study drug until last day of subject participation in the study.
- h) Serum sample.
- i) Urine sample or breathalyzer (alcohol screen).
- j) Via phone.
- k) Doses are to be administered at the clinic on Day1 (after completing other procedures) then on an outpatient basis.



- l) Stage 2 dosing begins on the day after the Week 4 visit.
- m) Includes concurrent medical and psychiatric history.
- n) [REDACTED]
- o) Use interactive response technology for study drug dispensing.
- p) 7 days post-therapy.
- q) Clinical labs should be fasted except for screening.
- r) Only if required for adverse event follow-up.
- s) Rating based on the past month
- t) Rating based on the past week
- u) Use “since last visit” version after screening.
- v) Women of childbearing potential.
- w) Remote visit (optional. Only if necessary, due to COVID-19 health emergency)
- x) Visit window is  $\pm 7$  days.

## 7.2. Scientific Rationale for Study Design

This Phase 2, proof-of-concept, placebo-controlled study uses a sequential parallel comparison design (SPCD), which is appropriate due to the short active treatment phases (two 4-week double-blind treatment periods) and the expected relatively meaningful placebo response rate. The purpose of conducting this exploratory study is to investigate the safety and tolerability of NYX-783 in a PTSD population, and to characterize the response profile of NYX-783 for the treatment of PTSD symptoms evaluated by the intrusion, avoidance, negative alterations in cognition and mood, and arousal & reactivity subscores as well as CAPS-5 total score. The results from this study will be utilized to inform the future NYX-783 development plans.

The SPCD is an adaptive design that allows for re-randomization of placebo subjects (both "non-responders" and "responders") from an initial stage of the study to placebo or active treatment in a subsequent stage of the trial. In this study, data from the "all-comers" population of Stage 1 arms [1 = placebo], [2 = NYX-783 50 mg QD], [3 = NYX 783 10 mg QD] and the subpopulations of "placebo non-responders" in Stage 2 arms [7 = placebo], [8 = NYX-783 50 mg QD], [9 = NYX 783 10 mg QD] are pooled to yield a single p-value for treatment comparison. Estimation of the treatment effect is enhanced by using data from the two study stages (Tamura 2011), and the SPCD design is appropriate for earlier stage trials, such as moderately-sized Phase 2 studies. (Tamura 2011, Baer 2013) The SPCD design aims to increase the probability of demonstrating a treatment effect, especially if the Stage 1 placebo response rate is high. (Chen 2011) The design reduces the impact of placebo response and thus improves the power of the study, which in turn allows for an adequately powered proof-of-concept trial with a smaller sample size and mitigation of the untoward effect of a placebo response. (Baer 2013) All efficacy analyses will be evaluated at the 0.10, one-sided  $\alpha$  level. One-sided is appropriate because the primary objective of the study is to demonstrate that NYX-783 is superior to placebo in reducing the severity of PTSD symptoms.

Beyond the primary objective, an SPCD can address useful exploratory aspects. In this study, for example, by providing information about the maintenance effect of NYX-783 treatment after re-randomization of subjects initially allocated to one of the active treatment arms [2] and [3] to placebo arms [10] and [11]. The re-randomization of both placebo-responders and placebo non-responders from Stage 1 will characterize the response profile of subjects with initial high vs. low placebo response rate in Stage 2 drug treatment arms [5], [6] vs. [8], [9] respectively.

Altered gene expression associated with PTSD has been characterized in peripheral blood samples collected from civilian and military populations in clinical trials. (Banerjee 2017) Analysis of RNA, including microRNA (miRNA) and/or messenger RNA (mRNA), provides a new opportunity to understand the biological pathways underlying PTSD, with the potential to establish biomarker-driven subgroups within the PTSD diagnosis, and/or to serve as a platform for pre-clinical mechanistic studies (Glatt 2013).

### 7.2.1. Subject Population Rationale

The goal of the study is to recruit subjects with a clearly identifiable primary traumatic event during adulthood.

Inclusion criterion #2 clarifies that subjects with delayed-onset PTSD (i.e., the full diagnostic criteria for PTSD are not met until at least 6 months after the event, although the onset and expression of some symptoms may be immediate) (APA 2013) will be allowed to enter into the study if the full diagnostic criteria for PTSD according to the DSM-5 are met at least 6 months prior to screening.

In delayed-onset PTSD, which occurs in about 25% of PTSD cases, (Utzon-Frank 2014) subjects might have experienced subthreshold PTSD symptoms after the trauma. Potentially, some additional trauma then impairs the individual's ability to cope with the index traumatic event. Consequently, subthreshold PTSD symptoms become more severe and evolve into clinically manifest PTSD. For inclusion into the study, at least 6 months of clinically manifest PTSD must be present prior to screening (inclusion criterion #2), with no further traumas in that same 6-month period (exclusion criterion #4).

Subjects meeting inclusion criteria #2, #3 and #4 enrich the study with a moderate-to-severe PTSD population. A score of 38 or higher on PCL-5 indicates likely PTSD in both civilian and veteran populations. (Blevins 2015, Hoge 2014) The timeframe associated with the primary traumatic event as defined by inclusion criterion #2 is designed to minimize spontaneous remission during the study, while exclusion criteria #1, #3, and #4 control for significant chronicity of the disease and minimize Axis-II disorders overlapping with associated complex traumas in childhood. Exclusion criterion #1 reduces the variability of the study population by excluding subjects with complex PTSD. Subjects with complex PTSD typically present with psychological symptoms related to prolonged, repeated trauma, and these are often present in addition to the more typical symptoms of PTSD, including changes in self-concept and response to stressful events. Although "complex PTSD" is not an established diagnostic category in the DSM-5, this protocol helps navigate identification of complex PTSD based on trauma type, age of subject when exposed to trauma, and certain phenotype (i.e., if affect dysregulation and interpersonal dysfunction are primary over other core PTSD symptoms).

Exclusion criteria #6, #7, #9, #14, and #16 minimize disease comorbidities, and #5 minimizes TBI consequences associated with the primary traumatic event. The purpose of exclusion criteria #10, #11, #12, and #13 is to exclude subjects taking psychoactive drugs due to either their potential influence on PTSD symptoms (different classes of antidepressants, sleep inducers with long half-life) or potential interference with NYX-783 influencing the effect of NMDA modulation on sleep architecture, interference with the pharmacology of NYX-783 (i.e., there is a possible interaction between gabapentinoids [pregabalin, gabapentin], interaction with and certain NMDA receptor complexes). (Chen 2018)

### **7.3. Justification of Dose**

The planned dose levels in this study are NYX-783 50 mg/day and 10 mg/day. Based on the preliminary PK results of the Phase 1, first-in-human study in healthy male and female volunteers (NYX-783-1002), the maximum plasma concentration ( $C_{max}$ ) of NYX-783 50 mg is expected to be approximately 0.8  $\mu\text{g/mL}$ . Total exposure (area under the plasma-concentration curve [ $AUC_{0-24}$ ]) is expected to be approximately 5.0  $\mu\text{g}\cdot\text{h/mL}$ .

In the contextual fear conditioning test, NYX-783, when administered to rats as a single oral dose of 1 mg/kg, facilitated fear extinction learning on extinction Days 1-4 and prevented the

re-consolidation of fear memory when retested on Day 14 compared to vehicle. At this pharmacologically-active dose (1 mg/kg) in rats, the plasma  $C_{max}$  is calculated to be 0.5  $\mu\text{g/mL}$ .

In addition, a single dose of NYX-783 in the fear conditioning stress model provided a long-term effect, preventing freezing behavior after one dose of drug in rodents and preventing recovery of fear after 14 days. One objective of this Phase 2 study is to confirm that the observed preclinical long-lasting effects are translatable to humans, i.e., once daily (QD) administration provides long-lasting benefits.

The pharmacologic effects of NYX-783 are currently believed to be driven primarily by  $C_{max}$ . Thus, the dose planned for the current clinical study is expected to result in human plasma concentrations that are within the  $C_{max}$  range that was associated with positive effects on -fear extinction measures.

The 50 mg dose is 1/12<sup>th</sup> of the maximum single dose (600 mg) and 25% of the maximum multiple dose (200 mg) given once daily for 7 days in the Phase 1 trial (NYX-783-1002), which were safe and well-tolerated. The 600 mg single dose as well as the 200 mg multiple dose were associated with PK exposures ( $C_{max}$  and AUC) below the NOAEL for both the dog and rat in the Good Laboratory Practice toxicology studies. In the Phase 1 trial, NYX-783 had a terminal elimination half-life ( $t_{1/2}$ ) of approximately 4 hours and demonstrated no accumulation with once daily dosing over 7 days. In all single-dose groups, approximately 57% of the administered dose was recovered as NYX-783 in the urine. Based on the available preclinical and preliminary clinical safety and PK data, the 10 and 50 mg dose strengths selected for this study are expected to be safe and well-tolerated.

## **8. SELECTION AND WITHDRAWAL OF SUBJECTS**

### **8.1. Subject Inclusion Criteria**

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Male and female subjects between the ages of 18 to 65 years (inclusive) at the time of signing the informed consent.
2. A primary diagnosis of PTSD (DSM-5 criteria, APA 2013) according to the CAPS-5 and SCID-5-CT, with the primary traumatic event occurring  $\geq 12$  months prior to screening. NOTE: In cases of “delayed--onset PTSD,” clinically manifest PTSD symptoms must be present for at least 6 months prior to screening.
3. PCL-5  $\geq 38$  at screening.
4. CAPS-5 total score  $\geq 30$  at screening.
5. Qualified to participate according to the SAFER interview.
6. Female subjects of childbearing potential must have a negative serum pregnancy test at screening and be practicing an adequate method of birth control (e.g., oral or parenteral contraceptives, intrauterine device, barrier, abstinence). Subjects may not be breastfeeding or plan to become pregnant or donate ova during the study and for 30 days after the last dose of study drug.
7. Male subjects who are sexually active with female partner(s) must agree to the following during the study and for 30 days after the last dose of study drug: a) use an acceptable method of birth control (condom with spermicide or surgical sterilization) and b) refrain from sexual activity with female partners who do not use an acceptable method of birth control. Barrier contraception (condom with spermicide) **must** be used by all male subjects who are not surgically sterilized at least 90 days prior to screening. Male subjects must also agree to refrain from sperm donation during the study and until 30 days after the last dose of study drug.
8. Ability to understand the requirements of the study, provide written informed consent, abide by the study restrictions, and agree to return for the required assessments.
9. Willing to use a smartphone-based video recording technology for monitoring compliance with study drug administration.
10. The subject has a stable social situation in the investigator’s opinion, e.g. no changes are expected during the course of the study in work, marital, or accommodation status.

### **8.2. Subject Exclusion Criteria**

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Complex PTSD, defined as a condition that may develop following exposure to an event or series of events of an extreme and prolonged or repetitive nature of which the subject experienced as extremely threatening or horrific and from which escape was difficult or

impossible (e.g., torture, slavery, genocide campaigns, prolonged domestic violence, repeated childhood sexual or physical abuse). If affect dysregulation and interpersonal dysfunction that are primary over other core PTSD symptoms, in the investigator's opinion, they should be considered as exclusionary criteria. NOTE: Non-interpersonal trauma (e.g. motor vehicle accident, mass transportation accident, domestic fire, mass shooting survivor) prior to 18 years of age are not exclusionary, and, the trauma can be considered the primary traumatic event (index trauma).

2. Trauma focused psychotherapies including, prolonged exposure therapy, cognitive behavioral therapy, eye movement desensitization and reprocessing therapy (EMDR) within 30 days prior to screening or during the study.
3. Primary traumatic event occurred prior to 2001.
4. Primary traumatic event was followed by further major traumatic life episodes in the 6 months prior to screening.
5. Primary traumatic event was associated with traumatic brain injury (TBI) categorized as Moderate-to Severe TBI, according to the Mayo TBI Severity Classification System. (Malec 2007) (See Appendix A)
6. Substance use disorder within the 6 months prior to screening. Use of any illicit drugs of abuse during the study period is prohibited.
7. History of alcohol abuse within the 6 months prior to screening. NOTE: Up to an average of 1 drink/day is allowed during the study.
8. Positive screen for alcohol or drugs of abuse, including phencyclidine, barbiturates, benzodiazepines, opiates, methadone, cocaine, cannabinoids, and amphetamines at screening or Day 1. Subjects who have a positive screen for cannabinoids or other medically prescribed drugs of abuse at screening, must have a negative screen at Day 1 and agree not to use during the study.
9. Any history of schizophrenia or other psychotic disorder, bipolar disorder, psychosis, or other Axis 1 disorder (except for major depressive disorder or anxiety disorders that followed exposure to the trauma or an anxiety disorder that showed a worsening after trauma). Anxiety disorders (such as selective mutism, specific phobia, social anxiety disorder, panic disorder, agoraphobia, and generalized anxiety disorder) are exclusionary only if the diagnosis preceded the primary traumatic event, and, the subject was seeking and received treatment for the symptoms prior to the primary traumatic event. NOTE: A current secondary diagnosis of major depressive disorder without full remission is not exclusionary; if treated, the dose of a permitted antidepressant must be stable for at least 30 days prior to screening and expected to continue during the study.
10. Clozapine use or electroconvulsive therapy within the 12 months prior to screening or during the study.
11. Current use of medications with primarily CNS activity (e.g., antipsychotics, anticonvulsant drugs [including gabapentinoids], psychostimulants, benzodiazepines, methylphenidate, doxazosin, prazosin, clonidine, first-generation sedating H1 antihistamines, quetiapine, eszopiclone, zolpidem extended-release, or any other sedative-hypnotic medications) within 3 days prior to screening or during the study. NOTE: Use of certain non-benzodiazepine sleep

- inducers (e.g., zolpidem immediate-release [up to 10 mg at bedtime], zaleplon [up to 20 mg at bedtime]), melatonin, or ramelteon for sleep are allowed.
12. Use of nefazodone, selective serotonin reuptake inhibitors (SSRIs, such as fluoxetine, sertraline, escitalopram, citalopram, paroxetine), serotonin and norepinephrine reuptake inhibitors (SNRIs, such as desvenlafaxine, duloxetine, venlafaxine), reversible and non-reversible monoamine oxidase inhibitors (such as selegiline, phenelzine, tranylcypromine), tricyclic antidepressants (such as amitriptyline, imipramine, nortriptyline), noradrenergic and specific serotonergic antidepressant (NaSSA) mirtazapine, serotonin modulator and stimulator antidepressants (such as vilazodone or vortioxetine), or the norepinephrine, serotonin and dopamine reuptake inhibitor bupropion within 30 days (90 days for fluoxetine) prior to screening or during the study. **NOTE:** Subjects on stable treatment ( $\geq 30$  days prior to screening) with St. John's Wort may continue this therapy during the study with no expected change in dose.
  13. Treatment with an injectable depot neuroleptic within 90 days prior to screening or during the study.
  14. Subjects who meet the criteria for suicidal intent, plan and/or behavior on the S-STS at screening or Day 1. These criteria are a score of 3 or 4 on questions 2 or 13, or 2 or higher on any of the following questions: 1a (only if 1b is coded YES), 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 14.
  15. In the process of litigating for compensation for a psychiatric disorder. Subjects who are in the process of applying for medical or VA benefits and/or those who have settled a disability claim prior to enrollment in the trial are eligible.
  16. History of Huntington's, Parkinson's, and/or Alzheimer's disease, multiple sclerosis, seizures (history of childhood febrile seizures are not exclusionary), epilepsy, or stroke.
  17. History of allergy, sensitivity, or intolerance to N-methyl-D-aspartate receptor (NMDAR) ligands, including ketamine, dextromethorphan, memantine, methadone, dextropropoxyphene, or ketobemidone, as well as current use of such agents.
  18. Received an investigational drug product or device within 30 days (or 5 half-lives, whichever is longer for a drug product) of dosing.
  19. Previously received NYX-783.
  20. Screening QT interval corrected for heart rate (HR) by Fridericia's formula (QTcF)  $>450$  (males) or  $470$  (females) milliseconds (msec) or an ECG that is not suitable for QT measurements (e.g., poorly defined termination of T-wave in the investigator's opinion).
  21. A known familial history or known presence of long QT syndrome, or a known history of past or current clinically significant arrhythmias or ischemic heart disease.
  22. Body mass index  $>35$  kg/m<sup>2</sup> at screening.
  23. HR  $\leq 45$  or  $>95$  beats per minute at screening.
  24. Uncontrolled Type I or Type II diabetes mellitus or uncontrolled hypertension.
  25. Estimated creatinine clearance  $<60$  mL/minute calculated by the Cockcroft-Gault equation at screening or history of renal disease, as assessed by the investigator or the sponsor-designated medical monitor.

26. Known diagnosis of a current infectious disease, including human immunodeficiency virus infection, hepatitis, or other ongoing infectious disease that the investigator considers clinically significant.
27. Current evidence of dysplasia or history of malignancy (including lymphoma and leukemia) in the last 5 years, with the exception of successfully treated non-metastatic basal cell or squamous cell carcinoma of the skin or localized carcinoma in situ of the cervix.
28. History of gastrointestinal disease or surgery (except simple appendectomy or hernia repair), leading to impaired drug absorption.
29. Known abnormal laboratory results, ECG results, medical history or concurrent conditions which, in the opinion of the investigator or sponsor, would preclude safe study participation or interfere with study procedures/assessments.
30. Uncorrected hypothyroidism or hyperthyroidism. NOTE: subjects with compensated hypothyroidism, with normal thyroid-stimulating hormone levels may be enrolled.
31. Impaired hepatic function characterized by a previous known diagnosis of chronic liver disease and/or the presence of direct bilirubin >1.5x, or, alanine transaminase, aspartate transaminase, alkaline phosphatase, or gamma-glutamyl transferase >2x the upper limit of normal at screening.
32. Current borderline personality disorder, antisocial personality disorder, or other personality disorder (e.g. subject showed pervasive pattern of disregard for and violation of the rights of others, or subject has had previous repetitive suicidal behavior [including aborted, interrupted or ineffective suicide attempts, gestures], self-mutilating behavior, or has ever been homicidal) of sufficient severity, in the investigator's opinion, to interfere with study participation.
33. Use of NMDAR-binding drugs (e.g., ketamine, dextromethorphan, memantine, methadone, lamotrigine, esketamine) within 60 days prior to dosing or during the study.
34. 3,4-Methylenemetamphetamine (MDMA)-assisted psychotherapy within 90 days prior to screening or during the study.
35. Expected to initiate any psychotherapy during the study.

### **8.3. Screen Failures**

Subjects who sign and date the ICF 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. Subjects may be rescreened if the investigator considers rescreening appropriate. If a screened subject is not eligible for randomization, the primary reason for failure should be recorded on the applicable eCRF. The primary reason for screen failure should be recorded according to one of the following categories: did not meet inclusion criteria; did meet exclusion criteria; protocol violation or subject noncompliance (specify); lost to follow-up; withdrew consent; other (specify).

### **8.4. Subject Withdrawal Criteria**

A 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 withdraw will not be replaced. For subjects who are lost to follow-up (i.e., those subjects whose status is unclear because of failure to appear for study visits without stating an intention to withdraw), the investigator should document in the source documents steps taken to contact the subject. Subjects are to be withdrawn from the study if any of the following criteria are met:

- Occurrence of an AE, intercurrent illness, or laboratory abnormality which, in the opinion of the investigator, warrants the subject's permanent withdrawal from the study for subject safety.
- Withdrawal of informed consent
- Noncompliance with protocol procedures
- Lost to follow-up
- Pregnancy
- Study termination by the sponsor
- Other: COVID-19 site response

## **8.5. Methods of Birth Control**

Female subjects of childbearing potential who are sexually active with a male partner must practice one of the following methods of birth control during the study and for 30 days after the last dose of study drug.

- Oral or parenteral contraceptives
- Intrauterine device (either hormonal or non-hormonal type acceptable)
- Barrier (condom, contraceptive sponge, diaphragm, or cervical cap with spermicide)
- Abstinence

Females not of childbearing potential are defined as those who have been surgically sterilized (hysterectomy, bilateral oophorectomy, or tubal ligation) or who are post-menopausal (amenorrhea) for at least 2 years.

Female subjects of childbearing potential must have a negative pregnancy test at screening and Day 1.

Male subjects who are not surgically sterilized must agree to use an acceptable method of birth control and refrain from sexual activity with female sexual partners who do not use an acceptable method of birth control during the study and for 30 days after the last dose of study drug. Male subjects who are not surgically sterilized for at least 90 days prior to screening, and who are sexually active with a female partner, must agree to use barrier contraceptives (condom with spermicide) during the study and for 30 days after the last dose of study drug. Male subjects must also agree to refrain from sperm donation during the study and until 30 days after the last dose of study drug.

## 9. SCHEDULE OF PROCEDURES

### 9.1. Screening Period (Day -28 to -14)

- Informed consent form (ICF) must be signed before any protocol-directed procedures are performed. A separate ICF for optional pharmacogenomics (PGX) and PAX blood sampling will also be administered at the screening visit.
- Inclusion/exclusion criteria
- Demographics and habits (alcohol and/or illicit drug use, employment history, smoking history, and index traumatic event)
- Medical and psychiatric history (including non-pharmacologic interventions)
- PTSD diagnosis, according to DSM-5 (SCID-5-CT to exclude co-morbidities and CAPS-5 to diagnose PTSD)
- LEC-5 (part of PCL-5 with LEC-5 and Criterion A)
- S-STS (last month version)
- Vital sign measurements (after at least 5 minutes of rest, sitting or supine)
- Height (calculate BMI)
- Body weight (calculate BMI)
- PE
- ECG (after at least 5 minutes supine rest)
- Laboratory tests:
  - Clinical chemistry (calculate estimated creatinine clearance)
  - Hematology
  - HIV, hepatitis B surface antigen, hepatitis C antibody
  - Serum pregnancy test for women of childbearing potential
  - Drug and alcohol screen (serum)
  - Urinalysis
- Clinical measures:
  - CAPS-5
  - PCL-5 (last month version)
  - HADS
  - CGI-S
  - PSQI
  - PSQI-A

○ [REDACTED]

- Prior and concomitant medications
- SAEs (collection begins after ICF signed)

### **9.2. Day -14 (±3 days)**

Subjects who meet eligibility criteria according to the PCL-5 ( $\geq 38$ ), SCID-5-CT, and CAPS-5 (total score  $\geq 30$ ) at screening will complete a SAFER remote phone interview. Those subjects confirmed to have met the study diagnostic and selection criteria as determined during the SAFER interview are then eligible to proceed in the study.

### **9.3. Stage 1: Day 1**

- Medical and psychiatric history updates (if applicable)
- Inclusion/exclusion criteria
- S-STS (since last visit version)
- Vital signs (after at least 5 minutes of rest, sitting or supine)
- Body weight
- ECG (after at least 5 minutes supine rest)
- Concomitant medications
- Laboratory tests
  - Clinical chemistry (fasted)
  - Hematology
  - [REDACTED]
  - [REDACTED]
  - Pregnancy test (urine) for women of childbearing potential
  - Drug (urine) and alcohol screen (breathalyzer)
  - Urinalysis
- Clinical measures (all measures except for CAPS-5 are not required if last measurement is within 14 days prior to Day 1)
  - CAPS-5
  - PCL-5
  - HADS
  - CGI-S
  - PSQI
  - PSQI-A

- [REDACTED]
- DES-II
- BAC-Symbol Coding
- Randomization
- Study drug dispensing, dose administration (in clinic and after completion of above-noted study procedures), and compliance monitoring
  - Medication adherence and reminder system training
- AEs (serious and non-serious)

#### **9.4. Week 2, Day 14 ( $\pm 7$ days)**

- S-STS (since last visit version)
- Vital sign measurements (after at least 5 minutes of rest, sitting or supine)
- Body weight
- Laboratory tests: clinical chemistry (fasted)
- Clinical outcome measures
  - CAPS-5
  - PCL-5
  - HADS
  - PSQI
  - PSQI-A
  - CGI-S
- Study drug accountability, compliance review, and dispensing
  - Medication adherence and reminder system review
- Concomitant medications
- AEs (serious and non-serious)

#### **9.5. Stage 2: Week 4, Day 28 ( $\pm 3$ days)**

Study drug (including extra kit) dispensed in Stage 1 may not be re-dispensed and/or administered in Stage 2.

- S-STS (since last visit version)
- Vital signs (after at least 5 minutes of rest, sitting or supine)
- Body weight
- PE
- ECG (after at least 5 minutes supine rest)
- Laboratory tests

- Clinical chemistry (fasted)
- Hematology
- Plasma concentration blood sample
- RNA (miRNA, mRNA) PAXgene® blood sample (optional)
- Pregnancy test (urine) for women of childbearing potential
- Drug (urine) and alcohol screen (breathalyzer)
- Urinalysis
- Clinical measures
  - CAPS-5
  - PCL-5
  - HADS
  - CGI-S
  - PSQI
  - PSQI-A
  - BAC-Symbol Coding
  - ██████
  - DES-II
- Randomization (for Stage 2)
- Study drug accountability, compliance review, and dispensing (for next day dosing)
  - Medication adherence and reminder system review
- Concomitant medications
- AEs (serious and non-serious)

### **9.6. Week 6, Day 42 (±7 days)**

- S-STS (since last visit version)
- Vital signs (after at least 5 minutes of rest, sitting or supine)
- Body weight
- Laboratory tests: clinical chemistry (fasted)
- Clinical outcome measures
  - CAPS-5
  - PCL-5
  - HADS

- PSQI
- PSQI-A
- CGI-S
- Study drug accountability, compliance review, and dispensing
  - Medication adherence and reminder system review
- Concomitant medications
- AEs (serious and non-serious)

**9.7. Week 8, Day 56 (±3 days)**

- S-STIS (since last visit version)
- Vital signs (after at least 5 minutes of rest, sitting or supine)
- Body weight
- PE
- ECG (after at least 5 minutes supine rest)
- Laboratory tests
  - Clinical chemistry (fasted)
  - Hematology
  - Plasma concentration blood sample
  - [REDACTED]
  - Drug and alcohol screen (serum)
  - Pregnancy test (urine) for women of childbearing potential
  - Urinalysis
- Clinical outcome measures
  - CAPS-5
  - PCL-5
  - HADS
  - CGI-S
  - PSQI
  - PSQI-A
  - BAC-Symbol Coding
  - [REDACTED]
  - DES-II
  - [REDACTED]

- Study drug accountability and compliance review
  - Medication adherence system review and retrieval of device (if applicable)
- Concomitant medications
- AEs (serious and non-serious)

### **9.8. Early Termination Visit**

- S-STS (since last visit version)
- Vital signs (after at least 5 minutes of rest, sitting or supine)
- Body weight
- PE
- ECG (after at least 5 minutes supine rest)
- Laboratory tests
  - Clinical chemistry (fasted)
  - Hematology
  - Plasma concentration blood sample
  - [REDACTED]
  - Drug and alcohol screen (serum)
  - Pregnancy test (urine) for women of childbearing potential
  - Urinalysis
- Clinical measures
  - CAPS-5
  - PCL-5
  - HADS
  - CGI-S
  - PSQI
  - PSQI-A
  - BAC-Symbol Coding
  - [REDACTED]
  - DES-II
  - [REDACTED]
- Study drug accountability and compliance review
  - Medication adherence system review and device retrieval (if applicable)
- Concomitant medications

- AEs (serious and non-serious)

### **9.9. Safety Follow-up Visit (7 days post-therapy $\pm$ 3 days)**

The following safety assessments are to be performed only if required for AE follow-up:

- Vital signs (after at least 5 minutes of rest, sitting or supine)
- Body weight
- PE
- ECG (after at least 5 minutes supine rest)
- Laboratory tests
  - Clinical chemistry (fasted)
  - Hematology
  - Urinalysis

The following assessments are to be performed for all subjects at this visit:

- Concomitant medications
- AEs (serious and non-serious)

### **9.10 Remote Visits**

In the event that a subject cannot be physically present at the investigational site (due to the COVID-19 health emergency) within the time window of their scheduled visits, the following assessments will be completed remotely through videoconference or phone call with site staff:

- Reporting of AEs
- concomitant medication
- the evaluation of suicidality (SSTS), and the
- administration of the CAPS-5

Remote assessments can only be done for Week 2, Week 6, and Safety Follow-up visits. The mode of assessment will be captured in the clinical database. Baseline, Week 4 (re-randomization) and Final Visit Week 8/ET must be completed at the site. The rest of the safety procedures such as blood and urine sampling, vital signs, ECG and physical exam must be done within 7 days of the remote assessment. Instances of remote assessment will be collected in the database. If these safety procedures cannot be completed in that timeframe, the subject may have to be discontinued from the study. Please consult with the [REDACTED] Medical Monitor as these issues arise.



## 10. STUDY TREATMENT

### 10.1. Dosing and Administration

The first dose of study drug in Stage 1 will be administered orally at the investigative site with water and supervised by blinded clinic personnel who will perform hand and mouth checks to confirm dosing. Subsequent study drug administration will be on an outpatient basis. Stage 2 dosing begins the day after the Week 4 visit.

Subjects will be instructed to take 1 capsule per day with water. The capsules should be swallowed whole and not opened.

Study drug provided at each visit will be sufficient to last until the next planned visit. [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

All study drug dispensed to the subject must be recorded in the eCRF and accountability logs. Subjects will be instructed to return all used and unused blister cards, including the extra blister card, at each subsequent study visit for accountability.

### 10.2. Randomization and Blinding

The study will be conducted as a placebo-controlled, double-blind study. The randomization schedule will be produced by the authorized sponsor designee.

On Day 1, subjects will be randomized to receive placebo, NYX-783 50 mg QD, or NYX-783 10 mg QD in a 4:1:1 ratio, respectively. Placebo responders and placebo non-responders (CAPS-5 total score reduction from baseline  $\leq 35\%$  and total CAPS-5 total score  $\geq 26$  at Week 4) will be identified at the completion of Stage 1 (Weeks 1-4) in a blinded fashion; then, each group (i.e., placebo responders and placebo non-responders) will be re-randomized in a blinded fashion to placebo, NYX-783 50 mg QD, NYX-783 10 mg QD in a 3:2:2 ratio, respectively, for Stage 2 (Weeks 4-8) treatment. Subjects who received active treatment during Stage 1 will receive placebo in Stage 2.

The authorized investigative site personnel will obtain blinded kit assignments using an interactive response technology (IRT) system. The blinded dose information may only be obtained by the investigator (or designee) in an emergency, where the information may have an impact on further treatment decisions or aid in the emergency treatment of the subject. Every effort must be made to contact the sponsor prior to unblinding.

### 10.3. Treatment Compliance

Blinded clinic personnel will observe dosing on Day 1 to ensure subject compliance. A smartphone--based video recording technology platform will be utilized for monitoring compliance with study drug administration. The platform uses artificial intelligence on smartphones to confirm medication ingestion. Additionally, built-in reminders and a communication system may allow for real-time intervention in case of study drug interruptions. Use of the platform will be required for all subjects. The platform will be provided to subjects preloaded onto a smartphone, or subjects will download the platform onto his/her personal mobile device during a study visit. Video recordings will be encrypted and transmitted to a secure centralized location for further analyses, including testing for duplicate enrollment at other investigative sites. The captured data and video will be reviewable through a roles and restricted system ensuring privacy of information. The system is compliant with the Health Insurance Portability and Accountability Act (HIPAA), which protects the privacy and security of healthcare information. Phone numbers of the subjects may also be collected and stored in an encrypted manner. Storing the phone numbers will allow for direct communication with subjects, including automated messaging from the Platform device and contact by healthcare providers or other monitoring personnel. At no time is the phone number visible to healthcare providers or sponsor monitoring personnel.

Investigative site personnel will also perform compliance calculations based on returned study drug. Noncompliance (missed doses) or partially administered doses must be documented in the eCRF.

### 10.4. Concomitant Medications

Concomitant medications used within 30 days prior to dosing and through the end-of-study assessments at the final follow-up visit must be documented on the concomitant medications eCRF. Additionally, use of any of the below medications within “90-day before Screening restrictions” must be documented on the eCRF.

The investigator (or designee) should instruct subjects to notify the study site about any new medications taken after the start of the study drug and prior to study discontinuation.

The following medication is prohibited within 12 months prior to screening and during the study:

- Clozapine

The following medications are prohibited within **30** days (90 days for fluoxetine) prior to screening and through the entire study period:

- Nefazodone
- Monoamine oxidase inhibitors such as selegiline, phenelzine, tranylcypromine
- SSRIs and SNRIs such as fluoxetine, sertraline, escitalopram, citalopram, paroxetine, desvenlafaxine, duloxetine, venlafaxine
- Tricyclic antidepressants such as amitriptyline, imipramine, nortriptyline
- NaSSA mirtazapine

- Serotonin modulator and stimulator antidepressants such as vilazodone or vortioxetine
- Norepinephrine, serotonin and dopamine reuptake inhibitor bupropion

**Initiation of current therapy** with the following medications is prohibited within **30** days prior to Screening or during the study:

- Other allowed antidepressants
- Hypericum perforatum (St. John's Wort)

However, St. John's Wort is **ALLOWED**, if the dose is stable (in the investigator's opinion) for at least **30** days prior to the start of screening and no change of dose occurs during the screening, treatment, or follow-up periods of the study.

Medications with primarily CNS activity are prohibited within **3** days prior to screening and through the entire study period. Examples of these medications include the following:

- Conventional or atypical antipsychotics, anticonvulsant drugs (including gabapentinoids), or psychostimulants
- Benzodiazepines
- Methylphenidate
- Doxazosin
- Prazosin
- Clonidine
- First generation sedating H<sub>1</sub> antihistamines
- Quetiapine
- Eszopiclone
- Zolpidem extended-release
- Other sedative-hypnotics

Use of certain non-benzodiazepine sleep inducers (e.g., zolpidem immediate-release [up to 10 mg at bedtime], zaleplon [up to 20 mg at bedtime]), melatonin, or ramelteon for sleep are allowed. **NOTE:** the non-benzodiazepine sleep inducers should be administered as a single dose immediately before bedtime and may not be re-administered during the night.

Use of NMDAR-binding drugs (e.g., ketamine, dextromethorphan, memantine, methadone, lamotrigine, esketamine) may not be used within 60 days prior to dosing or during the study.

Exceptions to these specifications must be approved by the sponsor designated medical monitor.

## **10.5. Study Drug Discontinuation**

Study drug must be discontinued for a given subject if the investigator determines that continuing it would result in a significant risk for that subject. Please also refer to protocol-specified Subject Withdrawal Criteria (see Section 8.4).

## 11. STUDY DRUG MATERIALS AND MANAGEMENT

### 11.1. Study Drug

NYX-783 is formulated for this study as 50 mg, and 10 mg capsules for oral administration. Matching placebo capsules for oral administration are also provided.

[REDACTED]

### 11.2. Study Drug Packaging and Labeling

Study drug will be packaged in kits containing blister cards of blinded NYX-783 50 mg, NYX-783 10 mg or placebo capsules. [REDACTED]

[REDACTED]

[REDACTED]. The labels will include, but will not be limited to, kit number, capsule count, storage conditions, sponsor name, and investigational use statement. The sponsor will provide the investigative site with sufficient amounts of study drug to conduct the study.

### 11.3. Study Drug Storage

Study drug should be stored in a locked, limited-access location according to label and under the direct responsibility of the investigator or designated pharmacist or clinic site staff. The capsules should be stored at 20°-25°C with excursions permitted between 15°-30°C. The investigative site should contact the sponsor for directions regarding non-permitted excursions.

### 11.4. Study Drug Preparation

The investigator may delegate study drug handling and accountability as appropriate. An interactive response technology (IRT) will specify to investigative site personnel which study drug kit to dispense. [REDACTED]

[REDACTED]. Refer to the Product Pharmacy Manual and/or IRT manual for additional instruction. The pharmacy manual includes instructions for registering product complaints, if required.

### 11.5. Study Drug Accountability

The investigator may delegate study drug accountability as appropriate. The investigative site personnel will receive, inspect, acknowledge study drug condition, document amount received/dispensed/returned, dispense, acknowledge shipments as instructed, and maintain study drug accountability records. A sponsor representative will inspect the study drug and accountability records. Site personnel will dispense (and administer if required) study drug to subjects, account for and document used/unused study drug, and return any unused study drug (if

applicable) to unblinded pharmacy personnel. Only subjects enrolled in this clinical study and eligible to receive treatment are to receive study drug material.

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

## **12. STUDY ASSESSMENTS**

### **12.1. Psychiatric Rating Scales/Outcome Measures/Test(s)**

Investigative sites will be required to identify and provide clinical raters with suitable experience and training to conduct interviews and/or clinician-rated scales. Remote rater qualification, certification, training, and surveillance will be required for all investigative site clinical raters. Enrollment may not begin at an investigative site until a clinical rater has been informed by the sponsor and/or designee that qualification, training, and/or certification requirements have been satisfied. If a clinical rater changes during the study, new rater(s) must complete qualification, training, and certification requirements and be deemed acceptable by the sponsor and/or designee prior to completing interviews and/or scales. Information from diagnostic interviews, medical/psychiatric and/or medication history, and rating scales may be requested by the sponsor or designee for data review and/or rater surveillance activities.

#### **12.1.1. Structured Clinical Interview for DSM-5 (SCID-5-CT)**

The Structured Clinical Interview for DSM-5, Clinical Trials Version (SCID-5-CT) is a semi-structured interview guide for making the major DSM-5 diagnoses. It is administered by a clinician or trained mental health professional who is familiar with DSM-5 classification and diagnostic criteria. ([APA 2013](#))

The SCID-5-CT modules will support assessing the mental disorders-related eligibility criteria.

#### **12.1.2. SAFER Remote Interview**

The SAFER remote interview is an independent psychologist- or psychiatrist-administered interview to assess whether a subject is appropriate for a study by confirming eligibility criteria. The acronym stands for the interview's attention to the following criteria: State versus trait; Assessability; Face validity; Ecological validity; and Rule of 3 Ps (pervasive, persistent, and pathological). ([Desseilles 2013](#)) The interview is intended to increase the quality of the study by restricting the sample to a more homogeneously defined PTSD population. As such, the SAFER interview can help in the selection of subjects who are good candidates for assessment of a treatment effect by minimizing the risk that factors unrelated to treatment will influence outcomes. The SAFER interview will be completed with the subject by phone.

## **12.2. Clinical Measures**

### **12.2.1. Clinician Administered PTSD Scale for DSM-5 (CAPS-5)**

The CAPS-5 is a 30-item structured interview for diagnosis and assessment of PTSD ([Weathers 2017](#)). In addition to assessing the severity and frequency of the 20 DSM-5 PTSD symptoms, questions target the onset and duration of symptoms, subjective distress, impact of symptoms on social and occupational functioning, improvement in symptoms since a previous CAPS-5 interview, overall response validity, overall PTSD severity, and specifications for the dissociative subtype (depersonalization and derealization). CAPS-5 total symptom severity score is calculated by summing severity scores for the 20 DSM-5 PTSD symptoms. Similarly, CAPS-5 symptom cluster severity scores are calculated by summing the individual item severity scores for symptoms corresponding to a given DSM-5 cluster: Criterion B, intrusions (items 1-5);

Criterion C, avoidance (items 6-7); Criterion D, negative alterations in cognitions and mood (items 8-14); and, Criterion E, alterations in arousal and reactivity (items 15-20). The assessor combines information on frequency and intensity of an item into a single severity rating (0-4) for each of the 20 DSM-5 PTSD symptoms, with a maximum possible score of 80 points. A higher score corresponds to more severe PTSD. (APA 2013)

#### **12.2.2. Hospital and Anxiety Depression Scale (HADS)**

The HADS is a self-assessment tool consisting of two subscales, one for anxiety (HADS-A) and one for depression (HADS-D). (Zigmond 1983, Bjelland 2002) Each subscale consists of 7 items that are scored from 0-3, for a maximum score of 21 for either anxiety or depression; higher scores represent more severe anxiety or depression.

#### **12.2.3. PTSD Checklist for DSM-5 (PCL-5)**

The PCL-5 is a 20-item self-report measure that assesses PTSD symptoms experienced over the last week or month according to DSM-5 criteria. Items assess symptoms across 4 symptom clusters of PTSD (re-experiencing, negative mood, avoidance, and hyperarousal) on a 0-4 point Likert scale. Total scores range from 0-80. The “since last week” version should be used after screening.

#### **12.2.4. Clinical Global Impressions-Severity (CGI-S)**

The CGI-S scale assesses the global severity of illness and change in clinical condition over time, and subjects are rated from 1 (normal, not at all ill) to 7 (among the most extremely ill subjects). (Guy 1976).

#### **12.2.5. Pittsburgh Sleep Quality Index (PSQI)**

The PSQI (Buysse 1989) is a questionnaire to assess sleep quality and disturbances over a 1-month time interval. A total of 19 items are self-rated, 15 objective (about frequency of sleep disturbances and subjective sleep quality) and 4 subjective (typical bedtime, wake-up time, sleep latency and sleep duration) items. These 19 items generate 7 component scores: subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medication, and daytime dysfunction, each of which ranges from 0: no difficulty to 3: severe difficulty. The PSQI Global Score is generated by addition of the 7 individual component scores and ranges from 0 to 21. A global score >5 indicates significant sleep disturbance. The additional 5 items rated by the bedpartner or roommate will not be collected for this trial.

#### **12.2.6. Pittsburgh Sleep Quality Index Addendum (PSQI-A)**

The PSQI-A (Germain 2005) is a self-report instrument to assess disruptive nocturnal behavior (DNB) in PTSD subjects, according to the following 7 types of behaviors: 1) hot flashes, 2) general nervousness, 3) memories or nightmares of traumatic experience, 4) severe anxiety or panic, not related to traumatic memories, 5) bad dreams, not related to traumatic memories, 6) episodes of terror or screaming during sleep without fully awakening and 7) episodes of acting out dreams, such as kicking, punching, running, or screaming. Each DNB is rated on a scale of 0-3 (0: not in the past month, 1: less than once a week, 2: once or twice a week, and 3: three or more times a week), with a global score range of 0-21.

[REDACTED]

[REDACTED]

#### **12.2.9. Dissociative Experiences Scale-II (DES-II)**

The Dissociative Experiences Scale-II (DES-II) (Carlson 1993) is a 28 item, self-report questionnaire that measures a wide variety of dissociative experiences, from normal (e.g., day-dreaming) to pathological (depersonalization). Subjects rate the percentage of time they have the experience, from 0% to 100% (in increments of 10).

#### **12.2.10. Life Events Checklist for DSM-5 (LEC-5)**

The LEC-5 (Weathers 2013) is a 17-item self-reporting tool to screen for potentially traumatic events in a patient's lifetime. The LEC-5 assesses exposure to 16 events that may result in PTSD or distress and one additional item that assesses for any other extraordinarily stressful event.

#### **12.2.11. Symbol Coding from the Brief Assessment of Cognition (BAC Symbol Coding)**

The Brief Assessment of Cognition (BACS, [Keefe et al., 2004](#)) is a pen-and-paper cognitive assessment used in hundreds of research studies and clinical trials. A tablet-based version of the BACS, the BAC App, was validated in 2016 to allow standardized presentation of task instructions and stimuli, audio-recording of responses, and automatized scoring and data management ([Atkins et. al., 2016](#)). BAC Symbol Coding is delivered on the Pathway tablet and offers a sensitive measure of processing speed. Participants are provided a key and asked to fill in the corresponding numbers beneath a series of symbols as quickly as possible within 90 seconds. The assessment takes approximately 3-5 minutes to complete, including instructions and practice.

### **12.3. Exploratory Assessments**

[REDACTED]





examination and assessment of the respiratory, cardiovascular, gastrointestinal, and musculoskeletal systems.

#### **12.4.4. 12-lead Electrocardiogram**

The ECG will include all 12 standard leads. Standard ECG parameters, including HR, RR, PR, corrected QT (QTc) intervals, and QRS duration, will be measured.

All ECGs should be collected after at least 5 minutes supine rest. Clinically significant, abnormal ECGs should be repeated. Any clinically significant ECG change should be recorded as an AE.

ECGs may be repeated for eligibility determination.

#### **12.4.5. Clinical Laboratory Assessments**

The investigator will review clinical laboratory test results as they become available for determination and documentation of clinical significance, if there are any out-of-range values. Abnormal laboratory results at Screening may be repeated for eligibility determination. Possibly drug-related or clinically-relevant abnormal values of uncertain causality must be repeated. Ongoing abnormal laboratory values should be followed at the investigator's discretion.

All women of child-bearing potential will receive serum pregnancy tests ( $\beta$ -HCG) and urine pregnancy tests.

Hepatitis and HIV screen includes: HIV, hepatitis B surface antigen, and hepatitis C antibody.

Hematology includes: White blood cell count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils); hemoglobin; hematocrit; mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); MCH concentration (MCHC); mean platelet volume (MPV); red blood cell count; red cell distribution width (RDW), and platelet count.

Clinical chemistry includes electrolytes (bicarbonate, calcium, chloride, phosphorus, potassium, sodium, magnesium), enzymes (ALP, AST, ALT, lactate dehydrogenase, gamma-glutamyl transferase), and other tests (albumin, bilirubin [total], direct bilirubin, indirect bilirubin, creatinine, glucose, protein [total], blood urea nitrogen, thyroid stimulating hormone [at screening only], and uric acid).

Qualitative urinalysis includes appearance, color, pH, specific gravity, glucose, ketones, protein, blood, leukocyte esterase, urobilinogen, bilirubin, and nitrites. Microscopy will include red blood cells, white blood cells, epithelial cells, casts, and bacteria.

Standard blood and urine drug panel includes analysis for benzodiazepines, opiates, cocaine, cannabinoids, amphetamines, barbiturates, methadone, and phencyclidine. Serum and alcohol breathalyzer testing will also be performed. The screening period may be extended to ensure washout, if needed, between screening and Day 1.

An estimated creatinine clearance will be calculated.

Refer to the laboratory sample collection, handling, and shipping specifications for additional instruction.

#### **12.4.6. Sheehan Suicidality Tracking Scale (S-STs)**

The standard version of the S-STs (Sheehan 2014[a]) 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” 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 tool is to be administered via interview with the subject (by a trained operator/interviewer). If possible, the same interviewer should be used throughout the study for the same subject. The S-STs accommodates a wide range of timeframes. 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 Columbia–Suicide Severity Rating Scale (Giddens 2014) and to all categories in the FDA Draft Guidance documents of both 2010 and 2012 and 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 and been shown to provide at least equal sensitivity to detecting suicidal ideation and behavior. (Sheehan 2014 [b])

**NOTE:** Suicidality identified after baseline will be recorded as an AE.

### **12.5. Adverse Events and Serious Adverse Events**

#### **12.5.1. Definition of an Adverse Event**

An AE is any untoward, undesired, or unplanned clinical event in the form of signs, symptoms, disease, or laboratory or physiological observations associated with the use of an intervention in humans participating in a clinical study, regardless of causal relationship.

Pre-existing conditions (i.e., ones that are present before study drug dosing) should be reported as an AE only if the frequency, intensity, or character of the condition worsens during the study. (Note: Pre-existing conditions are reported during the Screening period as part of the subject’s medical history.)

Laboratory or ECG abnormalities are not considered AEs 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 requires medical/surgical intervention or leads to study drug interruption or discontinuation, must be considered an AE.

#### **12.5.2. Definition of a Serious Adverse Event**

An SAE is any AE occurring at any dose that results in any of the following outcomes:

- Death  
“Death” is an outcome and is NOT the AE. In the event of death, the cause of death should be recorded as the AE. The only exception is “sudden death” when the cause is unknown.
- Is a life-threatening experience  
Life-threatening AEs 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 an SAE 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 (e.g., an allergic bronchospasm requiring intensive treatment in an emergency room or at home).

### **12.5.3. Adverse Event Reporting**

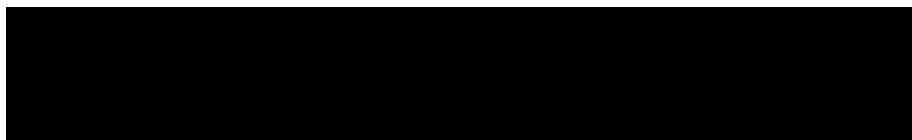
Adverse events will be collected from the time of study drug administration through the last day of the subject's participation in the study. Follow-up of AEs must be conducted in accordance with the investigative site's normal practice.

All AEs must be documented, evaluated, and reported in the source documents and electronic case report forms (eCRFs), and include the following information: duration (start and stop dates or if continuing at final visit), severity, relationship to study drug, action(s) taken, outcome, and whether it constitutes a serious adverse event (SAE). Diagnoses (rather than symptoms) should be recorded wherever possible. To avoid vague, ambiguous, or colloquial expressions, all AEs should be recorded in standard medical terminology rather than in the subject's own words. Symptoms related to lack of efficacy (in the investigator's opinion) will not be recorded as AEs unless there is a clinically significant safety concern (e.g., serious adverse event).

Subjects should be instructed to report all potential AEs to the investigator. Subjects should also be queried in a non-leading manner, without specific prompting (e.g., "How are you feeling?") about all potential AEs. 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 AEs.

### **12.5.4. Serious Adverse Event Reporting**

Serious adverse event (SAE) reporting will begin from the time of informed consent and will end 30 days after the last dose of study drug. SAEs must be reported to the sponsor or designee using the provided form either by email or fax to the following address or telephone number, respectively:



All SAEs that result in death or are life threatening, regardless of causal relationship, must be reported to the sponsor or designee within 24 hours of the site's knowledge of the event. A copy of the initial SAE report must be received within one (1) business day.

All other SAEs or other events reportable to FDA and/or institutional review board (IRB) will be forwarded to the sponsor or designee within one (1) business day.

The SAE report should provide as much of the required information as is available at the time. The following minimum information is required for reporting an SAE: subject identification, reporting source, and event outcome. Supplemental information may be transmitted using a follow-up report and should not delay the initial report. Aptinyx (or its authorized representative) 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 an SAE, the information will be treated as an SAE for the purposes of this protocol.

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

#### **12.5.5. Relationship to Study Drug**

For each reported AE/SAE, the investigator must assess the relationship to study drug using the following categories:

##### **Unrelated**

- Does not follow a known response pattern to the suspect study drug (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 study drug suggests that a relationship is unlikely.
- Follows a response pattern that is unlike that of the suspect study drug (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 study drug.
- May follow a known response pattern to the suspect study drug (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 study drug.
- 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 study drug, if applicable.

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

### **12.5.6. Severity of Adverse Events**

All AEs must 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 AE 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).

### **12.5.7. Action Taken for Adverse Events**

For each reported AE, the action taken must be evaluated and recorded on the eCRF according to the following criteria:

- No action taken
- Concomitant medication taken
- Hospitalization or prolongation of hospitalization
- Discontinued study
- Non-drug therapy
- Other (specify)

The investigator must also document on the eCRF the action taken with study drug (as a result of a given AE) according to the following criteria:

- Dose not changed
- Study drug withdrawn

- Not applicable
- Unknown

#### **12.5.8. Outcome for Adverse Events**

For each reported AE, 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

#### **12.5.9. Other Reportable Events**

Reports of overdose (with or without an AE), abuse, dependency, inadvertent or accidental exposure, pregnancy, and unexpected therapeutic benefit should be forwarded in the same time frame as an SAE. 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 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.

## **13. STOPPING RULES AND SAFETY MONITORING**

### **13.1. Stopping Rules for Individual Subjects**

Any subject may withdraw consent at any point during the study. The investigator can discontinue a subject at any time if it is deemed medically appropriate, or for subject noncompliance with study requirements. Subjects will be withdrawn from the study if any of the following criteria are met:

- Occurrence of an AE, intercurrent illness, or laboratory abnormality which, in the opinion of the investigator, warrants the subject's permanent withdrawal from the study for subject safety
- Repeated results (i.e., values are confirmed by repeat testing as soon as feasible) showing any of one following laboratory findings:
  - Total bilirubin  $\geq 2.5$  mg/dL
  - ALT or AST  $> 3$  x ULN
  - ALT or AST  $\geq 3$  x ULN and total bilirubin  $\geq 2$  x ULN
  - ALT or AST  $\geq 3$  x ULN, with the appearance of fatigue, nausea, vomiting, right upper quadrant pain, or tenderness, fever, rash, or eosinophilia ( $> 5\%$ )
  - Alkaline phosphatase  $\geq 2$ x ULN
- 2 consecutive ECGs showing a new absolute corrected QT interval (QTc)  $\geq 500$  milliseconds (msec) that was not present on the corresponding baseline ECG, or a QT/QTc interval  $> 60$  msec over the corresponding baseline value. The QT interval should be corrected by Fridericia's (QTcF) formula.

### **13.2. Stopping Rules for Study**

The DSMB will review safety data at regular intervals, as outlined in the DSMB charter. If the individual stopping criteria are met for five or more subjects receiving one of the treatment regimens with active study drug, dosing should be stopped based on unblinded review of data by the DSMB. The DSMB should assess the potential increase of risks associated with psychiatric symptoms, such as change in suicidality measured by the S-STS and change in dissociative symptoms measured by the DES-II. The study may be terminated or suspended if new concerning information regarding the safety of the study drug appears during the conduct of the study.

### **13.3. Data and Safety Monitoring Board (DSMB)**

The sponsor or designee will appoint a Data and Safety Monitoring Board (DSMB) for the periodic review of available study data. The DSMB is an independent group of experts that advises the sponsor and the study investigators. The members of the DSMB serve in an individual capacity and provide their expertise and recommendations. The primary responsibilities of the DSMB are to (1) periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and (2) make recommendations to the sponsor concerning the continuation, modification, or termination of the trial. The DSMB



considers study-specific data as well as relevant background knowledge about the disease, test agent, or patient population under study. The DSMB is responsible for defining its deliberative processes, including event triggers that would call for an unscheduled review, stopping guidelines, unmasking (unblinding), and voting procedures prior to initiating any data review. The DSMB is also responsible for maintaining the confidentiality of its internal discussions and activities as well as the contents of reports provided to it. Refer to the DSMB charter for additional details.

## 14. STATISTICAL METHODS

Detailed methodology for summary and statistical analyses of the data collected in this study will be documented in a statistical analysis plan (SAP), which will be finalized before study data are unblinded. Additional statistical analyses other than those described in this protocol may be performed if deemed appropriate and will be described in the SAP.

### 14.1. General Principles of Statistical Analysis

Categorical data will be summarized using counts and percentages. Continuous data will be summarized using descriptive statistics (number of subjects, mean, standard deviation [SD], median, minimum, and maximum). Unless otherwise specified, tests of treatment effects will be conducted using one-sided tests with a significance level of  $\alpha = 0.10$ .

### 14.2. Sample Size Determination

Response to treatment will be measured using the CAPS-5 score change from baseline to Week 4 (Stage 1) and from Week 4 to Week 8 (Stage 2). If the effect size of NYX-783 is in line with the effect sizes observed in similar historical studies, this study is powered at approximately 80% to reject the null hypothesis at a one-sided  $\alpha = 0.10$  level for any single specified efficacy comparison. The sample size estimate assumes a 15% dropout during stage 1 and 11% dropout during stage 2. The sample size may be adjusted if the actual dropout rate exceeds this assumption.

### 14.3. Analysis Populations

**Intention-to-treat (ITT):** The ITT population will consist of all randomized subjects. Subjects in the ITT population will be analyzed according to the treatment they are assigned at randomization. The primary efficacy analysis will be performed on the ITT population. Unless otherwise specified, all other efficacy analyses also will be conducted on the ITT population.

Efficacy data for the 50 mg QW group will be summarized and shown in the listings but will not be included in the formal efficacy comparisons between treatment groups.

NOTE: Any additional study populations for analysis of efficacy will be defined in the SAP.

**Safety Population:** The safety population consists of all randomized subjects who receive at least one dose of study drug (active or placebo). Subjects in the safety population will be analyzed according to the treatment they actually received. Unless otherwise specified, all safety analyses will be conducted on the Safety population.

### 14.4. Analysis of Efficacy Variables

#### 14.4.1. Methods of Analysis

Treatment arms [1 = placebo], [2 = NYX-783 50 mg QD], [3 = NYX-783 10 mg QD] and [7 = placebo], [8 = NYX-783 50 mg QD], [9 = NYX-783 10 mg QD] will be analyzed for the primary and secondary efficacy variables. Treatment arms [4 = placebo], [5 = NYX-783 50 mg QD], [6 = NYX-783 10 mg QD], [10 = placebo], and [11 = placebo] will be utilized for exploratory efficacy analyses to keep integrity of the trial.

**14.4.1.1. Primary Efficacy Endpoint Analysis**

The ITT population will be the primary efficacy analysis population. The data from the NYX-783 treatment groups (using both Stage 1 and Stage 2) will be used for the comparison to placebo. This consists of subjects (randomized to placebo and NYX-783 from treatment arms [1], [2] and [3] for Stage 1 and treatment arms [7], [8], and [9] for Stage 2.

The primary efficacy endpoint will be evaluated using the weighted combination of the estimated treatment effects from the stage-specific mixed models for repeated measures (MMRM). The MMRM models will include variables for treatment group, visit, treatment-by-visit interaction term, baseline CAPS-5 score and baseline-by-visit interaction. An unstructured covariance matrix will be used. In the case of non-convergence, alternative covariance structures will be used. The sequence of alternative covariance structures will be specified in the SAP. The Kenward-Roger approximation will be used to adjust the denominator degrees of freedom. The treatment effect will be assessed as the differences in least-squares mean (LS mean) changes from baseline to Week 4 (Stage 1) and from Week 4 to Week 8 (Stage 2) for the NYX-783 50 mg QD, NYX-783 10 mg QD, and placebo groups, pooled across Stages 1 and 2 using prespecified 0.6/0.4 weighting for Stage 1/Stage 2.

Subgroup analyses will be conducted with visit, treatment, investigator, treatment-by-visit interaction, the corresponding baseline score, and the baseline-by-visit interaction – and, if applicable – the corresponding change from screening to baseline will be included in the model.

**14.4.1.2. Secondary Efficacy Endpoints Analyses**

The ITT population also will be utilized for analysis of the secondary efficacy endpoints. Analysis of change from baseline in the secondary efficacy variables, including PCL-5, HADS-A, HADS-D, PSQI, PSQI-A, CGI-S and BAC symbol coding will use similar MMRM models to those used for analysis of the primary efficacy analysis, and include baseline severity score as a covariate.

In addition to comparing the individual active dose groups vs. placebo, similar analyses using analogous models will be done using the combined QD dose groups vs. Placebo.

[REDACTED]

Further efficacy analysis details will be provided in the SAP.

#### **14.4.2. Handling of Missing Data**

Missing data will be handled using MMRM's maximum likelihood. Any other missing data analyses to support the primary efficacy analysis will be described in the SAP.

#### **14.4.3. Supportive Analyses**

Sensitivity analysis of the primary endpoint may be conducted using alternative weights for the stage-wise treatment effects. The population(s) to be evaluated in the sensitivity analysis will be defined in the SAP. Additional post-hoc analyses may be completed.

### **14.5. Subject Disposition**

The summary of subject enrollment and disposition will display the number of subjects who are included in each analysis population and number of subjects who completed the study vs. discontinued early from the study. Specific reasons for discontinuation will be summarized according to the reasons listed on the eCRF. All subject disposition data and analysis population details will be presented in the data listings.

### **14.6. Demographic/Baseline Data**

#### **14.6.1. Demographics and Other Baseline Characteristics**

Demographic and other baseline characteristic data including gender, age, height, weight, and BMI will be summarized using descriptive statistics. Categorical data for race and ethnicity will be summarized by counts and percentages. Similar descriptive statistics will be used to summarize the baseline clinical and other efficacy outcome characteristics.

Treatment differences in subject characteristics at baseline will be assessed using Fisher's exact test for categorical variables and Type III sums of squares analysis of variance (ANOVA) for continuous variables.

The demographics and other baseline characteristics summaries will be provided for both the safety and ITT populations by treatment groups and overall. Data listings will be provided for all demographics and baseline characteristics data.

#### **14.6.2. Other Baseline Data**

Medical history, prior medications, and any other baseline data collected will be presented in separate data listings by the subject number.

### **14.7. Study Drug Exposure**

All study drug dosing information collected on the eCRF will be summarized and presented in the data listings.

### **14.8. Inclusion/Exclusion Criteria**

All inclusion/exclusion criteria not met will be presented in separate data listings by subject number.

## **14.9. Efficacy Data Listings**

Supporting data related to the efficacy and exploratory assessments will be presented in separate data listings by subject number, study stage, and treatment assignment.

## **14.10. Analysis of Safety**

The safety population will be used for the analysis of all safety variables.

### **14.10.1. Adverse Events**

Adverse events will be categorized by system organ class (SOC) and preferred term with the Medical Dictionary for Regulatory Activities. Summary tables for TEAEs will include numbers and percentages of subjects experiencing AEs by SOC and preferred term. If a subject has more than 1 AE 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 AE within an SOC category, the subject will be counted only once in that SOC category. The following summary tables will be included in the clinical study report for each treatment and overall: summary of AEs, relationship of AEs to study drug, severity of AEs, SAEs, and AEs leading to study drug discontinuation.

Data listings will be provided for all AEs, AEs leading to study drug discontinuation, and SAEs.

### **14.10.2. Clinical Laboratory Tests**

Individual results of clinical laboratory tests from hematology, serum chemistry, and urinalysis that are outside of the normal range will be flagged in the data listings. Results and change from baseline will be summarized by treatment arm and scheduled visit using descriptive statistics for numeric parameters and counts and percentages for categorical parameters.

Clinical laboratory data will be presented in the data listings.

### **14.10.3. Electrocardiogram**

Results and change from baseline will be summarized by treatment and scheduled visit and time point using descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum) for each ECG parameter assessment. All ECG data will be provided in the data listings.

### **14.10.4. Vital Sign and Weight Measurements**

Results and change from baseline will be summarized by treatment and scheduled visit and time point using descriptive statistics (number of subjects, mean, standard deviation, median, minimum, and maximum) for each measurement. All vital sign and weight data will be provided in the data listings.

### **14.10.5. S-STS**

Data collected for S-STS will be summarized by dose group and visit/time point. Change from baseline, if applicable, will also be calculated and summarized in the same manner. All S-STS data will be provided in the data listings.

**14.10.6. Other Safety Data**

Physical examination findings, concomitant medications and other safety data will be presented in the data listings.

**14.11. Interim Analysis**

No interim analyses are planned for this study.

## **15. ADMINISTRATIVE**

### **15.1. Source Documents**

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

### **15.2. 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 for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (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.

### **15.3. Case Report Forms**

An electronic CRF (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 sub-investigator listed on the Form FDA 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).

### **15.4. 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 submitted to the IRB by the investigative site for review and approval.

### **15.5. Audits and Inspections**

During the study, or after completion of the study, the 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. If the investigator receives notification of a regulatory inspection for a different study during the clinical conduct for this study, the investigator must notify the sponsor.

## **15.6. Institutional Review Board (IRB)**

This protocol, the informed consent document, and all relevant supporting data must be submitted to the IRB for approval. IRB approval of the protocol, informed consent document, 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 AEs 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.

## **15.7. Compliance with Regulatory Requirements**

This study 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.

## **15.8. Informed Consent**

Written signed informed consent must be obtained from potential study participants prior to the initiation of non-routine study-related tests. The original signed ICF for each participating subject must be filed with records kept by the investigator(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. ICF revisions must be obtained in a timely manner.

## **15.9. 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, and regulatory documents (IRB approvals/correspondence, study logs, FDA 1572 forms, financial disclosures, clinical study material records, study drug accountability records, and medical records).

## **15.10. Study Completion**

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

## **15.11. 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 (PHI), and in accordance with applicable national and/or local laws and regulations on PHI 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.

#### **15.12. Compensation, Insurance, and Indemnity**

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

#### **15.13. Financial Disclosure**

The investigator(s) are responsible for providing financial disclosure(s) in covered clinical studies. Principal investigators and sub-investigators 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.

#### **15.14. Records Retention**

According to US Investigational New Drug regulations (21 CFR 312.62), records and documents pertaining to the conduct of this study and the distribution of study drugs including, but not limited to, source documents, case report forms, ICFs, clinical laboratory test results, and drug inventory records must 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.

#### **15.15. Publication Policy**

The publication policy is outlined in the Clinical Trial Agreement.

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## **17. APPENDIX A: MAYO TBI SEVERITY CLASSIFICATION SYSTEM**

A. Classify as Moderate-Severe (Definite) TBI if one or more of the following criteria apply:

1. Death due to this TBI
2. Loss of consciousness of 30 minutes or more
3. Post-traumatic anterograde amnesia of 24 hours or more
4. Worst Glasgow Coma Scale full score in first 24 hours <13 (unless invalidated upon review, e.g., attributable to intoxication, sedation, systemic shock)
5. One or more of the following present:
  - Intracerebral hematoma
  - Subdural hematoma
  - Epidural hematoma
  - Cerebral contusion
  - Hemorrhagic contusion
  - Penetrating TBI (dura penetrated)
  - Subarachnoid hemorrhage
  - Brain Stem injury

B. If none of criteria A apply, classify as Mild (Probable) TBI if one or more of the following criteria apply:

1. Loss of consciousness of momentary to less than 30 minutes
2. Post-traumatic anterograde amnesia of momentary to less than 24 hours
3. Depressed, basilar or linear skull fracture (dura intact)

C. If none of Criteria A or B apply, classify as Symptomatic (Possible) TBI if one or more of the following symptoms are present:

- Blurred vision
- Confused (mental state changes)
- Dazed
- Dizziness
- Focal neurologic symptoms
- Headache
- Nausea