EN20-01: A 24-WEEK STUDY TO EVALUATE THE SAFETY AND EFFICACY OF CNTX-6970 IN SUBJECTS WITH MODERATE TO SEVERE KNEE OSTEOARTHRITIS PAIN.

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Maurizio Fava, MD (Sponsor-Investigator)
Psychiatrist-In-Chief
Director, Division of Clinical Research of the MGH Research Institute
Executive Director, Clinical Trials Network and Institute (CTNI)
Massachusetts General Hospital (MGH)
55 Fruit Street, Bulfinch 351
Boston, MA 02114

Robert Edwards, PhD (PPI)
Associate Professor of Anesthesia, Pain Management Center
Brigham and Women's Hospital
850 Boylston Street
Chestnut Hill, MA 02467

Thaddeus Tarpey, PhD (Statistician)
Professor & PhD Program Director
NYU Grossman School of Medicine
180 Madison Avenue, 4th Floor, Suite 4-53
New York, New York 10016

EPPIC-Net Clinical Coordinating Center Key Contacts

Address: MGH Clinical Trials Network and Institute
1 Bowdoin Square, 9th Floor
Boston, MA 02114

Study Contact/Project Manager:
Name: Allison Campbell
Email: acampbell17@mgh.harvard.edu
Phone: (781) 422-4120

Study Contact/Site Manager:
Name: Megan Bubello
Email: mbubello@mgh.harvard.edu

Medical Monitor:
Name: Mark Matza, MD
Email: mmatza@mgh.harvard.edu

Lead Clinical Research Associate (CRA)/Monitor:
Name: Miranda Williams, MS
Email: MWilliams93@mgh.harvard.edu
Phone: (863) 276-1178

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Signatory of Protocol/Amendments:
Name: Maurizio Fava, MD

Signature:

Signatory of Protocol/Amendments:
Name: Robert Edwards, PhD

Signature:

Signatory of Protocol/Amendments:
Name: Thaddeus Tarpey, PhD

Signature:

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BACKGROUND AND INTRODUCTION

Chemokines are small chemotactic peptides that control the trafficking of leukocytes, and in particular monocytes, to their target tissue. Chemokine receptors (CCRs, CXCRs, or XCRs) form a growing family of receptors (CCR1 up to CCR10, CXCR1 up to CXCR7, CX3CR1, XCR1, etc.) (Hughes and Nibbs, 2018). Most of the receptors have several ligands, and therefore show considerable cross-talk and redundancy, making pharmacological intervention challenging. The study drug, CNTX-6970, is a highly selective CCR2 and CCR5 antagonist (Centrexion Investigator's Brochure, 2019); it is not an antagonist of chemokine receptor subtypes CCR3, CCR7, or CXCR4. This property makes it appealing as a targeted treatment for persistent pain conditions characterized by inflammatory processes. The main ligand of CCR2 is interchangeably called chemokine ligand-2 (CCL2), or monocyte chemoattractant protein 1 (MCP-1) (Koelink et.al, 2012). The endogenous ligand for the CCR5 receptor or RANTES is regulated upon activation, normal T-cell expressed, and secreted (Koelink et.al, 2012). The pharmacological effect of CNTX-6970 is expected to come primarily from blockade of CCR2, although higher concentrations will also inhibit CCR5 as demonstrated in the Phase 1 single ascending dose (SAD) study in *ex vivo* assays.

Preclinical data suggest that CCR2 and MCP-1 are upregulated in cells responsible for the modulation of pain signals in peripheral nerves, dorsal root ganglia, dorsal horn of the spinal cord, and microglia (Bhangoo et al, 2007, White et al, 2009). Similar observations have been made for CCR5 in animal models of neuropathic pain (Matsushita et al, 2014). The MCP-1/CCR2 axis appears also to play a role in the signaling of pain at the level of the joint.

In mild and moderate osteoarthritis (OA), which is both quite common and highly disabling, the symptom of pain is the major contributing factor to functional impairment (Hunter et al, 2019). With an aging and increasingly obese population, this condition is becoming even more prevalent than in previous decades (Hunter et al, 2019). The expectation is that CCR2 antagonism will not only reduce pain, but also lead to improvement of function, and thus be relevant for treating signs and symptoms of OA. Therefore, pain and function will be the primary therapeutic targets in this development program. The leading symptom of OA is chronic pain with acute flares, particularly activity-related flares (Parry et al, 2017). CNTX-6970 has been shown to be effective in several animal models of OA, as demonstrated by increased tolerance of weight bearing in an experimentally injured knee or paw. These models may indicate analgesic effects and impact on function. On the other hand, no effect of CNTX-6970 on pain thresholds in healthy tissues, or on models of acute pain, have been observed, supporting the view that it will not interfere with protective pain reflexes and sensation of heat.

Based upon the underlying pathophysiology, two main types of pain can be distinguished: neuropathic pain and nociceptive/inflammatory pain. The most pronounced effects of CNTX-6970 pertain to nociceptive/inflammatory pain, which is mediated by pain receptors (nociceptors) that activate afferent somatic or visceral pain pathways via afferent nerves. Chronic somatic nociceptive/inflammatory pain conditions include OA and rheumatoid arthritis (RA). While the symptoms of inflammation seem to be the primary cause and are most pronounced in RA, they are clearly important as well in most patients with OA. As it is unclear whether CNTX-6970 will result in relevant systemic anti-inflammatory effects in RA patients, OA is most suitable for initial evaluation of symptomatic treatment with CNTX 6970.

The hallmark of OA is the damage and eventual loss of articular cartilage (Hunter et al, 2019) However, this damaged tissue is both aneural and avascular, making it an unlikely source of pain. Recent advances in

the understanding of OA have led to an appreciation that this disease involves all tissues of the synovial joint, not just the articular cartilage itself. Pain is likely generated in neurovascularized tissues within the joint, in particular the synovium and other soft tissues surrounding the joint (capsule, tendons, ligaments, muscles, and subchondral bone) (MacDonald et al, 2018). Although pain is considered the main source for functional impairment, it does not necessarily correlate well with degeneration and loss of function. At end stage degeneration, pain can be less pronounced, and functional impairment due to mechanical reasons is the leading symptom. Additionally, psychosocial comorbidities such as depression and anxiety commonly accompany chronic pain and can affect both the pain perception and tolerance thresholds. Additional biopsychosocial factors and personality traits may also contribute to perception of pain, which suggests that comprehensive phenotyping and careful selection of patients should be important components of osteoarthritis trials. This multifaceted pain matrix in OA (Read et al, 2008) may explain the limited effect sizes that common analgesics show, and open opportunities for individualized pharmacological approaches.

Given that the pathophysiology of OA is poorly understood, there are currently no curative or disease-modifying treatments approved for patients who are not yet at a stage of their condition at which total joint replacement is being considered. Overall, there are a few compounds and nutraceuticals for which there is questionable evidence of disease-modifying properties, highlighting the need for novel effective treatments in patients with this painful condition.

The MCP-1/CCR2 axis appears to play a role in the egress of macrophages from the bone marrow to the periphery, in addition to the recruitment of macrophages to the site of inflammation (Tsou, 2007). This role is supported by the clinical data from other CCR2 antagonists (Kalliomaki et al, 2013a; Kalliomaki et al, 2013b). In toxicological testing of CNTX-6970 in rats, decreases in peripheral monocytes were observed. It is therefore likely that CNTX-6970 will show a similar effect in humans in clinical testing. Nonclinical evidence points to a role of CCR2 in the defense against microbial pathogens (Serbina et al, 2008). However, the clinical relevance of a CCR2 antagonist mediated reduction in peripheral monocyte count may be limited in the absence of impaired monocyte function. Importantly, activity is dependent on a mechanism that does not require penetration into the central nervous system.

CCR2 antagonism has not been associated with any of the known safety issues of nonsteroidal anti-inflammatory drugs (NSAIDs). One of the appealing features of CNTX-6970 is that it does not significantly cross the blood brain barrier and therefore is not expected to have any of the central nervous system side effects associated with opioids and many other analgesics. Importantly the liability for addiction is very low.

Although the pharmacological effect of CNTX-6970 is expected to come primarily from blockade of CCR2, at higher concentrations, CNTX-6970 also exhibits CCR5 antagonism (Centrexion Investigator's Brochure, 2019), which may contribute to some degree to its putative analgesic effects.

A.1. Preclinical Experience

A comprehensive summary of the pharmacology, absorption, distribution, metabolism and excretion, toxicology, and nonclinical experience with CNTX-6970 to date is provided in the Investigator's Brochure (2019).

CNTX-6970 is a highly selective antagonist for CCR2 from humans, rats, and monkeys. CNTX 6970 is also an antagonist to human and monkey CCR5, but with a higher inhibitory constant (Ki). CNTX-6970 is

not an effective CCR5 antagonist in the rat. However, in the toxicology program, concentrations exceeded the rat Ki at the no observed adverse effect level (NOAEL). Therefore, risks associated with CCR5 binding were covered with the high doses in the rat toxicology program.

In rat models of pain varied with the model and was achieved in the dose range of 1 to 30 mg/kg. In models of inflammatory or neuropathic pain, the minimum effective doses were in the range of 1 to 10 mg/kg.

No relevant off-target pharmacological effects have been identified within the effective dose range. There was no evidence of a proarrhythmic risk of CNTX-6970 based on human Ether-à-go-go-related gene or papillary muscle studies. QTc prolongation was reported in the monkey at 250 mg/kg, but not at 50 mg/kg (maximum plasma concentration [Cmax] 5.67 µg/ml).

CNTX-6970 is a moderate clearance compound (17 to 34 ml/min·kg) with a high volume of distribution in rats and monkeys (4 to 6 L/kg). Oral bioavailability was high in female rats (95%) and monkeys (62%) and moderate in male rats (34%).

CNTX-6970 shows a moderate to low (~67 to 89%) binding to mouse, rat, cynomolgus monkey, and human plasma proteins.

CNTX-6970 was predominantly metabolized by CYP2C9 with a minor contribution of CYP3A4. Drug-drug interactions (DDIs) resulting from inhibition of transporters or cytochrome P450s (CYPs) are unlikely to occur in humans; the mean Cmax in humans after a single oral dose of 300 mg is at least 30-fold lower than the half maximal inhibitory concentrations for the transporters studied and the Ki for CYP2C9 inhibition (Ki = 29.9 µM).

In rats, CNTX-6970 was the dominant drug-related species in plasma, urine, feces, and bile. The major route of excretion for parent and metabolites was via bile and feces; urinary excretion only accounted for 9 to 18% of the administered dose.

In male and female rats, CNTX-6970 (20 mg/kg, 75 mg/kg, or 300 mg/kg) was given orally once daily (QD) for 4 weeks to assess toxicity and reversibility (4 weeks). At 300 mg/kg, gastric erosions and gastroesophageal reflux with lesions in the upper respiratory tract were considered related to the irritant potential of the test article. Foam cell aggregates (consistent with phospholipidosis) were identified in the gastrointestinal tract, liver, lungs, and mesenteric lymph nodes at 300 mg/kg. These changes were fully reversible with the exception of foam cell aggregates in the lungs of females. The NOAEL was 75 mg/kg where the mean Cmax was 3.97 and 6.61 µg/ml in males and females, respectively, and the mean area under the plasma concentration time curve (AUC) to the 24-hour time point (AUC0-24hr) was 15.8 and 32.6 µg·hr/ml in males and females, respectively, on Day 28.

In male and female monkeys, CNTX-6970 (10 mg/kg, 50 mg/kg, or 500/250 mg/kg) was given orally QD for 4 weeks to assess toxicity and reversibility (4 weeks). In the high-dose group (500 mg/kg), morbidity leading to euthanasia of 2 monkeys was caused by marked renal toxicity; following an off-dose period of 3 days, there was a dose reduction to 250 mg/kg on Day 15 in the remaining monkeys in this group. Generalized dermal exfoliation was apparent in this group from Week 3 onwards and resolved after 3 weeks without treatment. Reversible increased neutrophil counts were recorded in individual high-dose animals after 4 weeks as well as individual increases in urea nitrogen and creatinine, perturbations in electrolytes, decreases in albumin, and increases in hepatic transaminases. Histologically, minimal tubular casts were

observed in 1 female at 4 weeks and minimal tubular degeneration/regeneration was noted in 1 male after the recovery period. Foamy macrophages were present in various lymphoid tissues (spleen, thymus, lymph nodes, gut-associated lymphoid tissue, sternal bone marrow, and jejunum), but were not seen in any tissues from recovery animals. Reversible epidermal hyperplasia and hyperkeratosis correlated with dermal exfoliation. No toxicity was observed in the lower dose groups. The NOAEL of 50 mg/kg CNTX-6970 was associated with combined gender mean Cmax of 6.18 µg/ml and a mean AUC0-24hr of 20.5 µg·hr/ml on Day 28.

Genotoxicity, phototoxicity, and human epidermal irritation studies were all negative.

A. 2. Clinical Experience

CNTX-6970 has been evaluated in two Phase 1 studies in healthy volunteers.

A.2.1 First in Human Study: Single-Ascending Dose in Healthy Males

The first in human study was entitled: "*Safety, tolerability and pharmacokinetics of single rising oral doses of CNTX-6970 (BI 416970) in healthy male volunteers in a partially randomized, single-blind, placebo-controlled trial."*

The objective of this study was to investigate the safety, tolerability, and pharmacokinetics (PK), including dose proportionality, of a single ascending dose (SAD) of 10mg, 25mg, 50mg, 100mg, 200mg, 400mg, or 600mg of CNTX-6970 in 60 healthy male subjects.

Results: In total, 11 subjects (18.3%) reported adverse events (AEs) during the on-treatment period of the study. No dose-related trends were apparent for any AE and no major difference was observed when compared with placebo. No serious adverse events (SAEs) and no AEs leading to study discontinuation occurred.

No clinically relevant findings in laboratory evaluations, physical examinations, vital signs, or electrocardiogram (ECG) measurements were reported.

A.2.2. Single- and Multiple-Ascending Dose in Healthy Subjects

The second Phase 1 study (CNTX-HV-102) was entitled: "*A placebo-controlled, two-part study with single dose and multiple ascending oral dose to evaluate the safety, pharmacokinetics, and pharmacodynamics of CNTX-6970 in healthy subjects."*

This was a two-part study with a primary objective to evaluate the safety, PK, and PD of CNTX 6970 following repeated daily doses, and to determine if any age, sex, food, or formulation effects exist.

The first part of the study evaluated the effects of food on single doses of enteric coated 100-mg tablets of CNTX-6970. This part of the study also evaluated the effect of enteric or film coating on the absorption and PK of the 100-mg dose of the drug in healthy subjects.

The second part of the study evaluated the safety, PK, and PD of repeated doses of 100-mg, 300-mg, and 600-mg tablets administered once daily (QD), and 300-mg tablets administered twice daily (BID) for 10 days. The impact of age and sex on the PK of CNTX-6970 following repeated doses was also studied.

The PD measures in this study included the effects of repeated doses of CNTX-6970 on selected target systems (CCR-2 binding on CD14+ cells and CCR-5 binding on CD4+ cells) and the effects of repeated doses of CNTX-6970 on peripheral monocyte counts.

Results: No subjects experienced an SAE or AE that led to discontinuation from the study. There were no deaths in the study. There were no clinically meaningful changes from baseline in laboratory parameters, 12-lead ECGs, or physical examination findings observed in the study.

MCP-1/CCR-2 receptor binding inhibition by CNTX-6970 was observed to be concentration-dependent. Based upon the PD maximum effect, the greater extent of MCP-1/CCR-2 binding inhibition was observed as the dose increased in the once daily cohorts on both Day 1 and Day 10. Correspondingly, the PD area under the concentration-time curves (AUCs) increased as the dose increased.

The mean inhibition on Day 1 was 80% for Cohort 3 (100 mg, once daily), 89% for Cohort 4 (300mg, QD), 94% for Cohort 5 (600mg, QD), 90.6% for Cohort 6 (300mg, BID), and 91% for Cohort 7 (300mg, QD [elderly group]). The mean inhibition for the pooled placebo group was 8.9%.

As expected, the RANTES/CCR-5 receptor binding inhibition by CNTX-6970 was not as robust as the CCR-2 inhibition. The mean inhibition on Day 1 was 45.6% for Cohort 3 (100mg, QD), 79% for Cohort 4 (300mg, QD), 82% for Cohort 5 (600mg, QD), 89.6% for Cohort 6 (300mg, BID), and 72.7% for Cohort 7 (300mg, QD [elderly group]). The mean inhibition for the pooled placebo group was 3.4%.

Collectively, the results of these Phase I studies suggest that CTNX-6970 shows a favorable safety profile, produces minimal adverse events, and demonstrates substantial inhibition of the binding of monocyte chemoattractant protein-1 to its CCR-2 receptor in a concentration-dependent fashion across a range of daily doses.

STUDY RATIONALE

CNTX-6970, a novel potent antagonist of CCR2 with lesser effects on CCR5, is being developed as a new treatment for chronic pain, including painful osteoarthritis of the knee. Preclinical studies with CNTX-6970 demonstrate potent analgesia in multiple pain models including the CFA model of inflammatory pain, the MIA model of osteoarthritis pain, and the PNL model of nerve injury pain. In addition, the Phase 1 clinical studies have demonstrated excellent and dose-proportional target engagement and there have been no emergent safety issues observed in the SAD and MAD trials. CCR2 antagonists are under development for multiple indications including cancer, autoimmune disorders, atherosclerosis, stroke, and neurodegenerative diseases (Fantuzzi et al, 2019). The rationale for developing CNTX-6970 for the management of painful OA stems from mechanisms directly related to the affected joint as well as effects on neural signaling.

The dose selected for this Phase 2 trial is based on the findings from the Phase 1 studies detailed above. A dose of 300mg BID demonstrated good tolerability and safety, as well as over 90% inhibition of the binding of monocyte chemoattractant protein-1 to its CCR-2 receptor. Moreover, this dose produced nearly 90% binding inhibition at the CCR-5 receptor as well.

STUDY OBJECTIVES

The **first objective** of this study is to evaluate the safety and efficacy of CNTX-6970 for the treatment of pain related to OA of the knee. One dose of CNTX-6970 will be tested, which has been previously studied in healthy volunteers. No adverse effects were noted during the short-term (10 days) administration of this dose. The dose studied in this investigation is 300mg BID. This objective is addressed by Aims 1 and 2.

The **second objective** of this study is to evaluate the effects of CNTX-6970 on general pain-related measures, such as physical and psychosocial functioning, as well as on biomarkers of pain and inflammation. This will offer a fuller efficacy profile of the CNTX-6970 dose, characterizing its effects on a wide range of patients' experiences related to pain due to OA of the knee. This objective is addressed by Aims 3, 4, and 5.

The **third objective** is to obtain deep phenotyping of the target population both prior to study enrollment, during treatment with the experimental agent, and during treatment with placebo and to identify moderators and mediators (mechanisms) of treatment effects, including biomarkers for treatment response. The features that we will assess include inflammatory markers in synovial fluid, inflammatory markers in plasma, and daily assessments of patients' pain and functioning. This will allow us a) to study variability of characteristics associated with pain over time with active and placebo treatments as functions of CNTX-6970; b) to evaluate the effect of CNTX-6970 on different features individually and jointly; and c) to discover biomarkers for treatment response by exploring the effects of the identified measures on the efficacy of CNTX-6970 evaluated under the first two objectives. Combined with the first two objectives, this would move the field a step closer to personalized treatment and management of pain. This objective will be addressed by Aims 6 to 9.

C.1. Primary Objective

The primary objective of this study is to evaluate the safety and efficacy of 300mg BID CNTX-6970 for the treatment of pain related to OA of the knee compared to placebo. This objective will be accomplished through the following specific aims:

Aim 1: Assess the safety and tolerability of CNTX-6970 (300mg BID), and placebo.

There will be no hypothesis testing under Aim 1. To achieve Aim 1, we will tabulate all AEs and SAEs and will classify them by severity and relatedness to treatment. We will estimate the frequency of AEs and SAEs in the active and placebo groups with point estimates and 95% CIs. Similarly, we will summarize relevant laboratory measures with appropriate estimates.

Aim 2: Assess the efficacy of 300mg BID CNTX-6970 in comparison to placebo. The primary outcome measure used to assess efficacy will be patient-reported knee pain using the WOMAC Part A (Bellamy, et al., 1988).

HYPOTHESIS 1. CNTX-6970 300mg BID will be more effective than placebo with respect to pain as measured by WOMAC-A (primary outcome measure).

C.2. Secondary Objective

The second objective of this study is to evaluate the effect of CNTX-6970 (300mg BID) on general pain-related measures, including physical and psychosocial functioning, as well as biomarkers of pain and inflammation. This objective is addressed by the following specific aims:

Aim 3: Assess the efficacy of CNTX-6970 (300mg BID) in comparison to placebo with respect to secondary outcome measures related to OA on the knee: (a) WOMAC-C (function subscale) (Bellamy, et al., 1988); (b) Hospital Anxiety and Depression Scale (HADS) (Zigmond et al., 1983); (c) Patient Global Impression of Change (PGIC) (Kroenke et al., 2019); (d) PROMIS Sleep Disturbance Scale – 6A (Yu et al., 2011); (e) Sleep Duration Question; and (f) Daily Knee Pain Intensity on a 0-10 Numeric Rating Scale (NRS).

HYPOTHESIS 2: CNTX-6970 300mg BID will be superior to placebo with respect to symptoms measured by (a) WOMAC-C (function subscale); (b) Hospital Anxiety and Depression Scale (HADS); (c) Patient Global Impression of Change (PGIC); (d) PROMIS Sleep Disturbance Scale– 6A (Yu et al., 2011); (e) Sleep Duration Question; and (f) Daily Knee Pain Intensity on a 0-10 NRS.

Aim 4: Assess the efficacy of CNTX-6970 (300mg BID) in comparison to placebo with respect to general outcomes of pain: (a) Pain Catastrophizing Scale –Short Form 6 (Sullivan et al., 1995); (b) PROMIS Physical Functioning Short-Form 6b (Schalet et al., 2016); (c) Patient Health Questionnaire – 2 item scale (PHQ-2) – Depression (Arroll et al., 2010); (d) Generalized Anxiety Disorder – 2 item scale (GAD-2) (Kroenke et al., 2007; Plummer et al., 2016); (e) Tobacco, Alcohol, Prescription medication, and other Substance use Tool (TAPS-1) (Gryczynski, et al., 2017); and (f) Opioid Use Questionnaire (OUQ).

HYPOTHESIS 3: CNTX-6970 300mg BID will be superior to placebo with respect to outcomes measured by (a) Pain Catastrophizing Scale –Short Form 6; (b) PROMIS Physical Functioning Short-Form 6b; (c) Patient Health Questionnaire (PHQ) – Depression; (d) Generalized Anxiety Disorder – 2 item scale (GAD-2) and (e) Tobacco, Alcohol, Prescription medication, and other Substance use Tool (TAPS-1); and (f) Opioid Use Questionnaire.

Aim 5: Assess the effect of CNTX-6970 (300mg BID) in comparison to placebo with respect to biomarkers of pain and inflammation: (a) Staircase-Evoked Pain Assessment; (b) serum and synovial fluid levels of chemokines and cytokines; and (c) synovial monocyte chemoattractant protein-1/CCR-2 receptor binding inhibition in blood and synovial fluid.

HYPOTHESIS 4: Compared to placebo, CNTX-6970 300mg BID will result in greater improvement with respect to (a) Staircase-Evoked Pain Assessment; (b) serum and synovial fluid levels of chemokines and cytokines; and (c) synovial monocyte chemoattractant protein-1/CCR-2 receptor binding inhibition in blood and synovial fluid.

C.3. Tertiary Objective

The third objective of this study is to obtain deep phenotyping of the target population both prior to study enrollment and during treatment, and to identify biomarkers for treatment response. This objective will be accomplished through the following specific aims:

Aim 6. If 300mg BID CNTX-6970 is more effective than placebo, evaluate the following characteristics of its effect: (a) onset of action; (b) carryover effect after treatment discontinuation.

Aim 7. Identify biomarkers related to pain from OA of the knee and to response to treatment with CNTX-6970 at 300mg BID.

Aim 8: Explore sociodemographic and clinical predictors of response to CNTX-6970.

C.4. Note on Previous Protocol Versions

The design for previous protocol versions included two arms, 100 mg BID CNTX-6970 and Celecoxib (as an active comparator), that have been discontinued in this version of the protocol (n=22). The total number randomized overall will therefore be n=72 (up to 77) to include the discontinued arms. These two arms were removed due to study drug supply issues and to aid recruitment feasibility. The previous protocol stipulated that the analysis of data from these arms were to be analyzed separately and thus removal of these two arms does not impact the current study objectives (except one exploratory aim in the previous protocol versions did propose examining a possible dose response between the 100 mg and 300 mg CNTX-6970 doses).

The participants in these two arms will complete the trial and will be monitored for safety events. A safety analysis for individuals in these two arms will be performed the same as for the 300 mg CNTX-6970 arm. For the efficacy objectives, exploratory analyses will be performed on the data from these two arms by computing summary statistics (e.g., means and standard deviations) comparing 100 mg CNTX-6970 to placebo (and comparing Celecoxib to placebo) for the different periods of the crossover study for the outcome measures. Formal modeling of the data from these two arms will either not be possible or will provide only limited information due to the small sample size available in these two arms. In the event that data becomes available from the biorepository samples from these two arms, that data will also be analyzed in an exploratory fashion.

STUDY DESIGN

D.1. Overview

The study will employ a randomized, allocation-concealed, multicenter, placebo-controlled, multi-period crossover design (Schmid et al, 2018). This multi-period crossover randomized, controlled trial allows comparability and assessment of efficacy through repeated exposures within each subject to the active treatment and a control (placebo) in randomized sequence. Such multi-period crossover designs are ideal for treatments with rapid onset of action and short half-life such as the asset under study here. We have strived to minimize the complexity of this powerful design by using only 2 blocks with 2 periods each. The modest additional complexity of the proposed multi-period crossover design, compared to a parallel-groups design, is justified by the marked improvement in efficiency. The gains in efficiency afforded by the multi-period crossover design allow a substantial reduction in sample size without sacrificing statistical power. For example, our simulation experiments (with sample sizes ranging from 30 to 50, carryover effects ranging from 0 to 0.2, and an effect size of 0.4) indicated that the parallel design yields statistical power ranging from 0.20-0.25, whereas our proposed 2-block multi-period crossover design yields power ranging from 0.9-1.0.

The trial will compare an active treatment vs. placebo. The study will employ a multi-period crossover design with two blocks. Each block will consist of two treatment periods with each period lasting 6 weeks. Treatment assignments (active drug versus placebo) will be randomized for each patient to the two periods within each block. The period length of 6 weeks was chosen based on several considerations: (i) Most efficacious analgesic drugs demonstrate separation from placebo by 6 weeks; (ii) The decision to move CNTX-6970 forward to Phase 3 will require a clinically meaningful separation from placebo by 6 weeks; and (iii) In this Phase 2 study, implementing a treatment block longer than 6 weeks would make the overall design more challenging and burdensome by extending the duration of overall testing beyond 6 months.

In this study, the placebo will consist of inactive tablets identical to the active treatment tablets. Treatment assignments (active drug versus placebo) will be randomized for each patient to the two treatment periods within each block. (see Section F.1.6 for details about the randomization process; the unblinded randomization schema is available to unblinded study team members in Appendix I.

This study is part of Early-Phase Pain Investigation Clinical Network (EPPIC-Net), under The Helping to End Addiction Long-term^{SM} Initiative, or NIH HEAL Initiative^{SM}.

D. 2. Study Endpoints

D.2.1. Primary Safety Endpoint

The primary safety endpoint is the incidence of treatment emergent adverse events (TEAEs), reported between the administration of study drug on Day 1 and the completion of the study at Week 24 or Early Termination.

D.2.2. Primary Efficacy Endpoint

The primary efficacy endpoint is pain in the index knee, measured weekly using the WOMAC-A (Pain subscale), (Bellamy et al, 1988). We will use the numerical rating scale version of the WOMAC-A, with the subject assessing each of 5 questions using an 11-point (0 to 10) scale; the total score is the sum of the individual item scores (range 0-50). A higher WOMAC-A score represents worse symptom severity.

All WOMAC subscales show strong evidence of validity and reliability (Salafi et al, 2003), and are the most frequently used primary endpoints for OA trials because of their strong assay sensitivity (Jung et al, 2018). Assessments during the last 2 weeks of each treatment period will be used to test the primary hypotheses in order to avoid possible carryover effects of unknown duration. All weekly measurements of WOMAC-A will be used to study the course of OA pain symptoms during treatment, including onset of action and carryover effects.

D.2.3. Secondary Endpoints

The following measures are considered secondary outcomes related to pain due to OA of the knee. They will be assessed at Baseline and at study visits through Week 24. See Section F.1.1 Schedule of Events for full description of procedure timepoints. The Daily Knee Pain Intensity on a 0-10 NRS which will be recorded daily the week prior to each study visit. For a full description of secondary endpoint scales and assessments, see Section F.2.4 and F.2.5.

- Daily Knee Pain Intensity on a 0-10 Numeric Rating Scale (NRS).
- WOMAC-C (Function subscale)
- Hospital Anxiety and Depression Scale (HADS)
- PROMIS Sleep Disturbance Scale – 6A ***NIH HEAL Initiative CDE*
- Patient Global Impression of Change (PGIC) ***NIH HEAL Initiative CDE*

The following measures are part of the NIH HEAL Initiative CDEs and are considered general pain-related outcomes. They will be assessed at Baseline and at Week 24/ET. For a full description of the CDE scales and assessments, see Section F.2.5.

- Pain Catastrophizing Scale – Short Form 6

- PROMIS Physical Functioning Short-Form 6b
- Sleep Duration Question.
- Pain Health Questionnaire (PHQ) – Depression
- Generalized Anxiety Disorder – 2 item scale (GAD-2)
- Tobacco, Alcohol, Prescription medication, and other Substance use Tool (TAPS-1)
- Opioid Use Questionnaire (OUQ)

The following are physiological measures related to pain. They will be considered both as secondary outcomes and as potential biomarkers for pain and treatment response. For a full description of physiological pain scales and assessments, see Section F.2.

- Staircase-Evoked Pain Assessment
- Serum levels of cytokines and chemokines.
- Monocyte chemoattractant serum protein-1(MCP-1)/CCR-2 receptor binding inhibition by CNTX-6970
- Synovial fluid levels of cytokines and chemokines. These will be assessed at the end of the first treatment period (week 6). A specific list is provided in Appendix II.
- Monocyte chemoattractant protein-1 (MCP-1)/CCR-2 receptor binding inhibition by CNTX-6970 in synovial fluid. These will be assessed at the end of the first treatment period (week 6).

STUDY POPULATION

This study is designed to evaluate changes in pain related to primary OA of the knee. Eligible subjects will have evidence of chronic knee OA with a history of moderate to severe pain in the designated index knee for a minimum of 6-months prior to Screening. Although this condition commonly affects multiple joints and subjects may have bilateral OA of the knee(s), the efficacy assessments will be conducted only on the index knee.

E.1. Recruitment Feasibility

Patients will be recruited through advertisement, as well as clinician referral and self-referral. Self-referred patients will call the study sites and will be screened over the phone by the study coordinators. Those deemed potentially eligible will be scheduled for a screening visit with one of the study physicians at the sites. Based on clinical and human subject protection considerations, a potential subject will, under no circumstance, be advised to taper his/her current medication regimen prior to the screening visit.

Subjects will also be recruited through physician referral. Each site will recruit subjects via referral from primary care and specialty clinics from the communities surrounding each study site. Each site will utilize the experience of the MGH CCC to conduct outreach activities to community organizations, colleges, and other resources. Sites will regularly collaborate on strategies to recruit women and minorities, and sites will employ strategies specific to their local community, in ways that are culturally sensitive and specific to the local minority population. Retention will be enhanced with fair reimbursement (as deemed appropriate by the local IRB) for subjects' time. Site specific recruitment details are located in each site's recruitment plan.

E.2. Number of Subjects

We expect to screen approximately 300 patients to randomize up to 55 patients.

When the randomization target has been met, patients who have been screened but not yet randomized will be allowed to continue in the study unless they fail to meet randomization criteria.

E.3. Nature of Populations

Subjects must meet all of the following inclusion and none of the exclusion criteria (Section G.4.) to be eligible for participation in the study.

Protocol waivers for eligibility will not be granted under any circumstances. If, during the course of a subject's post-randomization participation in the trial, it is discovered that the subject did not meet all eligibility criteria, the justification to allow the subject to continue in the trial will be made by the PPI, with medical input from the Medical Monitor and the site's investigator(s), and will be documented in the patient's record. Whether or not the participant is allowed to remain in the trial, this will be reported as a major protocol deviation and not a waiver.

E.4. Subject Eligibility

E.4.1. Inclusion Criteria

A subject will be eligible for study participation if they meet all of the following criteria:

1. Individuals between 40 and 90 years of age (inclusive) at the time of the Screening Visit.
2. Willing to use a mobile smart device during the study period. Individuals who do not have access to a mobile device will be provided with one for the duration of the study and trained in its use.
3. Can understand the nature of the study and protocol requirements and is willing to comply with study drug administration requirements and discontinue prohibited concomitant medications before Baseline.
4. Consents to participate in the study.
    a. Provides written informed consent using the current form approved by the institutional review board (IRB).
5. Is capable of communicating with the site personnel, able to complete subject-reported outcome measures and can be reliably rated on assessment scales (in the opinion of the investigator).
6. Radiography of both knees with a posterior-anterior, fixed-flexion view taken during the Screening visit. The Index knee must show evidence of chronic OA with a K-L Grading Scale of 1, 2, 3, or 4. Such evidence will be provided by a central reading of the radiography of both knees from an expert radiologist of the CCC of EPPIC-Net.
7. Moderate to severe pain in the Index knee associated with OA and stable for a minimum of 6 months prior to Screening in the opinion of the investigator.
8. Confirmation of OA of the index knee: American College of Rheumatology (ACR) diagnostic criteria.

9. Subjects must have failed 2 or more prior therapies for knee OA pain. Failure is deemed to be inadequate relief in the opinion of the investigator. A knee OA pain therapy may be deemed to have been inadequate because of one or more of the following:
    a. Unacceptable adverse events (AEs)
    b. Inadequate response, or loss of response for chronic OA knee pain or
        - pain in the index knee was minimally improved, not improved, or was worse and/or
        - function, and/or stiffness in the index knee was minimally improved, not improved, or was worse
    c. Medical condition resulting in contraindication to the standard of care appropriate to the severity of the index knee OA pain. "Therapies" include, but are not limited to, each of the following:
        - nonsteroidal anti-inflammatory drugs (NSAIDs) (including topical), opioids, duloxetine, other systemic therapy, intraarticular (IA) corticosteroids, IA viscosupplements
        - physical therapy or bracing.

10. A mid-to-high level summed pain score (0-50) for the Index knee on the WOMAC-A pain subscale, which will be completed by the subject every 3 days (+/-1 day window) during the Screening Period. Subject must complete at least 4 WOMAC-A pain subscales.

    Note: Criteria for pain intensity requirements are specified in Appendix I: Blinded Information. This is only available to unblinded study team members and will be assessed centrally. Additionally, eligibility per study pain intensity requirements will be confirmed by the CCC before a State, Assessibility, Face and Ecological Validity and the Rule of the 3 Ps [persistent, pervasive, pathological] (SAFER) interview is scheduled.

11. Body mass index (BMI) of ≤ 40 kg/m^2.

12. Females are at least one of the following:
    a. Not of childbearing potential - defined as post-menopausal for at least 1 year, or surgically sterile (bilateral tubal ligation, bilateral oophorectomy, or hysterectomy); or
    b. Totally abstinent from heterosexual intercourse since the last menses before study drug administration; or
    c. Agree to concurrent use of at least two approved methods of birth control throughout their study participation. Approved methods of contraception are those that, alone or in combination, result in a low failure rate (i.e., less than 1% per year) when used consistently and correctly, listed below:
        1. Hormonal methods such as oral, implantable, injectable, or transdermal contraceptives for a minimum of 1 full cycle (based on the subject's usual menstrual cycle period) before study drug administration.
        2. Intrauterine device (IUD)
        3. Barrier method (Condoms, sponge, cap, diaphragm with or without spermicidal jellies or cream)
        4. Withdrawal (coitus-interruptus)
        5. Periodic abstinence (calendar, symptothermal, post-ovulation methods)
        6. Lactation amenorrhea method (LAM)
        7. Vasectomized partner (at least 3 months post-vasectomy)

13. Males are at least one of the following:
    a. Incapable of fathering a child – defined as surgical sterility (at least 3 months post-vasectomy); or
    b. Totally abstinent from heterosexual intercourse beginning at study screening; or
    c. Agree to use of at least one of the following medically acceptable methods of birth control throughout their study participation:
        1. Barrier method (condoms, sponge, diaphragm).
        2. Male condoms with or without a spermicide coating are acceptable, as some women are allergic to spermicide.

14. Willing and able to understand the study requirements, abide by the study restrictions, complete the study procedures, independently record responses on the pain scales and make daily/weekly entries using the NEForm (section K1), and independently communicate meaningfully with study personnel.

15. Willing to refrain from illicit drug use during the study, and to have illicit drug testing at screening and at later time points, if illicit drug use is suspected during the study. Illicit drug use is defined as the use of any medication or substance that is prohibited by law and is outside the prescribed use of a medical professional (or equivalent), including medications that are prescribed but are not being used as prescribed.

16. Complete the SAFER (State, Assessibility, Face and Ecological Validity and the Rule of the 3 Ps [persistent, pervasive, pathological]) interview, administered via teleconference by an MGH psychiatrist or psychologist, and receive a passing score which confirms pain history with knee OA medication history and a HADS score of <11 on both the depression and anxiety subscales.

17. The End of Day Pain Ratings are completed on at least 5 days of the Screening Period.

18. Patient-reported daily NRS (for average pain over the last 24 hours) meets the criteria specified in "Appendix I: Blinded Information" during the screening period. The algorithm is only available to unblinded study team members and will be assessed centrally. Additionally, screening NRS scores will be confirmed by the CCC before a SAFER interview is scheduled.

E.4.2. Exclusion Criteria

A subject will be excluded from the study if they meet any of the following criteria:

1. Any form of joint replacement surgery (partial or total) of the index knee, or joint replacement surgery of the non-index knee within 12 months of Screening.

2. Open surgery or arthroscopic surgery of the index knee/knee joint within 12 months of Screening.

3. Any painful condition(s) of the index knee due to disease other than OA. For example, periarticular or referred pain involving the index knee, or from joint disease other than OA associated with the index knee.

4. Other chronic pain anywhere in the lower extremities (e.g. hips, legs, feet) that is equal or greater in intensity or impairment than index knee pain. This includes radicular low back pain with radiation to the knee.

5. Secondary OA of the index knee due to acute or recurrent traumatic injury.
6. Significant instability (e.g., cruciate ligament tear or rupture or previous repair) within the past 5 years or current misalignment (>10 degrees varus or valgus) of the index knee.
7. Documented history of neuropathic arthropathy in the knee.
8. Imaging finding of bony fragmentation in the index knee (radiographic, computed tomography, or magnetic resonance imaging).
9. Physical/occupational/chiropractic therapy for the lower extremities or acupuncture for the lower extremities within 30 days of Screening or need for such therapy during the study.
10. Plans to have surgery, invasive procedures, or intra-articular (IA) injections of the index knee or procedure or surgery otherwise contraindicated for study participation while in the study.
11. Use of pain medication (including non-steroidal anti-inflammatory drugs [NSAIDs]) less than 5 days before Baseline or any time throughout the study, with the following exceptions (see Appendix III for additional information and MOP for maximum dosing and frequency):
    a. Concomitant Medications for Pain –
        i. Continuous use of one of the following medications prescribed for pain: tramadol, gabapentin, duloxetine, pregabalin, milnacipran, or tricyclic antidepressants that is:
            a. Chronic for at least 12 weeks; and
            b. At a stable dose for at least 4 weeks before Screening
        ii. Intermittent use of opioids that is:
            a. Ongoing for at least 4 weeks before Screening;
            b. At a frequency no more than 4 days/week; and
            c. Not be taken within 24 hours of a study visit
        iii. As needed use of acetaminophen
        iv. Continuous use of medical marijuana (or equivalent) that is chronic for at least 12 weeks and at a stable dose for 4 weeks
        v. Topical creams (includes CBD topicals)
            a. Continuous use allowed if chronic and stable for at least 12 weeks
            b. Intermittent use allowed if at a frequency of no more than 4 days/week
    b. Concomitant Medications for Non-Pain Indications That May Impact Pain –
        i. Continuous use of medication for non-pain indications that are known to potentially impact pain, e.g. duloxetine for depression, that is at a stable dose for at least 12 weeks prior to Screening.
        ii. Low-dose aspirin for the purposes of heart disease prophylaxis
12. Corticosteroid injection in the index knee within 30 days of Screening (unless the injectable is a long-acting agent such as triamcinolone acetonide extended-release injectable suspension (Zilretta) in which case the injection cannot be within 90 days of screening)
13. Received IA viscosupplementation (e.g., Synvisc®, Hyalgan®) within 90 days of Screening

14. Knee effusion requiring aspiration of the index or contralateral knee at time of screening.
15. Radiofrequency ablation (RFA) of the knee within 6 months prior to Screening.
16. Presence of any medical condition or unstable health status (including chronic or active infections) that, in the judgment of the investigator, might adversely affect the safety of the subject or the conduct of the study, or negatively affect the resulting data, including chronic conditions that are likely to alter the rate of healing or are likely to result in safety complications unrelated to the study medication, or significant compromise to key organ systems.
17. Has a current malignancy or has received treatment for malignancy at any time within 5 years prior to Screening.
    a. History of resected basal cell carcinoma and squamous cell carcinoma of the skin is not exclusionary, so long as treatment is not currently ongoing.
18. Ulcer or open wound anywhere in the region of the index knee.
19. Clinically significant abnormal laboratory results at the Screening Visit (in the opinion of the investigator), or significant organ disease that would put the subject at undue risk or affect the ability of the subject to participate in the trial.
20. Use of an investigational treatment, including investigational device(s), within 30 days of Screening, or 5 pharmacokinetic or pharmacodynamic half-lives (whichever is longer) or scheduled to receive such an agent while participating in the current study.
21. Has any of the following characteristics:
    a. Active or historic substance use disorder within the previous year as defined by the Diagnostic and Statistical Manual for Mental Health Disorders, fifth edition, or
    b. Urine drug screen is positive for an illicit substance and subject is unable to provide a valid prescription from a medical professional.
22. Has persistent moderate to severe depression or anxiety, as indicated by a score ≥ 11 on either subscale of the Hospital Anxiety or Depression Scale (HADS) at Screening.
23. Female has a positive pregnancy test at the Screening or Baseline Visit, is planning to become pregnant, or is breastfeeding.
24. Has ongoing litigation for workers' compensation.
25. Known diagnosis of long QT syndrome.
26. Male has a QRS interval ≥120 ms *and* QTcF ≥460 ms OR female has a QRS interval ≥120 ms *and* QTcF ≥480 ms at Screening.
27. Has moderate to severe congestive heart failure (New York Heart Association [NYHA] class III and class IV).
28. Has a history of symptomatic cardiovascular disease.
29. Current therapy with any immunosuppressive therapy, including corticosteroids (>5 mg/day of prednisone).

30. Moderate to severe hepatic impairment at the Screening Visit, as defined by any of the following:
    a. AST **and** ALT values that are ≥1.5 times ULN
31. Moderate to severe renal impairment at the Screening Visit, as defined by either:
    a. An estimated glomerular filtration rate [eGFR] < 45 mL/min; or
    b. A serum creatinine level > 1.3 mg/dL.
32. Use of CYP3A4 inhibitors (e.g., grapefruit juice, pantoprazole) or CYP3A4 inducers (e.g., omeprazole, carbamazepine, rifampin, St. John's wort) within 7 days prior to the Baseline Visit. Please see Appendix III. Concomitant Medications.
33. Use of CYP2C9 inhibitors (e.g., amiodarone, fluconazole) or CYP2C9 inducers (e.g. carbamazepine, rifampin, St. John's wort) within 7 days prior to the Baseline Visit. Please see Appendix III. Concomitant Medications.
34. Use of P-glycoprotein inhibitors (e.g., atorvastatin, azithromycin, carvedilol) within 7 days prior to the Baseline Visit or any time during study participation, unless taken for at least 3 months prior and at a stable dose for at least 1 month prior to the Baseline Visit. Please see Appendix III. Concomitant Medications.
35. Is classified as a poor metabolizer via the CYP2C9 pathway as determined by genetic testing (CYP2C9 genotyping).
36. Has clinically significant pulmonary disease such that chronic, daily use of medical treatment is required (e.g. COPD, asthma).
37. Has any of the following blood counts:
    a. Monocyte count < 1 % of white blood cells (e.g., for WBC = 4,500/μL, exclude patient if monocyte count < 0.01 * 4,500 or 45/μL; for WBC = 11,000/μL, exclude patient if monocyte count < 0.01 * 11,000 or 110/μL)
    b. Platelet count, or
        i. Females (sex at birth): < 125,000/μL
        ii. Males (sex at birth): < 150,000/μL
    c. Hemoglobin:
        i. Females (sex at birth): < 11.5 grams/dL
        ii. Males (sex at birth): < 13.0 grams/dL

E.5. Rationale for the Inclusion/Exclusion Criteria

The main rationale for inclusion and exclusion criteria is to enroll Subjects who are generally in good health, with chronic pain related to stable clinical course of osteoarthritis of the index knee, and who will be able to participate in this study for the duration of 24 weeks. Subjects that show with renal or hepatic impairment or are poor CYP2C9 metabolizers are excluded for safety reasons. Subjects in this study must be able and willing to provide multiple assessments about their pain and function at home via smartphone using the New England Survey Systems (NESS) app and via tablet at the time of in-person clinic visits. Individuals without a smartphone can be lent one from NESS.

STUDY ASSESSMENTS – PLAN AND METHODS

F.1. Study Conduct

***Table 1**: Schedule of Events*

Study Phase	**Screening Period (14-28 days before Baseline)**		**Baseline (Day 0)**	**Clinic Visits scheduled every 3 weeks (+/- 4 days)**															
Study Week	Screen Visit	Screen Period	Wk 0	Wk 0-3	Wk 3	Wk 3-6	Wk 6	Wk 6-9	Wk 9	Wk 9-12	Wk 12	Wk 12-15	Wk 15	Wk 15-18	Wk 18	Wk 18-21	Wk 21	Wk 21-24	Wk 24 Final Visit/ET[1]
Study Visit	**Screening Period**		**V1**		**V2**		**V3**		**V4**		**V5**		**V6**		**V7**		**V8**		**V9**
Clinic (C) Visit or Mobile (M) Visit	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C	M	C
Informed Consent	X																		
Review Eligibility Criteria	X	X	X																
Randomization			X																
IXRS Drug Dispensing			X		X		X		X		X		X		X		X		
ASSESSMENTS																			
Radiograph Imaging – Chest[2]	X																		X
Radiograph Imaging – Both Knees[3]	X																		
K-L Grade Confirmation by Central Radiologist[3]	X																		
12 – lead ECG	X		X		X		X		X		X		X		X		X		X
CYP2C9 Genotyping	X																		

Study Visit	Screening		V1		V2		V3		V4		V5		V6		V7		V8		V9
Physical Examination	X																		X
Vital Signs	X		X				X				X				X				X
Height and Weight BMI	X																		X
Contraceptive Use	X																		
Adverse Events	X		X		X		X		X		X		X		X		X		X
Medical History, OA History	X																		
Demographics	X																		
Analgesic Rescue Medication Use[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications and Therapies[5]	X		X		X		X		X		X		X		X		X		X
SAFER Interview[6]		X																	
Drug Accountability and Compliance			X		X		X		X		X		X		X		X		X
PROTOCOL PATIENT MEASURES – NEForm (NESS)																			
Distribute Smart Device (if applicable)	X																		
NEForm App Subject Training - Collection Procedures & Compliance (Retrain as Needed)[7]	X	X	X		X		X		X		X		X		X		X		X
Placebo Response Mitigation Training	X										X								
WOMAC – A (Pain Scale)[8]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hospital Anxiety and Depression Scale (HADS)[9]	X						X				X				X				X
Numeric Rating Scale (NRS) - Remote[10]		X		X		X		X		X		X		X		X		X	
WOMAC – C (Functional Scale)			X		X		X		X		X		X		X		X		X

Study Visit	Screening		V1		V2		V3		V4		V5		V6		V7		V8		V9
Staircase-Evoked Pain Assessment			X				X				X				X				X
Collect Smart Device (if applicable)																			X
NIH HEAL Initiative COMMON DATA ELEMENTS (CDEs)[11]																			
Pain Catastrophizing Scale – Short Form 6			X																X
PEG Scale			X																X
PROMIS Sleep Disturbance Scale – 6A			X				X				X				X				X
Sleep Duration Question			X																X
PROMIS Physical Functioning Short-Form 6b			X																X
Patient Health Questionnaire (PHQ) – Depression			X																X
Generalized Anxiety Disorder- 2 item scale (GAD-2)			X																X
Patient Global Impression of Change (PGIC)					X														X
Tobacco, Alcohol, Prescription medication, and other Substance use Tool (TAPS-1)			X																X
Opioid Use Questionnaire (OUQ)			X																X
LABORATORY COLLECTION (BLOOD & URINE)																			
Clinical Blood Lab Testing-Hematology[12]	X						X				X				X				X
Clinical Blood Lab Testing - Chemistry[12]	X		X		X		X		X		X		X		X		X		X

Study Visit	Screening		V1		V2		V3		V4		V5		V6		V7		V8		V9
Urine Drug Screen[13]	X																		X
Urine Pregnancy Test[14]	X		X								X								X
Urine Dipstick/ Urinalysis[15]			X		X		X		X		X		X		X		X		X
Blood for Biomarkers – Cytokines & Chemokines			X				X				X				X				X
Blood Sampling - PK Analysis							X				X				X				X
BIOBANKING SAMPLES For Future Testing [OPTIONAL] (Subject must have consented to Biobanking)[16]																			
Collect Whole Blood for Biobanking[17]			X																X
Leftover Plasma & Serum Sent to Biobank - Exploratory Biomarkers[18]			X				X				X				X				X
Collect Stool Sample for Biobanking[19]			X																
SYNOVIAL FLUID [OPTIONAL] (Subject must have consented to Synovial Fluid Collection)[20]																			
Synovial Fluid Collection (Cytokines & Chemokines) [21]							X												
Leftover Synovial Fluid Sent to Biobank - Exploratory Biomarkers[21]							X												

Notes:

1. If a subject is discontinuing the study on or before Week 24, the assessments listed for an Early Termination visit should be completed.
2. Chest radiography should be posterior-anterior view
3. Radiographs of both knees (posterior-anterior, fixed-flexion views) will be obtained to confirm the diagnosis of OA of the knees, as part of assessing the Kellgren-Lawrence (K-L) grade of the identified index knee during the Screening Period. Quality of radiographs will be assessed, and radiographs may need to be repeated at the direction of the central imaging reader. Only central reading of the K-L grade will be used to determine index knee eligibility, K-L grade of 1, 2, 3, or 4.
4. Rescue medication use will be recorded daily by the subject using the NEForm (section K1) (number of tablets/dose taken each day) and provided to the study staff during each clinic visit. Allowable rescue medications are outlined in Exclusion Criteria and Appendix III.

5. Concomitant medication use will be reviewed and captured at study visits. Subjects will be informed at the Screening visit that they will have to stop taking any analgesic medications other than those allowed, per Appendix III, for the duration of the trial.

6. An independent reviewer will conduct a remote SAFER interview to assess a patient's history of pain with OA, treatment history, and HADS score.

7. Subjects should try and complete at least 80% of remote procedures in the NEForm app

8. The WOMAC-A scale will be used to determine subject eligibility for enrollment. During the Screening period, the WOMAC-A will be completed every 3 days during the Screening period. After screening, the WOMAC-A will be completed weekly (± 2 days), with every third assessment being conducted at the in-person clinic visit.

9. A score ≥ 11 on either subscale of the HADS will exclude a participant from enrolling in the study. After randomization, investigators should monitor any HADS subscale scores ≥ 11; they should use clinical judgement to determine whether the participant should continue in the trial and whether intervention or treatment is needed. HADS may be repeated per PI discretion and MM approval.

10. During the screening period, the Knee Pain Intensity NRS (0-10) will be collected once per day following the Screening Visit. After Baseline, the NRS will be completed daily for the seven days immediately preceding all study visits (e.g. Week 3, Week 6). Subjects should be instructed to record their average daily knee pain intensity each evening using the NEForm app.

11. NIH HEAL CDEs can be collected from participant remotely to reduce in-person clinic time. This is optional.

12. Clinical laboratory tests will include:
    a. **Chemistry:** sodium, potassium, chloride, calcium, phosphate, magnesium, bilirubin (total and direct), AST, ALT, GGT, ALK phosphatase, creatinine, eGFR, uric acid, BUN/Urea, glucose, amylase, lipase, total protein, and albumin
        i. eGFR value will be calculated automatically in the EDC system, once lab results have been entered.
    b. **Hematology**: White cell count with differential (absolute values of neutrophil, lymphocyte, monocyte, eosinophil, and basophil), red cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH Concentration (MCHC) and platelets count

    If a laboratory result comes back as abnormal and/or exclusionary, the laboratory test can be repeated per PI discretion and Medical Monitor approval before the participant is screen failed or discontinued from study participation.

13. Urine drug screen test results must be confirmed as negative for drugs of abuse prior to randomization. Use the drug screen kits provided by the study, testing for amphetamines, barbiturates, buprenorphine, benzodiazepines, cocaine, ecstasy, methamphetamine, methadone, marijuana, opiates, oxycodone, phencyclidine.

    If the subject tests positive for a substance but is able to provide a valid prescription from a medical professional, the Medical Monitor may give permission for the subject to enroll in the study. For this study medical marijuana (or equivalent) use is allowed depending on regional legality.

14. Subjects will be tested for pregnancy if they are women of childbearing potential. A serum pregnancy test will be performed if the urine pregnancy test is positive. Subjects with positive serum pregnancy test results will be discontinued from study participation.

15. At Baseline, all subjects will have a full urinalysis panel completed. After baseline, a urine dipstick analysis will initially be done to assess urine health. Microscopic examination (urinalysis) will **only** be performed if the dipstick test is abnormal for leukocyte, blood, nitrite or protein. If results come back as abnormal, the laboratory test can be repeated per PI discretion and Medical Monitor approval

16. Confirm whether subject has signed consent for the optional biobanking sub-study.

17. 10 ml of blood will be collected and banked at NYU biorepository for the EPPIC-Net repository (see instructions for how to collect blood samples).
18. Additional blood will not be collected. Leftover serum from the 8.5 ml Biomarker Blood Analysis (cytokines and chemokines) and left-over plasma from PK collection will be banked at each of the "Blood for Biomarkers" time points. Plasma not collected at V1. Leftover biomaterial from the Biomarker collection will be sent to NYU biobank.
19. Stool will be collected and shipped by the patient directly to the NYU CBRD biobank
20. An optional synovial fluid tap will be conducted on subjects who consent to the tap at the Visit 3 (week 6) clinic visit. Approximately 1 cc of synovial fluid will be drawn for biomarker analysis. Sites who have opted out of collecting synovial fluid will not complete this. The WOMAC-A must be completed by the subject prior to the synovial fluid procedure.
21. Additional synovial fluid will not be collected. If leftover synovial fluid is available, it will be sent to the biorepository if the subject consented to biobanking. Sites who have opted out of collecting synovial fluid will not complete this.

F.1.2. Study Assessment Overview

Subjects must provide written informed consent before any study-related procedures are initiated, including the cessation of prohibited concomitant therapy.

For the timing of assessments and procedures throughout the study, refer to the Schedule of Events (Section F.1.1). Throughout the study, every reasonable effort should be made by study personnel to follow the timing of assessments and procedures in the schedule of events for each subject. If a subject misses a study visit for any reason, the visit should be rescheduled as soon as possible.

For cases of extreme circumstances where subjects are unable to make in person clinic visits, the site will thoroughly document the situation and collect data for use of rescue medication, concomitant medications, adverse events, and compliance with study medication via the telephone or video conference. Subjects will be asked to complete subject assessment surveys on their device using NEForm (See Section K.1). Study medication will be shipped to the subject per the site standard operating procedures.

As indicated in the Manual of Procedures (MOP), direct data entry (DDE) is required in the EDC for several electronic case report forms (eCRFs). Site staff performing certain study procedures, according to the site's Delegation of Authority (DOA) log, must enter the data directly into the electronic data capture (EDC) system at the time of the procedure.

Please refer to the MOP, Appendix AC. "Investigator Source Documentation & DDE Expectations" for full list of DDE expectations. In the event of a technical EDC issue, paper documentation is allowable.

DDE eCRFs Include:

1. Adult Demographics
2. Vital Signs
3. Contraceptive Form
4. Knee OA (med record if available – but some will be pt reported)
5. Physical Exam
6. Urine Drug
7. Urine Pregnancy
8. Urine Dipstick
9. Eligibility Question
10. Adverse Events
11. Protocol Deviations

F.1.3. Screening Period (two – four weeks)

The Screening Period will be 2 weeks in duration with a +14-day window (14-28 days). Radiographs of both knees (posterior-anterior, fixed-flexion views) will be obtained to confirm the diagnosis of OA of the knees, as well as the Kellgren-Lawrence (K-L) grade of the identified index knee during the Screening Period. Quality of radiographs will be assessed, and radiographs may

need to be repeated at the direction of the central imaging reader. Only central reading of the K-L grade will be used to determine index knee eligibility.

Subject eligibility for enrollment (moderate to severe knee pain) will be determined using the average WOMAC -A (pain subscale) scores across the Screening Period. The scale will be completed every 3 days (± 1 days) during the Screening Period(Screening Visit and Days 3, 6, 9, 12, etc.). A minimum of 4 WOMAC-A's need to be collected during screening to determine the index knee. The subject can continue to try to complete the WOMAC-A's until the required minimum of 4 WOMACs are obtained. An electronic device is used to enter data into NEForm (See Section K.1). The purpose of these assessments is to ensure that appropriate patients are entered into the study.

The investigator will determine that the patients meet eligibility criteria and will collect the demographic and medical data permitting full characterization of the patients. Screening evaluations are to be conducted as outlined in the Study Schedule of Events (Table F.1.1). The in-clinic screening visit procedures may be divided into two visits.

Screening Visit(s)

The screening visit should be conducted 14-28 days prior to the Baseline Visit. The in-clinic procedures can be split across two days, if necessary.

For all visits, NEForm will be used to record the Patient Reported Outcome (PRO) scales. All other data will be directly entered into the study database or collected externally and entered into the electronic data capture (EDC) system with source materials uploaded to Florence. Additional information and instructions on study activities can be found in MOP.

1. Written informed consent – NEForm on Tablet
    a. Informed Consent – documents patient's understanding and willingness to participate in the study; includes consent for optional biobanking and optional synovial fluid collection. Paper consent permitted if NEForm consent is not working properly.
    b. Participant Eligibility Affirmations – provides confirmation of patient-reported eligibility items, e.g. will refrain from using prohibited concomitant medications during the study.
    c. Post-Consent Checklist – documents appropriate consent process
2. Protocol Patient Measures
    a. NEForm App. Introduction to the remote procedures app and training on how to use it. Patients will be instructed to complete the End of Day Pain Ratings daily for the first week of screening using the NEForm app. If technical issues arise in the NEForm app, and the participant is unable to complete the at-home assessments during the first week of the screening period, they will be allowed to start the ePRO remote screening period once issues are resolved, as long as they are still within the screening period window. Patients will be prompted to record their pain and functioning as well as document use of rescue medication(s) each day.

b. PROs – NEForm on Tablet
    i. HADS
    ii. WOMAC-A (Pain Subscale)

3. Lab Collection
    a. Clinical blood labs – Chemistry, Hematology (Refer to Schedule of Events footnotes & F.2 Procedures for full list of labs to be collected)
    b. Central labs – CYP2C9 buccal swab, saliva, or blood sample (genotyping for poor metabolizers)
    c. Urine drug screen provided by the study
        i. If positive, work with MM to determine medical appropriateness of result, i.e. subject tested positive for a medication used as prescribed by a medical professional that is not otherwise excluded.
    d. Urine pregnancy test provided by the study, for women of childbearing potential (WOCBP)
        i. If positive, a serum pregnancy test will be done.
4. Other Assessments
    a. Radiograph image taken using fixed flexion radiographic imaging of both knees. Posterior-anterior only. The fixed-flexion method of imaging is used as it has been shown to be more accurate than the standing-extended view in assessing Kellgren-Lawrence (K-L) grading and joint space width. (Kan et al, 2017). Upload to NESS Image Portal.
    b. Confirmation of K-L grade (1, 2, 3, or 4) of the knees via central radiologist review in NESS Image Portal.
    c. Height and weight (BMI autocalculated by EDC).
    d. Review of analgesic rescue medication use, documented in EDC (Participant to document rescue medication daily in NEForm OA App)
    e. Demographics, documented in EDC.
    f. Contraceptive Use, documented in EDC
    g. Document Height & Weight – BMI in EDC
    h. Review of medical and OA history, including medical records and previous radiographic images of the knee, if available. Current and past knee OA treatments (including all previous pharmacologic, noninvasive, or invasive treatments) should all be documented in EDC.
    i. Vital signs – EDC.
    j. Complete physical examination – EDC.
        i. A pelvic or urogenital examination may be deferred, unless the investigator deems this to be clinically indicated.

k. Concomitant Medication
    i. the study team will complete the Concomitant Medication Log in EDC, using generic names for each medication listed, and including dose and frequency of use).

l. Placebo Response Mitigation Training, as a part of the NEForm (NESS) electronic assessments, patients will be presented with a placebo response mitigation transcript at the Screening and Week 12 visits. These instructions recommend patients answer questions while sitting alone in a quiet area free of distraction. The instruction also advises patients to focus only on pain in the index knee, ignoring pain in other areas and sensations in the index knee that are not painful (e.g. stiffness).

m. ECG (12-Lead) – ERT/Clario – Upload ECG to ERT Portal for central review. Site to download report, and investigator to review report, assess clinical significance, and sign ECG report and upload to Florence

n. Chest Radiograph – posterior-anterior only; upload report/image for remote monitoring review

o. Adverse event collection and documentation in EDC.

5. Review of inclusion/exclusion criteria.
6. Review NEForm app Instructions. Walk the patient through the steps and have them confirm that the device is set-up by completing the single-question eCRF via the app.
    a. This will verify the device is appropriately linked to the study and gives the patient a chance to try to use the app while site staff are present to assist.
7. Complete and send SAFER Scheduling Checklist to CCC

<u>Remote Screening Period</u>

The below list of assessments will be conducted remotely or via mobile device during the Screening Period. Detailed instructions and processes can be found in the study's MOP and its appendices.

1. Protocol Patient Measures: **Please educate patients on the importance of answering these prompts. Failure to do so during screening could result in ineligibility.** If technical issues arise in the NEForm OA app, and the participant is unable to complete the at-home assessments beginning on Day 0, they will be allowed to start the ePRO remote screening period once issues are resolved, as long as they are still within the screening period window. Sites should contact CCC to approve of change..
    a) PROs – Remotely via NEForm App
        1. End of Day Pain Ratings – NRS (0-10)
            - Completed <u>once daily</u> for the first seven days of the Screening Period
        2. WOMAC-A – completed on Screening Period days 3, 6, 9, and 12, etc. (±1 day). A minimum of 4 are required to determine the index knee.

b) SAFER interview by an MGH CTNI rater will be conducted to confirm that the subject meets the history and current requirements for pain criteria stated above.

1. Completed once by telephone; may only be scheduled after all Screening WOMAC-As have been completed. Contact CCC with scheduling concerns. The SAFER team will confirm the patient meets all other screening criteria, including blinded criteria prior to scheduling the patient for their SAFER interview.

Note: *eligibility criteria related to the remote measures, including pain intensity requirements are specified in Appendix I: Blinded Information and are only available to unblinded study team members.*

2. Other Assessments – captured once daily via NEForm App
   a) Concomitant rescue medication – capture use of analgesic medications

F.1.4. Re-Screening

Subjects may re-screen up to a maximum of two times, for a total of three Screening Visits. Each rescreen visit may only be initiated with the documented permission of the Medical Monitor in NESS. If a subject screen fails for knee radiography, rescreening is not recommended. For a full list of rescreening procedures and timelines, please see the protocol MOP.

F.1.5. Visit 1 – Baseline (Day 0)

For all visits, NEForm will be used to record the Patient Reported Outcome (PRO) scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review NEForm collection procedures/compliance with subjects

   Educate subjects to complete these forms. Subjects should try and complete at least 80% of remote procedures in NESS. If subjects fall below the 80% completion rate, retrain the subject at the next study visit.

2. Define index knee. Using the averaged screening period WOMAC-A scores by knee, the knee with the greater score at the end of screening is defined as the index knee going forward.

3. Review of inclusion and exclusion criteria to ensure that the patient meets the requirements for the study.
   a) At the beginning of the visit, make sure subject did not take any prohibited medications or therapy prior to baseline.
   b) At the end of the visit, confirm all criteria have been met prior to randomizing the subject.

4. Protocol Patient Measures – NEForm on Tablet
   a) PROs

- WOMAC-A (Pain Scale)
- WOMAC-C (Functional Scale)

b) Staircase-Evoked Pain Assessment

5. NIH HEAL Initiative CDEs for chronic pain – NEForm
   Optional: Participants and site staff may complete CDEs remotely to reduce time at the in-person clinic visit
   a) PEG Scale
   b) Pain Catastrophizing Scale – Short Form 6
   c) PROMIS Sleep Disturbance Scale – 6A
   d) Sleep Duration Question
   e) PROMIS Physical Functioning Short-Form 6b
   f) PHQ – Depression
   g) GAD-2
   h) TAPS-1
   i) OUQ

6. Lab Collection – enter data in EDC, upload reports for remote monitoring review
   a) Blood Draws:
      i. Chemistry (See Schedule of Events footnotes for full list)
      ii. Biomarkers – Cytokines & Chemokines (see section F.2.2)
   b) Urine Collection:
      i. Full Urinalysis Panel
      ii. Urine pregnancy test for WOCBP – EDC
         1. If positive, a serum pregnancy test should be performed before randomizing. If positive the subject should not randomize.

   c) Biobanking for Future Testing (See Section F.2.3 Biobanking – Blood and Stool Repository for Future Use; See MOP, Laboratory Manual).

      i. **If a subject has opted out of biobanking, DO NOT collect**.
      ii. Collect and freeze 10 ml whole blood for batch shipping to NYU's biorepository
      iii. Collect and freeze any leftover biomarker aliquots for batch shipping to NYU's biorepository
      iv. Give instructions for patient to collect and send standardized stool sample to NYU's biorepository (See MOP for patient-facing instructions)

7. Other Assessments
   a) Review concomitant medications (using generic names) in EDC.
   b) Review of analgesic rescue medication use since the last visit. This information is recorded on the NEForm app by the patient daily.
   c) Record new adverse events and provide updates for existing adverse events.
   d) Vital signs – EDC.

e) ECG (12-lead) – ERT/Clario; upload ERT report for remote monitoring review

8. Randomization (See Section F.1.6 below and MOP for additional details)
    a) If all criteria are met, the patient will be randomized via the Almac IXRS and a kit containing 2 bottles of study drug will be dispensed. The first dose of drug, which includes one tablet from each bottle, should be taken at the visit, to be sure that the patient understands the procedure. If the subject's first dose needs to be taken at home, the site will schedule a phone/teleconference meeting with the subject to ensure they understand the procedure before they take the first dose.
    b) Ensure Eligibility and Randomization eCRFs are completed in EDC.

Remote procedures to be completed between V1/Baseline & V2/Week 3:

9. Remote Protocol Patient Measures
    a) WOMAC-A – completed once weekly
    b) End of Day Pain Ratings – NRS (0-10)
        i. Completed <u>once daily</u> for the seven days prior to the next visit

10. Other Remote Assessments
    a) Concomitant Rescue Medication – captures use of acetaminophen and opioids daily
    b) Study Medication – captures time and confirms dosing twice daily
        i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.6. Randomization

For entry into the study and randomization, all eligibility criteria MUST be met (i.e., all inclusion criteria and no exclusion criteria). Patients who meet all eligibility criteria at the Baseline Visit (day 0) will be randomized. After documentation of written informed consent and the completed screening period, clinical sites will confirm eligibility and complete randomization by accessing Almac's centralized web-based system for randomization and drug management - the Interactive Web Response System (IXRS). Additional information and instructions can be found in the MOP and appendices.

Patients will receive treatments (from among 300mg CNTX-6970 and placebo) in randomly assigned sequences. A block randomization will be used, stratifying by K-L grade to achieve balance on sequences. The randomization will be implemented by Almac's IXRS in consultation with the study statisticians. Blinding of the randomization sequence from trial subjects, staff from the Clinical Sites, CCC, and DCC will be ensured through the use of the IXRS and a designated unblinded statistician.

After Randomization, drug will be dispensed.

F.1.7. Visit 2 (Week 3)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review remote forms completion rate with patient (confirm >80% forms completion rate or re-educate)
2. Protocol Patient Measures (PROs):
    a) WOMAC-A (Pain Scale)
    b) WOMAC-C (Functional Scale)

3. NIH HEAL CDE
    a) PGIC

4. Blood Collection – Lab Chemistry

5. Urine Collection – urine dipstick
    a) If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis.

6. Other Assessments
    a) Document concomitant medications (using generic names).
    b) Review of analgesic rescue medication with the patient
    c) ECG (12-Lead)
    d) Record adverse events
    e) Drug Accountability and Compliance

7. Use Almac's IXRS to get the next kit assignment.
    a) Dispensation of study drug (1 kit; 2 bottles)

Remote procedures to be completed between V2/Week 3 & V3/Week 6:

8. Remote Protocol Patient Measures – PROs – Use NEForm App
    a) WOMAC-A – completed once weekly
    b) End of Day Pain Ratings – NRS (0-10) - completed once daily for the week prior to the next visit

9. Other Remote Assessments – completed once daily
    a) Concomitant rescue medication – captures use of acetaminophen and opioids daily
    b) Study Medication – captures time and confirms dosing twice daily
        i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.8. Visit 3 (Week 6)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review remote forms completion rate with patient
2. Protocol Patient Measures
    a) PROs
        i. WOMAC-A (Pain Scale)
        Must obtain WOMAC-A before synovial fluid collection procedure
        ii. WOMAC-C (Functional Scale).
        iii. HADS
    b) Staircase-Evoked Pain Assessment.
3. Lab Draws/Collections
    a) Blood Draws:
        i. Chemistry
        ii. Hematology
        iii. PK Analysis (see section F.2.2)
        iv. Biomarker - Cytokines & Chemokines (see section F.2.2)
    b) Urine dipstick
        i. If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis.
    c) Synovial Fluid (Knee) Collection
        i. If subject opted out of synovial fluid DO NOT collect
        ii. Collect and ship to Myriad Lab for analysis
    d) Biobanking (See Section F.2.3; See MOP, Laboratory Manual)
        i. **If a subject has opted out of biobanking DO NOT collect**
        ii. Collect and freeze leftover blood and synovial fluid samples to batch ship to NYU's biorepository
4. NIH HEAL Initiative CDEs for chronic pain (Use NEForm)
    a) PROMIS Sleep Disturbance Scale – 6A.
5. Other Assessments
    a) Document concomitant medications (using generic names).
    b) Review of analgesic rescue medication
    c) Vital signs, as detailed in Section F.2.1
    d) ECG (12-Lead)
    e) Drug accountability and compliance.
    f) Record adverse events.
6. Use Almac's IXRS to get the next kit assignment.
    a) Dispensation of study drug (1 kit; 2 bottles)

Remote procedures to be completed between V3/Week 6 & V4/Week 9:

7. Remote Protocol Patient Measures
    a) WOMAC-A (Pain Scale) – completed once weekly
    b) End of Day Pain Ratings – NRS (0-10) – completed once daily for the seven days prior to the next visit
8. Other Remote Assessments – completed once daily
    a) Concomitant rescue medication – captures use of acetaminophen and opioids daily
    b) Study Medication – captures time and confirms dosing twice daily
        i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.9. Visit 4 (Week 9)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review remote forms completion rate with patient
2. Protocol Patient Measures - PROs
    a) WOMAC-A (Pain Scale).
    b) WOMAC-C (Functional Scale)
3. Blood Collection – Lab Chemistry
4. Urine Collection – Urine Dipstick
    a) If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis
5. Other Assessments
    a) Review concomitant medications (using generic names)
    b) Review of analgesic rescue medication
    c) ECG (12-Lead)
    d) Record adverse events
    e) Drug Accountability and Compliance
6. Use Almac's IXRS to get the next kit assignment.
    a) Dispensation of study drug (1 kit; 2 bottles)

Remote procedures to be completed between V4/Week 9 & V5/Week 12:

7. Remote Protocol Patient Measures
    a) WOMAC-A (Pain Scale) – completed once weekly
    b) End of Day Pain Ratings – NRS (0-10) - completed once daily for the seven days prior to the next visit

8. Other Remote Assessments – completed once daily
    a) Concomitant rescue medication – captures use of acetaminophen and opioids daily
    b) Study Medication – captures time and confirms dosing twice daily
        i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.10. Visit 5 (Week 12)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review remote forms completion rate with patient
2. Protocol Patient Measures
    a) PROs
        i. WOMAC-A (Pain Scale).
        ii. WOMAC-C (Functional Scale).
        iii. HADS
    b) Staircase-Evoked Pain Assessment.
3. NIH HEAL Initiative CDEs for chronic pain
    a) PROMIS Sleep Disturbance Scale – 6A.
4. Lab Draws/Collection
    a) Blood Draws:
        i. Chemistry
        ii. Hematology
        iii. Biomarkers - Cytokines & Chemokines (See Section F.2.2)
        iv. PK Analysis (See Section F.2.2)
    b) Biobanking (See Section F.2.3; See MOP, Laboratory Manual)
        i. If a subject has opted out of biobanking DO NOT collect
        ii. Send leftover blood samples to NYU biorepository for future testing
    c) Urine dipstick analysis
        i. If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis.
    d) Urine pregnancy test (kit provided by study) for WOCBP
        i. If positive, a serum pregnancy test should be performed.
5. Other Assessments
    a) Document concomitant medications (using generic names).
    b) Vital signs, as detailed in Section F.2.1
    a) Review of analgesic rescue medication with patient using data from NEForm.
    c) ECG (12-lead).
    d) Drug Accountability and Compliance.

e) Record adverse events.
f) Placebo Response Mitigation Training, as a part of the NEForm (NESS) electronic assessments, patients will be presented with a placebo response mitigation transcript at the Screening and Week 12 visits. These instructions recommend patients answer questions while sitting alone in a quiet area free of distraction. The instruction also advises patients to focus only on pain in the index knee, ignoring pain in other areas and sensations in the index knee that are not painful (e.g. stiffness).

6. Use Almac's IXRS to get the next kit assignment
    a) Dispensation of study drug (1 kit; 2 bottles)

Remote procedures to be completed between V5/Week 12 & V6/Week 15:

7. Remote Protocol Patient Measures
    a) WOMAC-A (Pain Scale) – completed once weekly
    b) End of Day Pain Ratings – NRS (0-10) -completed once daily for the seven days prior to the next visit
8. Other Remote Assessments – completed once daily
    a) Concomitant rescue medication – captures use of acetaminophen and opioids daily
    b) Study Medication – captures time and confirms dosing twice daily
        i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.11. Visit 6 (Week 15)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review remote forms completion rate with patient
2. Protocol Patient Measures -PROs
    a) WOMAC -A (Pain Scale)
    b) WOMAC-C (Functional Scale)
3. Lab Draws/Collections:
    a) Blood Draw – Lab Chemistry
    b) Urine dipstick Urine dipstick analysis
        i. If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis.
4. Other Assessments
    a) Document concomitant medications (using generic names).
    b) Review of analgesic rescue medication with patient using data from NEForm.
    c) Record adverse events
    d) ECG (12-Lead)

e) Drug Accountability and Compliance

5. Use Almac's IXRS to get the next kit assignment
    a) Dispensation of study drug (1 kit; 2 bottles)

Remote procedures to be completed between V6/Week 15 & V7/Week 18:

6. Remote Protocol Patient Measures
    a) WOMAC-A (Pain Scale) – completed once weekly
    b) End of Day Pain Ratings – NRS (0-10) - completed once daily for the seven days prior to the next visit

7. Other Remote Assessments – completed once daily
    a) Concomitant rescue medication – captures use of acetaminophen and opioids daily
    b) Study Medication – captures time and confirms dosing twice daily
        i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.12. Visit 7 (Week 18)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review forms completion rate with patient

2. Protocol Patient Measures
    a) PROs
        i. WOMAC-A (Pain Scale).
        ii. WOMAC-C (Functional Scale).
        iii. HADS
    b) Staircase-Evoked Pain Assessment.

3. NIH HEAL Initiative CDEs for chronic pain
    a) PROMIS Sleep Disturbance Scale – 6A.

4. Lab Draws/Collection
    a) Blood Draws:
        i. Chemistry
        ii. Hematology
        iii. Biomarkers – Cytokines & Chemokines (see section F.2.2)
        iv. PK Analysis (see section F.2.2)
    b) Biobanking (See Section F.2.3; See MOP, Laboratory Manual)

i. **If a subject has opted out of biobanking DO NOT collect**
ii. Send leftover blood samples to NYU biorepository for future testing

c) Urine dipstick
i. If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis.

5. Other Assessments
   a) Document concomitant medications (using generic names).
   b) Vital signs, as detailed in Section F.2.1.
   c) ECG (12-Lead)
   d) Review of analgesic rescue medication use
   e) Drug Accountability and Compliance
   f) Record adverse events.

6. Use Almac's IXRS to get the next kit assignment
   a) Dispensation of study drug (1 kit; 2 bottles) for Weeks 18-21

Remote procedures to be completed between V7/Week 18 & V8/Week 21:

7. Remote Protocol Patient Measures
   a) WOMAC-A (Pain Scale) – completed once weekly
   b) End of Day Pain Ratings – NRS (0-10) - completed once daily for the seven days prior to the next visit

8. Other Remote Assessments – completed once daily
   a) Concomitant rescue medication – captures use of acetaminophen and opioids daily
   b) Study Medication – captures time and confirms dosing twice daily
      i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.13. Visit 8 (Week 21)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities can be found in the MOP.

1. Review forms completion rate with patient

2. Protocol Patient Measures -PROs
   a) WOMAC-A (Pain Scale)
   b) WOMAC-C (Functional Scale)

3. Blood Collection – Lab Chemistry

4. Urine dipstick

a) If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis.

5. Other Assessments
    a) Document concomitant medications (using generic names).
    b) Review of analgesic rescue medication with patient using data from NEForm.
    c) ECG (12-Lead)
    d) Record adverse events
    e) Drug Accountability and Compliance

Remote procedures to be completed between V8/Week 21 & V9/Week 24

6. Remote Protocol Patient Measures –
    a) WOMAC-A (Pain Scale) – completed once weekly
    b) End of Day Pain Ratings – NRS (0-10) -completed once daily for the seven days prior to the next visit

7. Other Remote Assessments – completed once daily
    a) Concomitant rescue medication – captures use of acetaminophen and opioids daily
    b) Study Medication – captures time and confirms dosing twice daily
        i. Failure to document 6 consecutive doses (3 days) of study medication is grounds for early termination. Monitor for missed doses and reeducate patient as needed.

F.1.14. Visit 9 (Week 24 or Early Termination)

For all visits, NEForm will be used to record the PRO scales. All other data will be directly entered into the study database or collected externally and entered into the EDC with source materials uploaded to Florence. Additional information and instructions on study activities, can be found in the MOP.

1. Review forms completion rate with patient

2. Protocol Patient Measures – NEForm on Tablet
    a) PROs
        i. WOMAC-A (Pain Scale)
        ii. WOMAC-C (Functional Scale)
        iii. HADS
    b) Staircase-Evoked Pain Assessment

3. NIH HEAL Initiative CDEs for chronic pain – NEForm on Tablet
    a) PEG Scale.
    b) Pain Catastrophizing Scale – Short Form 6.
    c) PROMIS Sleep Disturbance Scale – 6A.
    d) Sleep Duration Question
    e) PROMIS Physical Functioning Short-Form 6b.
    f) PHQ – Depression.

g) GAD-2
h) PGIC
i) TAPS-1
j) OUQ

4. Lab Draws/Collection
    a) Blood Draws:
        i. Chemistry
        ii. Hematology
        iii. Biomarkers – Cytokines & Chemokines (See Section F.2.2)
        iv. PK Analysis (See Section F.2.2)
    b) Urine Dipstick
        i. If abnormal for leukocytes, blood, nitrite, or protein, complete full urinalysis.
    c) Urine Drug Screen – test kit provided by study
    d) Urine Pregnancy Test for WOCBP – if positive confirm with serum pregnancy test
    e) Biobanking (See Section F.2.3; See MOP, Laboratory Manual)
        i. If a subject has opted out of biobanking DO NOT collect
        ii. 10ml of whole blood will be collected and frozen to batch ship to NYU's biorepository
        iii. Freeze leftover blood samples to batch ship to NYU's biorepository

5. Other Assessments
    a) Physical examination.
        i. A pelvic or urogenital examination may be deferred unless the investigator deems this to be clinically indicated.
    b) Document Concomitant medications (using generic names).
    c) Review of analgesic rescue medication use.
    d) Chest Radiograph - posterior-anterior only
    e) ECG (12-Lead)
    f) Vital signs, as detailed in Section F.2.1.
    g) Drug Accountability and Compliance
    h) Record adverse events.

6. If applicable, collect NESS loaner device from subjects.

F.2. Procedures

F.2.1. Laboratory/Diagnostic Procedures

As outlined in Table 1, after the patient has signed the informed consent document, laboratory procedures will be conducted regularly to confirm subject safety. Urine and blood tests may be repeated per PI discretion and Medical Monitor approval before a subject is screen failed (during screening) or discontinued from study participation.

1. Urine Pregnancy Test: A urine pregnancy test will be performed at the Screening Visit, Baseline Visit, Week 12 (Visit 5), and Week 24/ET (Visit 9) using the test kit provided by the study.
    - In the event of a positive urine pregnancy test, a serum pregnancy test will be conducted to confirm.
        - If a urine pregnancy test is positive at the Baseline Visit, hold randomization until the serum pregnancy test can verify the results.
    - Subjects with a positive serum pregnancy test at Screening or Baseline will be excluded from the study. Subjects with positive serum pregnancy test results after Baseline will be discontinued from study participation.
    - Additional serum or urine pregnancy tests may be performed as determined necessary by the Investigator or required by local regulation, to establish the absence of any pregnancy any time during study participation.
2. Urine Drug Screen: A urine drug screen will be conducted at the Screening Visit and Week 24/ET (Visit 9) using the test kit provided by the study. The urine drug screen will include amphetamines, barbiturates, buprenorphine, benzodiazepines, cocaine, ecstasy, methamphetamine, methadone, marijuana, opiates, oxycodone, and phencyclidine.
    - If positive, the subject will have the opportunity to provide evidence of medical necessity for their result, e.g. positive for oxycodone due to prescribed intermittently for their OA pain. The Medical Monitor will make the final decision regarding medical support for any positive results.
    - For this study, continuing intermittent opioid use is allowed as a rescue analgesic medication. See Appendix III and MOP for additional information.
    - For this study, medical marijuana (or equivalent) use is allowed for use depending on regional legality.
3. ECG: 12-lead ECGs will be measured at all in-clinic visits.
    - All ECG tracings should be checked for completeness and potential artifacts during the visit. If poor quality or otherwise indicated, additional ECG assessments can be made at the discretion of the investigator.
    - All ECG tracings will be uploaded to ERT (central ECG laboratory) and read at the scheduled time points by a central reader. The site will download the ECG report results from the ERT portal.
    - An investigator at the site is required to review all ECG reports to assess for potential safety concerns or exclusionary reasons. Any abnormalities noted by the central reader must be assessed by the Investigator for clinical significance (CS/NCS) on the report with a date and signature or initials:
        - Abnormal and Not Clinically Significant (NCS)
        - Abnormal and potentially Clinically Significant (CS)

- Any ECG results that are judged by the Investigator to be potentially CS will be recorded on the ECG report and the eCRF. This would constitute an AE and should be entered on the Adverse Event Form and monitored as described in Section F.4.8.1 Adverse Event Reporting. Repeat or unscheduled samples may be taken for safety reasons or to address technical issues with the samples.
- The site will upload the report with the investigator's signature and assessment to Florence within 3-business days of receipt of the ERT report. If technical issues arise, please refer to the ERT manual for other methods of transmission. See **MOP Appendix AC. Investigator Source Expectations and DDE Expectations** for investigator requirements.

4. Clinical Blood Lab Tests: Chemistry will be obtained at each in-clinic visit. Hematology will be obtained at the Screening Visit, and at the Weeks 6, 12, 18, and 24/ET Visits.

    - **Chemistry**: Sodium, potassium, chloride, calcium, phosphate, magnesium, glucose, urea nitrogen (BUN/Urea), creatinine, uric acid, bilirubin (total and indirect), alkaline phosphatase, AST, ALT, GGT, eGFR, amylase, lipase, total protein and albumin.
        - Estimated glomerular filtration rate [eGFR] will be calculated in the EDC system using Modification of Diet in Renal Disease (MDRD) Study equation and must be assessed by the Investigator for clinical significance (CS/NCS).
    - **Hematology:** White cell count with differential (absolute values of neutrophil, lymphocyte, monocyte, eosinophil, and basophil), red cell count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH Concentration (MCHC) and platelets count
        - The actual dates and times of sample collection must be recorded in the subject source notes or the laboratory requisition form. All clinical laboratory test results outside the reference range must be assessed by the Investigator for clinical significance (CS/NCS) on the report with a date and signature or initials: Abnormal and Not Clinically Significant (NCS)
        - Abnormal and potentially Clinically Significant (CS)

    Any laboratory test results that are judged by the Investigator to be potentially CS will be recorded on the /lab report and the eCRF. This would constitute an AE and should be entered on the Adverse Event Form and monitored as described in Section F.4.8.1 Adverse Event Reporting. Repeat or unscheduled samples may be taken for safety reasons or to address technical issues with the samples.

    The site will upload the lab report with the investigator's signature and assessment to Florence within 3-business days. See MOP **Appendix AC. Investigator Source Expectations and DDE Expectations** for investigator requirements.

5. Vital Signs: Oral temperature, pulse, respiratory rate and blood pressure will be recorded at the Screening Visit, Baseline Visit, and at the Weeks 6, 12, 18, and 24/ET Visits.
    - Patients should be **seated** for 2 minutes prior to collecting pulse and blood pressure.

6. Physical Exam: At the Screening Visit and Week 24/ET (Visit 9), patients will have a physical examination.
    - Genitourinary evaluations will not be conducted, unless deemed necessary by the investigator.
7. CYP2C9 Genotyping: At the Screening Visit, a buccal swab, saliva, or blood sample will be collected to identify and exclude poor CYP2C9 metabolizers. The results must be reviewed before a subject is randomized.
8. Urine Testing:
    - At Baseline, a full urinalysis panel will be done for all subjects, including color, clarity, specific gravity, pH, leukocyte esterase, protein, glucose, ketones, bilirubin, blood, nitrite, urobilinogen.
    - After Baseline, a Urine Dipstick will be completed at all in-clinic visits and will include Calcium, Glucose, Protein, pH, Bilirubin, Blood, Urobilinogen, Micro albumin, Ascorbate, & Specific Gravity. If the dipstick test is abnormal for leukocyte, blood, nitrite or protein, a sample should be sent for a full urinalysis panel.

The required laboratory tests are listed in Table 2, below. Laboratory tests will be performed at the site, and each site will provide their laboratory certificates and normal ranges to the coordinating center. Reference ranges have been standardized across study sites for lipase and amylase due to institutional variability and are as follows, please refer to protocol ***Appendix IV Amylase and Lipase Normal Ranges Memo_12Jan2024*** for additional information.

F.2.1.1. Table 2: Frequency of Laboratory & Diagnostic Procedures

Laboratory Test	**Frequency**
Hematology (local lab) #	Screening and Week 6, 12, 18, and 24/ET
Chemistry (local lab) #	Screening, Baseline, Weeks 3, 6, 9, 12, 15, 18, 21, and 24/ET
Urine Dip Stick ^	Weeks 3, 6, 9, 12, 15, 18, 21, and 24/ET
Urinalysis (UA) ^	Complete UA panel at Baseline; for abnormals at Weeks 3, 6, 9, 12, 15, 18, 21, and 24/ET
Pregnancy test (urine) using kit provided *	Screening, Baseline, and Weeks 12 and 24/ET
Drug Screen (urine) using kit provided	Screening and Week 24/ET
CYP2C9 Genotyping	Screening

[#] For a full list of tests conducted, please see Section F.2.1.
^ Microscopic examination will only be performed if the dipstick test is outside of the reference range for leukocyte, blood, nitrite, or protein.
* Only for WOCBP. If urine pregnancy test is positive, a serum pregnancy test will be conducted.

F.2.2. Blood and Synovial Fluid Biomarkers (Cytokines and Chemokines)

At Baseline and Week 6, 12, 18, and 24/ET visits, 8.5 ml blood samples will be drawn. Serum will be separated and a commercially-available multiplex assay (the Myriad Human Inflammation MAP® 1.0) will be used to evaluate multiple inflammatory markers. In addition, we will assess:

- Monocyte chemoattractant protein-1 (MCP-1)/CCR-2 receptor binding inhibition by CNTX-6970 (Baseline and Weeks 6, 12, 18 and 24).
- Synovial fluid levels of cytokines and chemokines, assessed at the end of the first treatment period (Visit 3).
- Monocyte chemoattractant protein-1 (MCP-1)/CCR-2 receptor binding inhibition by CNTX-6970 in synovial fluid, assessed at the end of the first treatment period (Visit 3).

Extra (left over) blood samples not required to be sent to the lab for analysis will be frozen and batch shipped to NYU biobanking for future exploratory analysis. IF A SUBJECT HAS OPTED OUT OF SPECIMEN SAMPLING DO NOT SEND. See MOP for additional details.

An optional synovial fluid tap will be conducted on the index knee on subjects at the week 6 clinic visit. The synovial fluid tap is optional for both sites and participants. Approximately 1ml of synovial fluid will be withdrawn for biomarker analysis. Synovial fluid collection will be performed by a physician with training and experience. Synovial fluid analysis will include cytokines and chemokines as a part of establishing biomarker for treatment of OA pain with CNTX-6970.

Extra (left over) Synovial fluid from the sample that is not required to be sent to the lab for analysis will be sent to NYU biobanking for future exploratory analysis. IF A SUBJECT HAS OPTED OUT OF SPECIMEN SAMPLING DO NOT SEND.

F.2.2.1. <u>Blood Test for Pharmacokinetics (PK analysis at Weeks 6, 12, 18, and 24/ET [V3, V5, V7 and V9)</u>

Four (4) ml of Blood will be drawn. Pharmacokinetic/pharmacodynamics PK/PD analysis of this study will be conducted to determine dose relationship of CNTX-6970 and its analgesic effect in patients with OA. This determination will be obtained at the time when patients are at the steady state of CNTX-6970. Pharmacokinetic samples will be obtained at Week 6, Week 12, Week 18, and Week 24/ET Visits.

Extra (left over) blood samples, beyond what was required to be sent to the lab for analysis, will be frozen and batch shipped to NYU biobanking for future exploratory analysis. IF A SUBJECT

HAS OPTED OUT OF SPECIMEN SAMPLING DO NOT SEND. See MOP for additional details.

F.2.3. Biobanking – Blood and Stool Repository for Future Use

The Informed Consent Form (ICF) will inform whether or not the subject has opted out of specimen biobanking. If the patient has opted out, no blood or stool sample for biobanking will be obtained.

F.2.3.1. Whole Blood (Baseline and End of Study)

The EPPIC-Net DCC's NYU Center for Biospecimen Research and Development (CBRD) will store and manage biological samples (biosamples) collected in this clinical trial. The samples will be used for the present study and also for potential future research as permitted by the study-specific informed consent form. Biosamples stored for this study may include, but are not limited to: whole blood, plasma, serum, stool, synovial fluid and/or derivatives of these specimens. The samples will be stored only for the period defined in the informed consent form, which may be indefinite. Biospecimens may be shared in accordance with the protocol-defined data and sample sharing plan and the informed consent form.

Biosamples will be documented in LabVantage, a secure network linking biospecimens to corresponding clinical and pathological data. LabVantage does not include any identifying personal health information (PHI). The CBRD and LabVantage meet all Good Laboratory Practice (GLP) and FDA guidelines.

F.2.4. Protocol Patient Measures

1. WOMAC-A (Pain subscale). Pain in the index knee, measured weekly by the WOMAC-A (Pain subscale). We will use the numerical rating scale version of the WOMAC, with the subject assessing each of 5 questions using an 11-point (0 to 10) scale, and the total index score is the sum of the individual item scores (range 0-50). A higher WOMAC-A pain score represents worse pain.

   The WOMAC-A will be used to study the course of OA pain symptoms during treatment, including onset of action and carryover effects.

2. WOMAC-C (Function subscale). The WOMAC-C physical function subscale contains 17 items assessing daily functioning, each using an 11-point (0 to 10) numerical rating scale. The total index score (0-170) is the sum of the items. A higher WOMAC-C function score represents worse functioning and less ability to engage in daily activities.

3. Daily Knee Pain Intensity on a 0-10 Numeric Rating Scale (NRS). Pain intensity is reported by patients with chronic pain as one of the most important targets of treatment, and daily pain intensity ratings are a recommended core outcome measure for clinical trials of treatments for chronic pain. Daily ratings are preferable to ratings of recalled pain over longer time periods such as a week, as daily ratings minimize the influence of recall biases (Dworkin et al., 2005). Subjects provide one-daily reports (at the end of the day) of their

average knee pain intensity on a 0-10 pain intensity NRS over the course of a week, and those daily ratings are averaged to compute a mean knee pain intensity score.

4. HADS (Hospital Anxiety and Depression Scale). The HADS is a 14-item self-report questionnaire designed to assess symptoms of anxiety and depression in those with medical illness (Norton et al, 2013). It has well-established reliability and validity in the assessment of symptoms of depression and emotional distress, and it has been used in numerous clinical trials. It does not include somatic symptoms, such as fatigue and sleeplessness, which may otherwise be attributable to physical illness, and it has been standardized among large community samples. It has also been validated in several medical illness populations with good sensitivity and specificity for predicting DSM-IV major depression or generalized anxiety disorder diagnoses. The HADS has been recommended as a psychosocial phenotyping measure in clinical trials of treatments for chronic pain (Edwards et al, 2016).

5. Staircase-Evoked Pain Assessment: This procedure consists of stepping fully up and down onto an 8in (20.32cm) high platform with both feet a total of 24 times. The lead leg is alternated between each up/down cycle. Subjects are instructed to use their normal gait for completing this task and are encouraged to complete the task despite increasing pain, without stopping if possible. The procedure is timed, and current knee pain intensity on a 0-10 NRS is assessed immediately before and following the procedure while the subject is in a seated, resting position.

6. SAFER Interview: The SAFER Interview (Targum et al, 2008) is a validated, structured, clinician-rated scale that is used to facilitate the identification of appropriate and valid patients for clinical trials. The interview will confirm that identified patients have symptoms that reflect the current state of illness and that these symptoms can be assessed with the appropriate rating instruments. The SAFER and the HADS will be administered to the patients remotely via telephone by psychiatrists and psychologists from the CCC.

   After the assessments are evaluated, the CCC will notify the study center, indicating whether the patient meets all criteria and is eligible to continue. Study center staff will schedule the telephone assessment (SAFER interview) with staff at the CCC. Patients who do not meet the SAFER criteria will be discontinued from the study and considered a screen failure. The information collected by the CCC clinicians will be copied and transmitted to the study centers to retain as source documentation.

F.2.5. NIH HEAL Initiative Common Data Elements (CDEs)

1. PEG Scale. The PEG consists of 3 items assessing pain intensity, interference of pain with enjoyment of life, and interference of pain with general activity. PEG scores range from 0-10, with higher scores representing a greater burden of pain.

2. Pain Catastrophizing Scale – Short Form 6. Catastrophizing is a pain-specific psychosocial construct comprising cognitive and emotional processes such as helplessness, pessimism,

rumination about pain-related symptoms, and magnification of pain reports. The short-form Pain Catastrophizing Scale is a 6-item, self-report measure of catastrophic thinking associated with pain (McWilliams, et al, 2015). Scores range from 0-24, with higher scores indicating more catastrophizing.

3. The Patient-Reported Outcomes Measurement Information System (PROMIS) Physical Functioning Short-Form 6b. The PROMIS Physical Functioning short form is a 6-item scale which is widely used in pain research. It is a unidimensional scale that shows broad coverage of the physical function construct, good construct validity, and high levels of temporal stability (Schalet et al, 2016). The PROMIS Physical Function Scale is expressed as a T-score, with a population mean of 50 and SD of 10. Higher scores represent better physical functioning; possible T scores in this distribution range from 21 to 59 (http://www.healthmeasures.net/images/PROMIS/manuals/PROMIS_Physical_Function_Scoring_Manual.pdf).

4. PROMIS Sleep Disturbance Short Form. The PROMIS Sleep Disturbance short form is a convenient 6-item scale that correlates strongly with the longer forms. It shows greater measurement precision for assessing sleep disturbance than other commonly-used (and much longer) questionnaires such as the Pittsburgh Sleep Quality Index and the Epworth Sleepiness Scale; its brevity and convenience are a major advantage for both research and clinical settings (Yu et al, 2011). The PROMIS Sleep Disturbance Scale is expressed as a T-score, with a population mean of 50 and SD of 10. Possible T scores in this distribution range from 31.7 to 76.1; higher scores represent a greater disruption of sleep (i.e., lower sleep quality).

5. Patient Health Questionnaire (PHQ) – Depression. The 2-item PHQ-2 is a brief depression screening tool that correlates strongly with PHQ-9 scores and shows good sensitivity for identifying individuals with depressive disorders in the general population and in a variety of medical samples (Arroll et al, 2010). Scores range from 0-6, with higher scores indicating more depressive symptomatology.

6. Generalized Anxiety Disorder – 2 item scale (GAD-2). The GAD-2 is a 2-item screening tool that is widely used to screen for clinically significant anxiety symptoms and anxiety disorders in clinical settings. It shows good sensitivity and specificity as a screening tool for anxiety disorders (Kroenke et al, 2007). Scores range from 0-6, with higher scores indicating more anxiety symptomatology.

7. Tobacco, Alcohol, Prescription medication, and other Substance use Tool (TAPS-1). The TAPS-1 is the screening component of the TAPS tool and consists of a single stem question with four items covering frequency of past-12-month use of tobacco, alcohol, illicit drugs, and non-medical use of prescription medications. Scores range from 0-4; higher scores indicate a higher likelihood of problematic substance use. The TAPS-1 shows good sensitivity and specificity for identifying substance use disorders (Gryczynski, et al, 2017).

8. Opioid Use Questionnaire (OUQ). The OUQ is an indicator of past or present use of any of the listed opioid medications. There are a total of three yes/no items where a yes indicates the use of such medications.

F.2.6. Blinding Procedures

Eligible subjects will be randomized through an interactive web response system (IXRS). The randomization will be stratified by K-L grade. Blinding will be assured by restricting access of site and Sponsor personnel and/or designees to the treatment codes, and by providing identical tablets and packaging for the placebo and CNTX-6970 tablets. Subjects will be provided with 2 bottles (CNTX-6970 100mg or placebo; CNTX-6970 200mg or placebo) at each clinic visit.

For the final analysis, the treatment sequence codes for all subjects will be released after all subjects have completed the study and the clinical database is locked.

For the DSMB safety reviews, the treatment sequence codes will be released to an independent statistician and a programmer to produce unblinded reports. The Sponsor and the investigators will remain blinded.

Unblinding of individual treatment sequence during the study is discouraged. The PI at a site may break the blind for a given subject in the event of a medical emergency, where knowledge of the subject's treatment sequence must be known in order to facilitate appropriate emergency medical treatment. If reasonable, the investigator should attempt to contact the study Medical Monitor before unblinding a subject's treatment sequence identity in order to obtain concurrence that unblinding a subject's treatment sequence assignment is necessary. If not reasonable prior to unblinding, investigators should notify the Medical Monitor as soon as possible after an unblinding event. Details of the process to be followed are provided in a separate Almac IXRS manual. Once unblinded, a subject must be permanently withdrawn from study participation.

F.2.7. Safety Measures

Laboratory values will be recorded in the electronic case report form (eCRF). Abnormal laboratory values will be recorded as AEs only if deemed clinically significant. Abnormal laboratory values (clinically significant and not clinically significant), will be reviewed by the Medical Monitor and PPI for trends. The presence of any side effect or adverse event that would not be deemed as exclusionary will be carefully documented at screen (for the past week) and at every subsequent visit using the patient's self-report to the study staff. Reasons for premature discontinuation, including intolerable side effects, will be recorded.

The primary safety endpoint for the study is the following: Percent of adverse events and serious adverse events in the final 2 weeks of each treatment period. Vital signs (heart rate, blood pressure, etc.) will be obtained regularly throughout the study at the time of study visits. Height and weight will be collected during the screening period and at the subject's final study visit. Blood chemistry and hematology panels will also be obtained regularly at study visits. All ECGs will be documented by recording date, time, heart rate, QRS duration, PR interval, RR interval, QT, and QTcF on the eCRF. Paper strips will be maintained in the patient file. If indicated, additional ECG assessments can be made at the discretion of the investigator.

Because CNTX-6970 is metabolized by CYP2C9 and CYP3A4 (to a lesser extent), use of inhibitors and inducers of those two genes are excluded for the duration of participation in the trial. CYP2C9 genotyping will be used to exclude poor metabolizers. As a P-glycoprotein (P-gp) substrate, introduction of any new P-gp inhibitor is also exclusionary for the duration of study participation – P-gp use must have been chronic for at least 3 months and stable for at least 1 month prior to Baseline. Additionally, until more is known about the hepatic metabolization and renal excretion of CTNX-6970, individuals with moderate to severe impairment in hepatic or renal functioning will be excluded from participation.

ECGs will be done at each in-clinic visit to assess heart health. Urine dipstick/urinalysis and liver functioning tests (AST, ALT, GGT, total bilirubin, direct bilirubin, ALK phosphatase) will be collected at each in-clinic visit to monitor the subject's health throughout the study.

F.3. Discontinuation

F.3.1. Discontinuation of Subjects

In accordance with the Declaration of Helsinki and other applicable regulations, subjects have the right to withdraw from the study at any time, and for any reason, without prejudice to their future medical care. Subjects may be discontinued or withdrawn from the study for any of the following reasons:

- Serious adverse event.
- The investigator determines that continuation in the study would be detrimental to a subject's well-being, including criteria under F.3.1.1
- Subject fails to comply with protocol requirements.
- Subject misses more than 6 consecutive doses of study medication.
- Subject is lost to follow-up.
- Subject becomes pregnant.

F.3.1.1. Automatic Stopping Criteria for Subjects

If a subject meets any of the following criteria at any time during the study, they must be discontinued from the study drug and withdrawn from participation, in accordance with the procedures outlined in Section F.3.2.

a. 1) ALT and/or AST >3x ULN with total bilirubin >2x ULN (unless elevated bilirubin is related to confirmed Gilbert's Syndrome). Subjects with total serum bilirubin >2x above ULN will be followed until resolution. 2) Confirmed AST and/or ALT >5x ULN for more than 2 weeks.

b. Serum creatinine >1.5 × ULN or Grade 2 (moderate) value based on Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.

c. ECGs (minimum criteria): absolute QTcF ≥500 ms; absolute QTcF <320 ms (clinically significant QTc shortening); prolongation in repeated, averaged ECG measures of PR or QRS of >40 ms from the Baseline Visit.

d. Monocyte count <1% of white blood cells (e.g., for WBC = 4,500/μL, stop if monocyte count <0.01 * 4,500 or 45/μL), hemoglobin <11 g/dL (Grade 2 in females) or <12.4 g/dL (Grade 2 in males), platelets <100,000/μL on 2 consecutive blood draws. A confirmatory blood draw will be completed at an in-clinic unscheduled visit. Discontinuation will be confirmed by the consecutive blood draw at the next in-clinic visit.

F.3.2. Discontinuation of Subjects who Stopped taking Study Medication

Should a patient discontinue study medication and decide to withdraw consent, an EOS visit should be scheduled at the earliest convenience to complete the EOS study procedures, unless the subject agrees to schedule it for the next study visit.

Should a patient discontinue study medication, but not withdraw consent, best efforts should be made by the site to collect all study data at the regular study assessment times through the closest of the following study visits: Week 6, Week 12, Week 18 or Week 24/ET. At this clinic visit, all assessments outlined for the EOS visit should be conducted.

Should the subject decline to come into the site for an ET visit, an abbreviated version of the Week 24/ET visit should be performed over the telephone. Sites should complete as many as the patient is willing/able to complete remotely. If the subject is unable or unwilling to complete any of these assessments, it will not be considered a protocol deviation. The following assessments are listed in order of importance and should be completed in that order, dependent on the subject's willingness and capabilities:

1. WOMAC – A (pain scale)
2. WOMAC – C (functional scale)
3. Patient Global Impression of Change (PGIC)
4. Assessment of Analgesic Medication Use
5. Concomitant Medications and Therapies
6. Hospital Anxiety and Depression Scale (HADS)
7. Drug Compliance
8. Adverse Events
9. Pain Catastrophizing Scale – Short Form 6
10. PEG Scale
11. PROMIS Sleep Disturbance Scale – 6A
12. Sleep Duration Question
13. PROMIS Physical Functioning Short-Form 6b
14. Patient Health Questionnaire (PHQ)
15. Depression Generalized Anxiety Disorder – 2 item scale (GAD-2)
16. Tobacco, Alcohol, Prescription medication, and Other Substance use Tool (TAPS-1)
17. Opioid Use Questionnaire (OUQ)

The eCRF Completion Guidelines and MOP will provide further instructions on data collection for the end of study visits.

Failure to collect follow-up data despite best efforts will not be counted as a protocol deviation. Action items, instructions and script will be provided to site coordinators for tracking down and obtaining data from those patients who discontinued treatment. Logs will be kept for actions taken to reach each patient who discontinued treatment.

If a subject is lost to follow-up, every effort should be made to contact the subject by telephone. Two phone calls followed by a certified letter should be documented before considering the subject lost to follow-up.

For subjects who continue to be followed for safety, adverse events should continue to be reported as described in Section F.4.

F.3.3. Discontinuation of Study

This study may be suspended or discontinued in part or in whole, at any time. The study may be discontinued for a number of reasons, including but not limited to those listed below:

- Excessive AE(s) - serious [i.e., an SAE]
- It is determined that continuation in the study would be detrimental to subjects' well-being
- At the discretion of a study oversight body, such as NINDS, IRB, DSMB, etc.
- Loss of funding from the NIH

F.3.3.1. Automatic Pausing/Stopping Criteria for Study

If the study meets any of the following criteria at any time, the study must be stopped, and the data reviewed. These criteria will be monitored by the unblinded statistician or designee.

a. If ≥ 2 serious adverse events (SAEs) occur in the CNTX-6970 300 mg / placebo cohort that are deemed to be possibly, probably or definitely related to the study drug, dosing will be stopped and available safety information will be reviewed to evaluate whether dosing in that arm should continue.

b. If ≥ 2 subjects in the CNTX-6970 300 mg / placebo cohort experience Grade 3 or 4 AEs for vital signs, systemic effects, or laboratory abnormalities that are deemed to be possibly, probably or definitely related to the study drug as defined in the following guidance for industry, *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials*, available at, https://www.fda.gov/media/73679/download, dosing will be stopped and available safety information will be reviewed to evaluate whether the study should continue.

The table below should be referred to when determining severity for non-systemic effects or if an AE is not listed in the FDA guidance.

Grade	Grade Category	Intensity Definition
1	Mild	An AE that is usually transient and may require only minimal treatment of therapeutic intervention. The event does not generally interfere with usual activities of daily living.)
2	Moderate	An AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject
3	Severe	An AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention
4	Potentially Life Threatening: Emergency Room visit or hospitalization	

c. Clinical study will be terminated if any death attributable to the 300 mg CNTX-6970 treatment occurs.

F.4. Specification of Safety Parameters

F.4.1. Definition of an Adverse Event

An AE is defined as "any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related" (CDER, 2012). An AE can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality or seriousness. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

An adverse reaction means any adverse event that was likely to have been caused by a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For this study, all events, whether or not related to the study drug, will be captured.

AEs do not include the following:

- Stable or intermittent chronic conditions (such as myopia requiring eyeglasses) that are present prior to baseline and do not worsen during the study.
- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, transfusion). The condition that leads to the procedure is an AE if not present at baseline.

- Overdose of either study drug or concomitant medication without any signs or symptoms unless the subject is hospitalized for observation.
- Hospitalization for elective surgery planned prior to study (situation where an untoward medical occurrence has not occurred).
- Symptoms that are an expected part of knee OA, unless a significant change in severity or duration.
- Pregnancy will not be considered an AE, but if it occurs, it will be reported on a pregnancy form.

Adverse events will be recorded from the time informed consent is obtained. AEs should be assessed at each visit. All AEs for randomized patients must be either resolved or stable at the end of the study. If ongoing at the end of the study, the subject should be referred for appropriate treatment.

F.4.2. Definition of Serious Adverse Events

In addition to the severity rating, each AE will be classified by the investigator as "serious" or "not serious." The seriousness of an event will be defined according to the applicable regulations and generally refers to the outcome of an event. An SAE is one that meets one or more of the following:

- Is fatal
- Is immediately life threatening
- Results in disability or permanent damage
- Requires hospitalization
- Prolongs existing hospitalization
- Is a congenital anomaly or birth defect (in an offspring)
- Is medically significant
- *May also include any other event that the investigator or Medical Monitor judges to be serious or that suggests a significant hazard, contraindication, side effect, or precaution.*

F.4.3. Definition of Life-Threatening

A life-threatening event places the subject at <u>immediate</u> risk of death from the event as it occurred. This does not include an AE, which, had it occurred in a more severe form, might have caused death.

F.4.4. Definition of Hospitalization

Hospitalization is defined by NINDS as a full admission to the hospital for diagnosis and treatment. This includes prolongation of an existing in-patient hospitalization. Hospitalization may include social hospitalization, defined as inadequate family support or care at the subject's primary residence that results in the subject being admitted to the hospital (i.e., wound care, nutrition).

Examples of visits to a hospital facility that do not meet the serious criteria for hospitalization include emergency room visits that do not result in a full hospital admission, outpatient surgery, preplanned or elective procedures, and protocol procedures

F.4.5. Definition of Disability or Permanent Damage

Disability is defined as a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.

F.4.6. Definition of Medically Significant

Important medical events (medically significant events) that may not result in death, be life threatening, or require hospitalization may be considered to be an SAE when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization or development of drug dependency or drug abuse.

F.4.7. Classification of an Adverse Event

The description of each AE should use the following definitions:

F.4.7.1. Severity of Event – as determined by the Investigator

The severity of each AE will be graded on a 4-point scale and reported in detail as indicated on the eCRF. Vital signs, systemic effects, and laboratory abnormalities will be graded using the ***Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials***, available at https://www.fda.gov/media/73679/download (See MOP Appendix M).

As noted in the FDA guidance listed above, institutional laboratory reference ranges should be used when determining the severity of abnormal lab results, if appropriate. Due to variability in institutional laboratory reference ranges for amylase and lipase, ranges have been standardized across study sites.

Please refer to protocol *Appendix IV Amylase and Lipase Normal Ranges Memo_12Jan2024* for additional information.

The table below should be referred to when determining severity for non-systemic effects or if an AE is not listed in the FDA guidance.

Grade	Grade Category	Intensity Definition
1	Mild	An AE that is usually transient and may require only minimal treatment of therapeutic intervention. The event does not generally interfere with usual activities of daily living.)

2	Moderate	An AE that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the research subject
3	Severe	An AE that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention
4	Potentially Life Threatening: Emergency Room visit or hospitalization	

F.4.7.2. Relationship to Study Agent – as determined by the Investigator

The causality of each AE should be assessed and classified by the investigator as "related" or "not related." An event is considered related if there is "a reasonable possibility" that the event may have been caused by the product under investigation (i.e., there are facts, evidence, or arguments to suggest possible causation).

- Definitely related
- Probably related
- Possibly related
- Probably not related
- Definitely not related

Consider the following when assessing causality:

- Temporal associations between the agent and the event
- Response to cessation (de-challenge) or re-challenge
- Compatibility with known drug class effects
- Known effects of concomitant medications
- Pre-existing risk factors
- A plausible mechanism
- Concurrent illnesses

F.4.7.3. Duration

The duration of the AE should be recorded using the following criteria:

- Start: Date of the first episode of the AE or date of significant sustained worsening in severity
- Stop: Date when AE either ceased permanently or changed in severity
    - If AE has not resolved/recovered, document as ongoing

In the event that a subject is withdrawn from the study because of an AE, the subject should be followed and treated by the investigator until the AE has resolved, stabilized, or a new chronic baseline has been established.

F.4.7.4. Frequency

The frequency of the AE should be indicated according to the following definitions:

- Single: Experienced once, without recurrence
- Recurrent: More than one discrete episode with the same severity

F.4.7.5. Action Taken with Regard to Study Medication

- Dose not changed
- Drug withdrawn
- Drug interrupted
- Not applicable

F.4.7.6. Outcome

- Recovered/resolved
- Recovered/resolved with sequelae
- Not recovered/not resolved
- Fatal: Death related to adverse event
- Unknown

F.4.7.7. Seriousness

See Section F.4.2. for additional information about determining the seriousness of an AE.

- Not serious
- Serious

F.4.7.8. Expectedness – as determined by the Investigator

An AE is deemed unexpected (in terms of nature, severity, or frequency), given the information provided in research-related documents, such as the Investigator's Brochure (IB), and the characteristics of the subject population being studied.

An adverse event or suspected adverse reaction is considered "unexpected" if the event/reaction is:

- Not listed in the IB;
- Listed in the IB, but not at the specificity or severity that has been observed; or
- Not consistent with the risk information described in the general investigational plan or elsewhere in the current application (per 21CFR312.32).

F.4.8. Reporting Procedures

F.4.8.1. Adverse Event Reporting

The investigator or designee must record all observed AEs and all reported AEs. At each visit, the investigator or designee should ask the subject a nonspecific question (e.g., "Have you noticed

anything different since your last visit?") to assess whether any AEs have been experienced since the last report or visit.

All AEs, serious and not serious, will be recorded on the AE eCRF page using appropriate medical terminology. Severity and relationship to study drug will be assessed by the investigator as described above.

When possible, clinical AEs should be described by diagnosis and not by symptoms (e.g., "cold" or "seasonal allergies" instead of "runny nose").

All AEs, whether or not related to the study drug, must be fully and completely documented on the AE eCRF and in the subject's notes.

F.4.8.2. Serious Adverse Event Reporting

The reporting of serious adverse events (SAEs) to the NINDS, the IRB, and Food and Drug Administration (FDA) is a regulatory requirement. The EPPIC-Net CCC and DCC are responsible for reporting SAEs to NINDS. Each site is responsible for reporting SAEs at their site to the cIRB and their local IRB, as applicable. CCC is responsible for the reporting of SAEs to the FDA. Each Regulatory Authority has established a timetable for reporting SAEs based upon established criteria.

The investigator or designee will immediately report to the CCC any SAE, whether or not considered study intervention related. All SAEs, even those that are listed as expected in the protocol or IB, should be reported and must include an assessment of causality/relationship to study drug. The CCC will inform Centrexion of any SAEs that need to be reported for the Investigational New Drug (IND).

All SAEs will be followed until satisfactory resolution or until the investigator deems the event to be chronic or the patient is stable. Other supporting documentation of the event may be requested by the DCC, CCC, NINDS, or Centrexion and should be provided as soon as possible.

F.4.8.2.1. SAE Reporting Timeline

The EPPIC-Net CCC will be responsible for notifying:

- the FDA of any unexpected, fatal or life-threatening, suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after CCC's initial receipt of the information.
- the FDA and all participating investigators in an IND safety report of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the CCC determines that the information qualifies for reporting.

For this study, sites will complete the electronic SAE and/or Pregnancy eCRF for initial reporting and any subsequent follow-up information. The EDC system will send an automatic notification to the PI, the Medical Monitor, and any other study personnel, as required.

In the event of any SAE (other than death), the study subject will be instructed to contact the investigator (or designee) using the telephone number provided in the ICF. All subjects

experiencing an SAE will be seen by the investigator or designee as soon as is feasible following the report of the SAE.

SAEs that occur after the study follow-up period should be reported if in the judgment of the investigator there is "a reasonable possibility" that the event may have been caused by the product.

F.4.8.3. Unanticipated Problem Reporting

An unanticipated problem is any incidence, experience, or outcome that is:

- Unexpected (in terms of nature, severity, or frequency) given the information provided in research-related documents and the characteristics of the subject population being studied;
- Related or possibly related to participation in the research; and
- Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

Advarra uses the following to determine whether something is an unanticipated problem involving risk to subjects or others:

- Unanticipated or unexpected at the time of IRB approval;
- Involved new or increased risk to subjects or others; and
- Related to the research.

F.4.8.4. Elective Procedures and Surgeries

As a part of consenting, subjects are asked to affirm that are not planning to have any surgeries, invasive procedures, or IA injections of the index knee or any surgeries/procedures that are otherwise contraindicated during their participation the study (exclusion criterion #10).

For the purposes of this protocol, the following conventions will apply for SAE reporting of elective procedures and surgeries:

- A prescheduled elective procedure or a routinely scheduled treatment is not to be considered an SAE, even if the subject is hospitalized, provided the site stipulates that:
    - The condition requiring the prescheduled elective procedure or routinely scheduled treatment was present before and did not worsen or progress between the subject's consent to participate in the clinical trial and the time of the procedure or treatment.
    - The prescheduled elective procedure or routinely scheduled treatment is the sole reason for admission and intervention.
- An untoward medical event occurring during the pre-scheduled elective procedure or routinely scheduled treatment should be recorded as an AE or an SAE. Any concurrent medications should also be recorded on the eCRF.

F.4.8.5. Other Reportable Information

In addition, and for the purposes of monitoring, the following should be reported via the AE, SAE, and/or Pregnancy reporting forms, as appropriate:

- A new diagnosis of cancer
- Instances of overdose of study drug (where there are associated symptoms)
- Any occurrence of pregnancy (with or without AEs)

F.4.8.6. Reporting of Pregnancy

Women of childbearing potential are permitted in this study. Women of non-childbearing potential are defined as those who have been postmenopausal for at least 12 months, who do not have a uterus, have had a bilateral tubal ligation, have undergone bilateral salpingectomy, and/or have had both ovaries removed.

Any female subject who becomes pregnant during the study (with or without AEs) must be discontinued from study treatment and the pregnancy must be reported on the pregnancy form. Any female subject who becomes pregnant during the study will be followed through the first well-baby visit. Any AEs that are the consequence of pregnancy and that meet the criteria for serious should also be reported via the AE/SAE forms.

F.5. Protocol Deviations and Violations

No prospective entry criteria protocol deviations are allowed; all subjects must meet all eligibility criteria in order to participate in the study. Protocol waivers for eligibility will not be granted under any circumstances. If, during the course of a subject's post-randomization participation in the trial, it is discovered that the subject did not meet all eligibility criteria, s/he will be discontinued, unless the discontinuation presents an unacceptable medical risk. The justification to allow the subject to continue in the trial will be made by the study investigator, with medical input from the PPI and the Medical Monitor and will be documented. Whether or not the participant is allowed to remain in the trial, this will be reported as a major protocol deviation and not a waiver.

All follow-up safety assessments must be completed and documented as outlined in the protocol. Protocol deviations and violations will be recorded in the eCRF (non-patient specific deviations will be documented in a log on Florence) and a monthly report will be generated by the DCC for review.

[redacted]TREATMENT

G.1. Dosing Schedule

Study medication will be taken orally twice a day by the subject, with or without food. The study medication will consist of two tablets. Doses should be taken at least 8 hours apart, but as close to 12 hours apart as possible.

G.2. Study Drug Packaging, Labeling and Administration

CNTX-6970 will be provided as off-white, oval tablets in strengths of 100mg and 200mg and with matching placebo tablets.

Sites will be provided with two (2) different kit types, each kit containing two (2) 60-count bottles as follows:

Kit Type	Kit Contents 60-Count Bottles	
	Bottle 1	Bottle 2
Placebo	CNTX 100mg PTM	CNTX 200mg PTM
CNTX 300 mg BID	CNTX 100mg	CNTX 200mg

**PTM = Placebo to Match*

Each kit will contain the following information on the label:

- Study number and IND number
- Unique Kit number
- Drug treatment label (each kit will include two bottles):
  - Bottle 1: CNTX-6970 100mg or placebo
  - Bottle 2: CNTX-6970 200mg or placebo
- Route of administration
- Directions for use
- Storage conditions
- Space for investigator name, telephone, dispensing date and subject number
- Space for study subject identifier
- Label statement "Caution: New Drug-Limited by federal law to investigational use"
- Label statement "Caution: Keep out of reach of children"
- Name and address of study drug distribution center

Subjects will be instructed to take a tablet from each of the two bottles (bottles 1 and 2) twice a day with or without food.

The first dose of the drug will be administered on site, to ensure that the patient understands the procedure. If the subject cannot take the first dose on site due to drug supply issues, the first dose can be taken at home. The site will schedule a phone or video teleconference (preferred) to ensure the participant understand the procedure before taking the first dose.

G.3. Study Drug Receipt, Accountability, and Storage Conditions

Study drug, or Investigational Product (IP), supplies will be received by the investigator or other authorized study personnel at the study site, managed and stored safely and properly, and kept in a secured location with restricted access. Shipping records, signed receipts, and IP inventory records should be maintained for all shipments of IP. Study staff with responsibility for drug management will keep accurate and complete records of study drug received by the site, dispensed to study subject, and used and returned by study subject. If the site's local policies do not require use of the research pharmacy, IP may be stored and managed by trained study team members, so

long as storage conditions are restricted/locked access, temperatures are monitored regularly, and the IP-related documentation is maintained appropriately.

Study drug is to be stored at 20ºC to 25ºC (68ºF to 77ºF). Excursions are allowed between 15ºC to 30ºC (59ºF to 86ºF). If a temperature excursion lasts longer than 24 hours or exceeds the allowed-excursion range, the study drug should be quarantined and the study CCC Project Manager should be contacted immediately upon identification. The drug should remain in quarantine until it is confirmed by the CCC PM to be safe to use.

Study drug will be dispensed by appropriately qualified staff as designated by the PI's delegation of authority log. Subjects will self-administer the study drug at home following the instructions provided to the subject at the study visit. The PI or designee will retain returned study drug, or study drug supplies not dispensed to subjects, until the CCC provides written approval for disposition.

The study case report forms and source documents will include prompts to record study subject eligibility to receive study drug, study drug dispensed, dosing start date, dosing end date, missing doses, and study drug returned.

In accordance with local regulatory requirements, the investigator, designated site staff, or head of the medical institution (where applicable) must document the amount of investigational product dispensed and/or administered to study subjects, the amount received from the central pharmacy/site, and the amount destroyed upon completion of the study. If institutional policy does not allow the retention of IP for review prior to destruction, the site must have written approval from the CCC detailing how IP accountability will be handled in the absence of monitor review. The PI or designee is responsible for ensuring product accountability records are maintained throughout the course of the study. The inventory will include details of study drug received and dispensed to subjects. The site must receive written approval from the CCC prior to either destroying the remaining study drug or returning it to the Central Pharmacy or manufacturer. An accounting must be made of any drug deliberately or accidentally destroyed. Discrepancies between the amount of study drug received and dispensed drug must be reconciled.

G.4. Possible Drug Interactions

Drug-drug interactions (DDI) resulting from inhibition of transporters or cytochrome P450 (CYP) are unlikely to occur in humans; the mean maximum plasma concentration (Cmax) in humans after a single oral dose of 600mg is at least 18-fold lower than the concentration at 50% inhibition for the transporters studied. Additionally, CNTX-6970 was predominantly metabolized by CYP2C9 with a minor contribution of CYP3A4. Concomitant use of CYP2C9 and CYP3A4 inducers and inhibitors is prohibited within 7 days of Baseline and during participation in this trial. P-glycoprotein inhibitors are also prohibited, as CNTX-6970 is a P-glycoprotein substrate, unless use has been chronic for at least 3 months and stable for at least 1 month prior to Baseline.

G.5. Analgesic and Rescue Medications

Subjects may continue use of tramadol, gabapentin, duloxetine, pregabalin, milnacipran, or a tricyclic antidepressant, if prescribed for pain and use is chronic for at least 12 weeks and at a stable dose for at least 4 weeks prior to Screening. Other continuous, chronic analgesic treatments are exclusionary.

Some medications that may impact pain are used to treat non-pain indications. For this trial, continuous use of such medications, e.g. duloxetine for depression, may be continued throughout study participation, if the dose has been stable for at least 12 weeks prior to Screening. Additionally, continued use of low-dose aspirin for heart disease prophylaxis is allowed.

Starting with the first visit, the only allowable analgesic rescue medications that can be taken for pain are acetaminophen and opioids (see Appendix III and MOP for additional details, including maximum daily dosing). Intermittent use of opioids must have started at least four weeks prior to Screening; introduction of new opioid treatment during the study is prohibited. The amount of rescue medication(s) taken each day will be recorded by the subject in the NEForm app.

G.6. Concomitant Medications

For a full list of concomitant medications and their restrictions for use, please see Appendix III: Concomitant Medications.

All concomitant medications taken during the study will be recorded in the Concomitant Medication Log for each patient, along with dosage information and start and stop dates. Allowed concomitant medications include any prescription or over-the-counter medication not specifically excluded by the protocol. In the present study, acetaminophen, opioids, and select anticonvulsants are the only permitted concomitant/rescue analgesic medication. Patients requiring excluded drugs will be discontinued from the study. If there are any questions regarding concomitant medications, contact the Medical Monitor.

G.6.1. Prohibited Medications

Subjects will be prohibited from using any experimental or investigational drug and/or use an experimental or investigational medical device within 30 days, or within a period of less than 5 times the experimental drug's half-life, prior to screening, whichever is longer, or may still be experiencing pharmacodynamic effects from an experimental drug as stated in the inclusion exclusion criteria.

Subject will be prohibited from receiving therapy that is known to interfere significantly with drug metabolism or transport (within 7 days, or 5 half-lives (whichever is longer) of dosing with study drug. Exceptions will only be made if the rationale is clearly documented by the investigator, and discussed with the Medical Monitor.

Medications that are not included in the above, but that may reasonably be expected to interfere significantly with the metabolism or excretion of CNTX-6970, that may be associated with a significant drug interaction with CNTX-6970, or that may pose a significant risk to the subject's

participation in the study will be assessed on a case by case basis to determine, to the extent possible from the published literature, if they should be prohibited.

The direct FDA link for all CYP2C9, CYP3A4, and P-GP interactions is: Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers | FDA

ETHICAL CONSIDERATIONS

H.1. Risk/Benefit Assessment

Subjects must be capable of understanding the nature of this study and its potential risks, discomforts, and benefits. Study physicians will obtain consent after they have fully explained the study purpose and its procedures, and potential Subjects have demonstrated an understanding of the protocol, willingness to participate, and competency to consent.

H.2. Safety

H.2.1. CNTX-6970

The potential for reproductive and developmental toxicity with CNTX-6970 has been assessed in multiple species in several studies. All studies were negative indicating that there was no evidence of in vitro or in vivo genotoxicity. Similarly, studies conducted to evaluate potential for phototoxicity, dermal toxicity (sensitivity), immunotoxicity, and reproductive and developmental toxicity found no evidence for concern. In addition, there is little to no evidence of a proarrhythmic or cardiovascular risk of CNTX-6970. In human studies, CNTX-6970 was well tolerated at all doses tested and there were no potential or identified risks established for the drug. The most commonly reported adverse event was headache, which was mild in nature, and was reported by under 20% of Subjects. In the Phase I trials of CNTX-6970, no subjects experienced an SAE or AE that led to discontinuation from the study. There were no deaths in the study. There were no clinically meaningful changes from baseline in laboratory parameters, 12-lead ECGs, or physical examination findings observed in the study. Collectively, the safety profile of the drug appears to pose minimal risk.

H.3. Informed Consent

The investigator or delegated study team member must ensure that patients are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical trials in which they volunteer to participate. Preparation of the consent form is the responsibility of the PI and must include all elements required by 21 CFR §50.25 and the central IRB.

All subjects will receive the consent form for the study. These documents will be read by the patients and also reviewed by the patient with a delegated study team member on the research staff prior to participating in the study. Any questions, concerns, or ambiguities will be clarified by the PI or another study clinician prior to the patient signing consent. Subjects will sign informed consent and only then will begin participation in the study.

H.4. IRB Review

Before study initiation, the PI must have written and dated approval/favorable opinion from the central IRB for the protocol, consent form, patient recruitment materials/process (e.g., advertisements), and any other written information to be provided to patients.

The PI will ensure the central IRB is provided with reports, updates, and other information (e.g., Safety Updates, Amendments, and Administrative Letters) according to regulatory requirements and institutional procedures.

A detailed list of required regulatory documents also to be submitted to EPPIC-Net Clinical Coordinating Center will be sent upon final approval of the protocol.

DATA HANDLING AND RECORDKEEPING

I.1. New England Survey Systems (NESS) NEForm

Radiology image uploads, radiology image review data, SAFER Rater data, electronic Patient Reported Outcomes (ePROs), and electronic Informed Consent (eConsent/eICF) will be collected using a validated electronic data capture system (NEForm by NESS). NEForm has been successfully deployed in Medical/Dental Operations as well as Clinical Trials. NEForm is fully validated and compliant to 21 CFR Part 11, HIPAA, GDPR, and other standards.

Data collected on the secure mobile application are stored locally on the mobile device, synced to a central server, and backed up on an off-location server. Data collected on the secure website are stored on a central server and backed up on an off-location server. Data collected are encrypted and uploaded/synced via additionally encrypted transmission to secure servers where access is limited according to the protocol. Strict operating procedures are enforced to protect the confidentiality, integrity, and access of Protected Health Information (PHI) when it is stored, transmitted, and maintained. When any PHI is collected in NEForm and uploaded to NESS, such PHI data are ONLY accessible to designated staff at NESS and handled in accordance with NESS SOPs. Such data are also only accessible to designated staff at the sponsor or the sponsor's designee when such access is properly requested. In such cases, the sponsor or sponsor's designee would follow their own SOPs.

The ePROs will be programmed by NESS and will include a notification system to patients reminding them to complete study activities. Data will be maintained by NESS and provided to the DCC at specific time points throughout the study. NESS will provide a dashboard that sites may access in order to ensure that patients are completing PROs at the appropriate timepoints. Sites are only able to see their own data. The DCC and CCC will be able to review all sites' data.

Additionally, automated and text message reminders will be sent by NESS to remind study patients about off-site ePROs, rescue medication use, and IP compliance.

For all data expected to be entered directly into NESS, paper source is permitted in the event of technical difficulties or other approved situational events.

I.2. Completion of EDC Forms

An Investigator is required to prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the investigation on each individual treated with the investigational product or entered as a control in the investigation.

TrialMaster will be used as the Electronic Data Capture (EDC) system and managed by the DCC. The EDC system will include eCRFs designed to capture study information, which are completed by trained site staff. Patient reported outcomes and additional eCRFs will be captured in the NESS EDC system. Data entered directly into the eCRFs via a tablet or other data entry device will be considered as source. Specific details of the procedures for Data Management are found in the Data Management Plan.

For all data expected to be entered directly into TrialMaster, paper source is permitted in the event of technical difficulties or other approved situational events.

I.3. Procedure for Corrections

Query resolution will be performed and tracked within the EDC system, and completed by trained site staff. Specific details regarding EDC query flow are found in the Data Management Plan.

I.4. Record Retention

The PI must retain investigational product disposition records, case report forms, and source documents for the minimum period required by applicable regulations and guidelines or institutional procedures, and at least for 10 years. If the PI withdraws from the study (e.g., relocation, retirement), the records shall be transferred to a mutually agreed upon designee (e.g., another investigator, IRB, EPPIC-Net). Notice of such transfer will be given in writing to the EPPIC-Net CCC and NINDS.

I.5. Data Confidentiality

Potential risks to data confidentiality will be mitigated by requirements for the de-identification of all study data and by security protocols for all data capture systems. All users of the EDC system will be tracked and provided access in a secure fashion following established Standard Operating Procedures (SOPs) for this process.

As with all research data, information gathered by the study will be used only for aggregate analysis; it will not be released with any information that identifies research Subjects. The data managers, statisticians, and PPI do not have access to the identities of patients. That information is retained only at the clinical sites. Uses and risks related to data collection will be outlined in the informed consent form and reviewed with the subjects.

I.5.1. Certificate of Confidentiality

This research is covered by a Certificate of Confidentiality (CoC) from the National Institutes of Health. The CoC protects subjects from the release or use of information, documents, or samples

without permission, including federal, state, or local civil, criminal, administrative, legislative, or other proceedings. An example would be a court subpoena. The CoC does not prevent the subjects from willingly releasing information about their involvement in this research.

MONITORING AND OVERSIGHT

Central IRB approval will be obtained and documented prior to subject enrollment and screening.

J.1. Evaluation of Study Sites

Potential study sites will be evaluated by EPPIC-Net to determine suitability for the proposed study. Information reviewed will include, but is not limited to, facility details and site capabilities, past performance in similar studies, investigator and staff experience, ongoing studies at the site, projected enrollment in this study, and FDA or other agency audit findings. Study sites may be asked to complete a study-specific Site Selection Questionnaire and other documents for consideration for participation and EPPIC-Net or clinical study monitors may make a Site Qualification Visit prior to completing the evaluation process. Upon completion of this evaluation, EPPIC-Net will invite the chosen sites for participation.

J.2. Initiation of Study Sites

Prior to subject enrollment, a study initiation visit will be completed at each investigational site to ensure the following: central IRB approval has been obtained and documented prior to subject screening, the investigators and study personnel are appropriately trained and clearly understand the study, the investigators and study personnel accept the obligations incurred in undertaking this clinical investigation.

J.3. Periodic Monitoring Visits

Qualified clinical monitors or qualified contract monitors representing EPPIC-Net will conduct investigational site monitoring visits to ensure that all investigators conduct the study in compliance with the protocol and applicable regulations. The site will receive notification prior to each monitoring visit during the course of the study. It is expected that the PI and/or Sub-Investigator(s) and other appropriately trained study staff are available on the day(s) of the visit in case any questions might arise.

Periodic monitoring visits will be made in accordance with the approved monitoring plan at all active study sites throughout the clinical study to assure that the PI obligations are fulfilled and all applicable regulations and guidelines are being followed. These visits will assure that the facilities are still acceptable, the protocol and investigational plan are being followed, the central IRB has been notified of approved protocol changes as required, complete records are being maintained, appropriate and timely reports have been made to the EPPIC-Net and the central IRB, and the PI is executing all agreed activities.

EPPIC-Net retains the right to remove either the PI or the investigational site from the study for issues of non-compliance with the protocol or regulatory requirements, in accordance with its SOPs.

On one or more occasions, the study site may be inspected or audited by an EPPIC-Net representative or a third party. The PI will be informed in advance of this visit.

J.4. Risk-Based Monitoring

EPPIC-Net will employ a protocol-specific Risk Based Monitoring (RBM) plan to ensure a robust quality control method for determining and enabling dynamic management of study risks and facilitating ongoing improvement in the trial conduct and oversight. The Acuity RBM system uses tools and dashboards to identify Key Risk Indicators (KRIs), which highlight potential issues with (for example) trial conduct, safety, data integrity, compliance, and enrollment, that may require further monitoring from a qualified contract monitor.

J.5. Suspension and Early Termination of a Site

The policies and procedures for the suspension or early termination of a study at a Clinical Study Site (i.e. Hub or Spoke participating in a particular clinical trial) are described in EPPIC-Net SOP Suspension and Early Termination of a Site. Circumstances that may necessitate invoking early termination or suspension of a site include but are not limited to:

- Enrollment at a site below expectation
- Protocol violations at the study site that place study subjects at risk of serious harm or that render the study data from that site untrustworthy or invalid
- Continuing or serious noncompliance by study investigators with regard to the protocol or regulations
- Any other site event that increases Subject risk of serious harm or undermines the integrity of the study
- Request of the site

The study can be suspended or terminated unilaterally at a site by the NIH, FDA, the central IRB, the local IRB (if applicable), or upon recommendation from the NINDS Data and Safety Monitoring Board (DSMB). The EPPIC-Net Study Trial Committee will receive concerns about site study suspension or termination, investigate, and determine if study should be suspended or terminated at a site, based on the above criteria.

J.6. Direct Access to Source Data/Documents

Subcontracts with study sites will specify that EPPIC-Net or its representatives will have direct access to source data and documents for study monitoring. Additionally, the central IRB, NINDS, and FDA may review source data following appropriate guidelines for this process.

J.7. Interim Analysis

No interim analysis is planned for this study.

J.8. Medical Monitoring

The Medical Monitor for this trial will be designated by EPPIC-Net. The Medical Monitor will review all adverse events, assess the benefits and risks of protocols on an ongoing basis, and work

in collaboration with the PPI to identify safety signals and trends, in accordance with the approved medical monitoring plan.

In addition, the Medical Monitor will be available to sites for questions regarding inclusion/exclusion criteria, protocol conduct, and safety. Trained and qualified EPPIC-Net staff physicians will be available to provide coverage during times when the Medical Monitor is unavailable. Sites will be provided with the Medical Monitor's cell phone number for emergency situations. Otherwise, sites are instructed to contact the Medical Monitor through email. Each month the Medical Monitor will receive a listing of protocol violations for review and identification of possible trends.

Serious adverse events will be reported to EPPIC-Net by the site within 24 hours of the site's knowledge of the event. In addition, the Medical Monitor may request individual patient records, including laboratory data, clinical records, and other study related data, to evaluate these events against the known safety profile of the study treatment and the disease. Any records sent to the Medical Monitor by the site will be redacted for any identifying information before transmission. The Medical Monitor may recommend actions including partial or complete unblinding, and/or modifying or terminating the study. In addition to safety monitoring, the Medical Monitor may review enrollment data, demographic information, retention status, and other reports prepared by the study statistician that describe study performance and progress.

J.8.1. Urgent Clinical Situations

During the course of the study, a patient will be reassessed for continued participation based on clinical judgment of the investigators. All subjects will be offered access to 24/7 coverage for medical emergencies through the clinical staff at each site. Treatment options and their risks and benefits, including continued study participation and other treatment options, will be sensitively explained. All study investigators have extensive clinical experience in the treatment knee OA, and can make the decision (while blinded) as to removal of subjects from the trial, particularly in the event of clinical deterioration. When a patient is removed from the trial based on clinical judgment, investigators will implement an appropriate treatment plan and arrange for follow-up for the patient.

J.9. The Data and Safety Monitoring Board (DSMB)

The NINDS DSMB will review the progress of the study and will monitor patient intake, outcomes, adverse events, and other issues related to patient safety. Interim, independent, and unbiased reviews of the study's ongoing progress will be provided. The DSMB will consist of experts in the study's subject matter field(s), clinical trials, biostatistics, and ethics. These individuals will not be Subjects in the trial and will not have participated in the planning of the protocol. The DSMB will consider safety or other circumstances as grounds for early termination, including either compelling internal or external evidence of treatment differences or the infeasibility of addressing the study hypothesis (e.g., poor patient intake, poor adherence to the protocol).

At each of its meetings during the study period, the DSMB will review the randomization rates and assess the difference between the actual and the projected rates, as well as the impact of these

assessments on overall trial size. If the study enrollment is inadequate, the reasons for exclusion may be scrutinized and actions may be suggested. An assessment of whether the trial should be continued will be made. The DSMB makes recommendations after each meeting to the NINDS Director about whether the study should continue or be stopped.

J.10. The Central IRB (Advarra)

The Central IRB will be the IRB of record for all sites. They will monitor the study's serious adverse events and review deviations on a continual basis. They will conduct annual reviews of the study.

DATA ANALYSIS

K.1. General statistical considerations

The general approach for inference for most of our aims will utilize a mixed-effects multi-period crossover model with 2 blocks and 2 treatment periods per block (of length 6 weeks). This model will be used to access the efficacy CNTX-6970 (300mg BID). Data will be analyzed on an intent-to-treat (ITT) basis with a per protocol analysis set used as a supportive analysis. Additionally, modified ITT (mITT) data sets will be prepared for supportive analyses to access the impact of the changes in the eligibility criteria across the different protocol versions.

In order to account for possible carryover effects, the multi-crossover model will incorporate carryover effect terms when appropriate. Only carryover effects from active to placebo will be formally tested, but a carryover effect from placebo to active will be studied in an exploratory fashion.

A patient who remains in the study for the first two treatment periods or for all four treatment periods will receive a balanced exposure to treatments randomized to receive. If patients discontinue treatment prior to completing the entire 24 weeks of study, all their data (including weekly assessments) from their time in the study will be used in the analyses. Because the main analysis is based on maximum likelihood estimation using a mixed-effects multi-period crossover model, the inferences will be unbiased under missing at random assumptions. No imputation of missing data is planned for this study, unless intensive investigations indicate that reliable and valid data imputation is possible, in which case multiple imputation will be performed and the analysis of the imputed data sets will serve as a sensitivity check of the results from the primary analysis based on the mixed-effects models (van Buuren et al, 2011).

All analyses will adjust for age, sex, K-L grade, and site.

All hypothesis tests will be two-sided using 5% significance level, and *p*-values unadjusted for multiple testing will be reported. The justification for this decision is that this is a Phase II investigation and all assessed outcomes are of specific and unique interest. Much more important than testing hypotheses is the efficient estimation of all quantities of interest, and this will be carried out throughout the analyses. Point estimates, 95% confidence intervals and standardized effect sizes (e.g., Cohen's d) will be reported for all estimated effects, regardless of whether the null hypotheses were rejected or not.

K.2. Hypothesis testing

Aim 1: Assess the safety of CNTX-6970 (300mg BID).

There will be no formal hypothesis testing under Primary Study Aim 1. All AEs and SAEs observed during different treatment periods will be summarized based on severity and relation to the active treatment (CNTX-6970 300mg BID). The reports will include tabulation of the AEs and SAEs by period, block, and treatment; point estimates and 95% CIs will be reported. Similarly, we will summarize relevant laboratory measures (see Section F.2.1) with appropriate estimates.

Aim 2: Assess the efficacy of CNTX-6970 (300mg BID) in comparison to placebo with respect to the primary outcome measure for pain due to osteoarthritis of the knee - WOMAC Part A.

WOMAC Part A (WOMAC-A) assessed only in the last two weeks of each 6-weeks treatment period will be used for this primary analysis in order to avoid any carryover effects, which are expected to vanish after 4 weeks; the specific carryover effect of the two doses of CNTX-6970 will be studied under Aim 3.

HYPOTHESIS 1: CNTX-6970 300mg BID is superior to placebo with respect to symptoms measured by WOMAC Part A.

The hypothesis testing is based on the WOMAC-A assessed only in the last 2 weeks of each 6-week treatment period. This provides a more than adequate 4-week washout interval. The adequacy of this 4-week period is based on extensive pharmacokinetics (PK) and pharmacodynamics (PD) of the study agent. The PK/PD analyses were performed for single dose and multiple ascending dose (MAD) studies using both QD and BID dosing regimens. For the single dose study, the PK study showed that the mean half-life ranged from 15.8 to 16.6 hours. For PD, the CNTX dossier reports that "… based on the half-life of CNTX-6970 determined in humans it is estimated that the duration of the PD may be between 8-12 hours." Specifically, after two doses of 300mg the plasma level is 10ng/ml 36 hours after dosing. The PD effect is less than 50% at 10ng/ml based on the MCP-1 interaction with CCR2. Hence, we anticipate a more than adequate washout period. Additionally, under Aim 6 (see below), we explicitly study the potential carryover effects in the statistical modeling. The statistical models will utilize a standard approach to adjust for carryover effects in a multi-period crossover design when estimating treatment effects, and allows for testing for the existence of carryover effect (Chen and Chen, 2014).

Aim 3: Assess the efficacy of CNTX-6970 (300mg BID) in comparison to placebo with respect to secondary outcome measures related to OA of the knee: (a) WOMAC-C (function subscale), (b) Hospital Anxiety and Depression Scale (HADS), (c) Patient Global Impression of Change (PGIC), (d) PROMIS Sleep Disturbance Scale – 6A, (e) Sleep Duration Question, and (f) Daily Knee Pain Intensity on a 0-10 NRS.

HYPOTHESIS 2: CNTX-6970 300mg BID is superior to placebo with respect to symptoms measured by (a) WOMAC-C (function subscale), (b) Hospital Anxiety and Depression Scale (HADS), (c) Patient Global Impression of Change (PGIC), (d) PROMIS Sleep Disturbance Scale–6A, (e) Sleep Duration Question, and (f) Daily Knee Pain Intensity on a 0-10 NRS.

Outcomes (a) and (f) are assessed at baseline and each study visit through week 24. Outcome (b) will be assessed at each in-person visit except baseline. Outcome (c) will be assessed at week 3 and week 24 only. Outcome (d) will be assessed at baseline and each in-person visit through week 24. Outcome (e) will be assessed at baseline and week 24 only. The analysis plan for outcomes (a), (b), (d), and (f) will be similar to the analysis for aim 2 except that the model will adjust for possible carryover effects in placebo-treated periods that are preceded periods with the active drug treatment; the analysis plan for outcomes (c) and (e) will follow the same approach as in aim 4 below.

Aim 4: Assess the efficacy of CNTX-6970 (300mg BID) in comparison to placebo with respect to general outcomes of pain: (a) Pain Catastrophizing Scale –Short Form 6, (b) PROMIS Physical Functioning Short-Form 6b, (c) Patient Health Questionnaire (PHQ) – Depression, (d) Generalized Anxiety Disorder – 2 item scale (GAD-2); (e) Tobacco, Alcohol, Prescription medication, and other Substance use Tool (TAPS-1); and (f) Opioid Use Questionnaire.

HYPOTHESIS 3: CNTX-6970 300mg BID is superior to placebo with respect to outcomes measured by (a) Pain Catastrophizing Scale –Short Form 6, (b) PROMIS Physical Functioning Short-Form 6b, (c) Patient Health Questionnaire (PHQ) – Depression, (d) Generalized Anxiety Disorder – 2 item scale (GAD-2), (e) Tobacco, Alcohol, Prescription medication, and other Substance use Tool (TAPS-1), and (f) Opioid Use Questionnaire.

Outcomes (a)-(f) are assessed at baseline and week 24. Testing hypotheses for these outcomes will not benefit from the multi-period crossover design because they are assessed only at baseline and week 24. Instead, we will test the hypotheses by comparing subjects taking active drug versus placebo at the last treatment period ending on week 24, controlling for the baseline value of the outcome.

Aim 5: Assess the efficacy of CNTX-6970 (300mg BID) in comparison to placebo with respect to biomarkers of pain and inflammation: (a) Staircase-Evoked Pain Assessment, (b) Serum and synovial fluid levels of chemokines and cytokines; and (c) Synovial monocyte chemoattractant protein-1/CCR-2 receptor binding inhibition in blood and synovial fluid.

HYPOTHESIS 4: Compared to placebo, CNTX-6970 300mg BID will result in more improvement with respect to (a) Staircase-Evoked Pain Assessment, (b) Serum and synovial fluid levels of chemokines and cytokines; and (c) Synovial monocyte chemoattractant protein-1/CCR-2 receptor binding inhibition in blood and synovial fluid.

The Staircase-Evoked Pain Assessment will be assessed at Baseline and each in-person study visit through Week 24 (Week 3, 6, 9, 12, 15, 18, 21, and 24/ET visits).

Hypothesis 4 will be tested with the same approach as for testing the hypotheses under Aim 3.

K.3. Exploratory analyses

Aim 6. If CNTX-6970 is more effective than placebo, evaluate the following characteristics of its effect: (a) onset of action; (b) carryover effect after treatment discontinuation.

We will study the change of symptoms over time using a time trend in our statistical models within each of the 6-week treatment periods to model the weekly WOMAC-A measure.

Under this aim, the interest is not in hypothesis testing, but rather in understanding the progression and magnitude of onset of action and the carryover effects of the CNTX-6970 compound. This approach will allow an elucidation of the nature of action of the compound.

Aim 7. Identify biomarkers related to pain from OA of the knee, course of the disease, and response to treatment with CNTX-6970.

Statisticians on the study team have published methods for analysis of complex multimodal data, such as EEG and brain imaging for developing optimal treatment decision (e.g., Ciarleglio et al, 2018; Jiang et al, 2020; Park et al, 2020a; Park et al, 2020b; Petkova et al, 2020), one of the objectives of precision medicine. Although this previous work has been primarily motivated by mental health research (e.g., depression), the methodologies developed apply equally to pain and other medical conditions. As necessary, new methodologies will be developed for the joint analysis of outcome measures.

The results of these investigations are expected to be features (for example, the variability of pain across days) or combinations of features that constitute biosignatures for specific response to CNTX-6970, placebo response, or non-specific response to both an active agent and placebo. For all indices and estimates, we will perform extensive internal cross-validation. All results from these discovery analyses will be reported as exploratory and subject to independent validation. The interpretation of the results will also be in the context of all analyses that were performed, and will emphasize the discovery nature of the work.

Aim 8: Explore sociodemographic and clinical predictors of response to CNTX-6970.

The rich data collection will allow for exploration of the relationship between treatment with CNTX-6970 and placebo, and sociodemographic and clinical covariates. No hypotheses will be tested under this aim. Rather various research questions will be addressed, such as:

Q1: Do women show a greater reduction in pain ratings following CNTX-6970 compared to placebo, relative to men?
Q2: Do patients with high levels of psychological distress show a greater reduction of pain with CNTX-6970, relative to patients who do not have high levels of psychological distress?

K.4. Sample Size

The sample size for this study was determined based on the primary efficacy aim to detect a significant effect of each dose of CNTX-6970 compared to placebo in the multi-period crossover design model. Power was computed for effect sizes ranging from 0.25 to 0.50 using Monte Carlo simulation. Data were simulated from multi-period crossover models with block, period, and carryover effects, and varying within and between subject variability. Although carryover effects can be efficiently isolated and estimated in the analysis stage by using mixed-effects models, power for the primary hypothesis was computed using only WOMAC-A assessed during the last 2 of 6 weeks per treatment period to minimize carryover effects. The power of the test depends on

the relationship of the between-subject variability to the within-subject variability. In general, the between-subject variability is larger than the within-subject variability, but we investigated a range of Between/Within (B/W) variance ratios (5/1, 2/1). With a total sample size of *n*=55, there will be more than adequate power for the study using a two-sided Type I error rate of α=0.05. A power analsyis was conducted with n=50 and the power to detect an effect size (for an active treatment vs. placebo) of Cohen's d=0.35 is approximately 95% when the ratio B/W=2/1. Assuming a 15% early termination rate during each of the 4 periods will yield a sample size of n=26 with complete observations at all treatment periods for this simulation. If we conservatively assume that only subjects with complete data will be analyzed, the corresponding power with *n*=26 is approximately 81%. Because we will use all available data from all enrolled subjects in the analysis, the actual power in the case of 15% early termination rate per treatment period will be larger than 81%, and anywhere between 81% and 95%. If the ratio B/W is larger than 2, say B/W=3, 4 or 5, the power for detecting the treatment effects is much larger. For example, with only 26 patients, if B/W=5, the power to detect an effect of magnitude Cohen's d=0.35 is 0.96. Note that the reported test-retest reliability of the WOMAC-A measure is 0.86, which corresponds to a B/W=7.

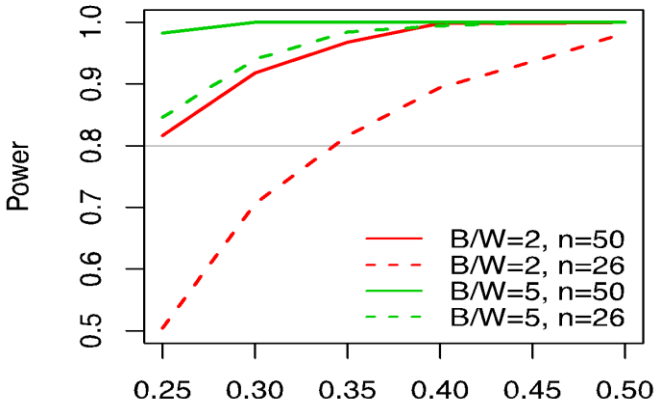

Figure 2: Power versus Cohen's d effect size. B/W indicates the ratio of between-subject to within-subject variance of the WOMAC-A measure. Assuming that only data from completers will be used in the analysis gives a lower bound of the power that can be achieved for the given range of effect sizes.

PUBLICATION POLICY

See EPPIC-Net Publication Policy.

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INVESTIGATOR STATEMENT

EN20-01: A 24-WEEK STUDY TO EVALUATE THE SAFETY AND EFFICACY OF CNTX-6970 IN SUBJECTS WITH MODERATE TO SEVERE KNEE OSTEOARTHRITIS PAIN
VERSION 9.0 22JAN2024

Principal Investigator Signature Page
I confirm that I have read the above EN20-01 protocol version 9.0, dated 22Jan2024. I understand it, and I will work according to the moral, ethical, and scientific principles governing clinical research as set out in the principles of GCP and as described in 21 CFR parts 50, 54, 56, and 312 and according to applicable local requirements. I will ensure all site staff delegated to the study are appropriately trained on this version of the protocol.

Confidentiality Statement
The confidential information in this document is provided to you as a Principal Investigator or Consultant for review by you, your staff, and the applicable Institutional Review Board/Ethics Committee. Your acceptance of this document constitutes agreement that you will not disclose the information contained herein to others without written authorization from the NINDS.

Principal Investigator:

_____ _____
Signature Date

APPENDICES

Appendix I: BLINDED INFORMATION

Available upon request.

Appendix II: Cytokines & Chemokines (from the Inflammation MAP Multiplex panel):

Adiponectin
Alpha-1-Antitrypsin
Alpha-2-Macroglobulin
Apolipoprotein(a)
Beta-2-Microglobulin
Brain-Derived Neurotrophic Factor
C-Reactive Protein
Complement C3
EN-RAGE
Eotaxin-1
Factor VII
Ferritin
Fibrinogen
Granulocyte-Macrophage Colony-Stimulating Factor
Haptoglobin
Immunoglobulin A
Immunoglobulin M
Intercellular Adhesion Molecule 1
Interferon gamma
Interleukin-1 alpha
Interleukin-1 beta
Interleukin-1 receptor antagonist
Interleukin-10
Interleukin-12 Subunit p40
Interleukin-12 Subunit p70
Interleukin-17
Interleukin-18
Interleukin-2
Interleukin-23
Interleukin-3
Interleukin-4
Interleukin-5
Interleukin-6
Interleukin-7
Interleukin-8
Macrophage Inflammatory Protein-1 alpha
Macrophage Inflammatory Protein-1 beta
Matrix Metalloproteinase-3
Matrix Metalloproteinase-9
Monocyte Chemotactic Protein 1
Myoglobin
Plasminogen Activator Inhibitor 1
Pulmonary and Activation-Regulated Chemokine
Serum Amyloid P-Component
Stem Cell Factor
T-Cell-Specific Protein RANTES
Thyroxine-Binding Globulin
Tissue Inhibitor of Metalloproteinases 1
Tumor Necrosis Factor alpha
Tumor Necrosis Factor beta
Tumor necrosis factor receptor 2
Vascular Cell Adhesion Molecule-1
Vascular Endothelial Growth Factor
Vitamin D-Binding Protein
von Willebrand Factor

Appendix III: Concomitant Medications

Subjects who take prohibited concomitant medications during the trial will be discontinued, unless the discontinuation presents an unacceptable medical risk. The justification to allow the subject to continue in the trial will be made by the Sponsor with medical input from a site investigator (PI, clinical Sub-I), and will be documented. Whether or not the participant is allowed to remain in the trial, this will be reported as a major protocol deviation and not a waiver.

The table below lists prohibitions and restrictions by medication class, including representative medications within class. Medications within each class include, but are not limited to, examples listed in this table. Any questions regarding prohibited and restricted medications should be discussed with the Medical Monitor or appropriate designee.

The direct FDA link for all CYP2C9, CYP3A4, and P-GP interactions is: Drug Development and Drug Interactions | Table of Substrates, Inhibitors and Inducers | FDA

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Analgesics	Y	Y	Acetaminophen, opioids, and select anticonvulsants are allowed as a rescue medication (as described in the respective sections). Other analgesics as specified below: Continuous use of one of the following medications prescribed for pain is allowed: tramadol, gabapentin, duloxetine, pregabalin, milnacipran, or tricyclic antidepressants. Use must be chronic for at least 12 weeks and at a stable dose for at least 4 weeks before Screening. Intermittent use of opioids is allowed if use is ongoing for at least 4 weeks before Screening, and at a frequency no more than 4 days/week, and the opioid is not taken within 24 hours of a study visit. As needed use of acetaminophen is allowed. Continuous use of medical marijuana (or equivalent) that is chronic for at least 12 weeks and at a stable dose for 4 weeks is allowed. Continuous use of topical creams (includes CBD topicals) is allowed if use is chronic and stable for at least 12 weeks. Intermittent use of topical creams is allowed if at a frequency of no more than 4 days/week. Other analgesics (for example, NSAIDS) are not allowed.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Anesthetics, general	Y	—	If procedures requiring general anesthesia are to occur/have occurred, please contact MGH CTNI to report the medical condition(s).
Anesthetics, local	Y	N	—
Anorexics	N	N	—
Antacids	Y	Y	—
Antiacne	Y	Y	Topical agents only, including topical antibiotics. Isotretinoin (Accutane) is not allowed.
Antianginal agents	N	N	Calcium agonists (e.g. diltiazem, nifedipine, verapamil) and beta blockers (e.g. carvedilol, propranolol, metoprolol) are excluded if used to treat angina.—
Antiarrhythmics	N	N	Amiodarone is excluded.
Antiasthma agents	Y	N	—
Antibiotics	Y	Y	Chronic use of topical antibiotics for acne is allowed, except for the MAOI linezolid (Zyvox) and isoniazid, which are not allowed. Erythromycin, clarithromycin, rifampin, and azithromycin are excluded.
Anticoagulants	N	N	Common drugs include: Warfarin (Coumadin) is not allowed. Antiplatelet agents are allowed (see “Antiplatelets”).
Anticholinergics	Y	Y	Except for scopolamine, which is not allowed.
Anticonvulsants	N	Y[b]	Gabapentin, and pregabalin are allowed. Other anticonvulsants are not allowed, including lamotrigine and carbamazepine.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Antidepressants	N	Y[b]	Monoamine oxidase inhibitors (which may have unknown drug-drug interactions) are excluded. Concomitant use of trazodone (up to 200mg daily), nefazodone or duloxetine are allowed No dose changes are allowed during the study.
Antidiarrheal preparations	Y	N	Only loperamide HCl (Imodium), bismuth subsalicylate (Pepto-Bismol), and kaolin preparations are allowed.
Antifungals, systemic	N	N	
Antifungals, topical	Y	Y	
Antihistamines	Y	Y	The use of combinations containing pseudoephedrine or phenylephrine is not allowed. Combination products containing the word nighttime or are specifically marketed for before sleep routinely include an antihistamine and are not allowed. Combination products ending in "-D" routinely contain a stimulant such as phenylephrine, and the appropriate limits above apply to them. (See "Cough and Cold Preparations" for combination products.)
Antihypertensives	N	Y	Diltiazem, verapamil are excluded
Anti-impotence medications	Y	Y	—
Anti-inflammatory drugs	Y	N	Indomethacin (Indocin) and systemic corticosteroids are not allowed for PRN or chronic use. NSAID use is exclusionary.
Antimigraine	N	N	Common one includes: Triptans not allowed.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Antinauseants/ Antiemetics	Y	N	Phosphoric acid preparations (Emetrol, Emecheck), bismuth subsalicylate (Pepto-Bismol), cola syrup, 5-HT_3 receptor antagonists (e.g., ondansetron), and prokinetic agents (metoclopramide) are allowed only for PRN. Scopolamine is not allowed for PRN or chronic use (see section on antihistamines).
Antineoplastics/ Immunosuppressant agents	N	N	Common drugs include: Interferons, methotrexate, and other immunosuppressant agents are not allowed. Call MGH CTNI for approval for certain cases in cancer remission maintaining therapy.
Antiobesity/ Appetite suppressants	N	N	Common drugs include: OTC Alli (Xenical), sibutramine (Meridia), and phentermine (Adipex-P and others) are not allowed.
Antiplatelet agents	N	Y[b]	Aspirin (maximum 325 mg/day) and clopidogrel (Plavix) are allowed. Note that use of an SSRI or of a triple uptake inhibitor may increase bleeding times and possibly prothrombin times.
Antipsoriatic treatments	Y	Y	Only topical agents are allowed. Acitretin (Soriatane) is not allowed.
Antipsychotics	N	N	—
Antismoking medications	N	N	Common drugs include: Varenicline (Chantix) is not allowed. Chronic nicotine replacement may be allowed in certain cases after review with MGH CTNI.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Antiviral agents	Y	Y	Only oral or topical agents are allowed. Only acyclovir, famciclovir, valacyclovir, penciclovir, docosanal, trifluridine, and vidarabine are allowed. Amantadine, rimantadine, indinavir, nelfinavir, ritonavir, saquinavir are not allowed. Tamiflu (oseltamivir phosphate), and Relenza (zanamivir) inhalants are permitted for influenza prophylaxis but use is limited to a 7- to 14-day course in accordance with prescribing information. Interferons are not allowed. Boceprevir (Victrelis®) is not allowed.
Anxiolytics	N	Y	Chronic, stable treatment with benzodiazepines is allowed. Stable in dosing at least four weeks prior to randomization is required.
Barbiturates	N	N	
Benign prostatic hyperplasia treatments	N	Y[b]	Male patients who have symptoms of obstructed voiding should not be included in the study. Surgically or medically treated patients must be asymptomatic and receiving a stable dosage of allowed medications (α-1 blockers, finasteride, or dutasteride) for 1 month before screening.
Buspirone	N	Y	Stable in dosing at least four weeks prior to randomization is allowed.
Cough/cold preparations	Y	N	Use of cough and cold preparations containing pseudoephedrine or dextromethorphan is not allowed, as are those containing phenylephrine. Combination products ending in "-D" routinely contain a stimulant such as phenylephrine, and the appropriate limits apply to them. (See "Antihistamines".)
Diuretics	Y	Y[b]	Episodic use of diuretics is restricted to treatment of premenstrual symptoms. For chronic use, medication and dosage should be stable for 1 month before screening.

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Dopaminergics	N	Y	Common drugs include: Dopamine agonists for restless leg syndrome are allowed for chronic use.
Gastrointestinal: ● H_2-blockers/ ● proton pump inhibitors/ ● prokinetic agents	Y	Y	Cimetidine (Tagamet),) metoclopramide, omeprazole and pantoprazole are not allowed within 7 days of Baseline.
Hormonal (noncontraceptive) therapies	N	Y	See below.
Hormone suppressants	N	Y[b]	Only finasteride (Proscar) and dutasteride (Avodart) are allowed for chronic use. Bromocriptine (Cycloset, Parlodel) is not allowed
Hormones: reproductive	N	Y	Systemic hormonal contraceptives (oral contraceptives of estrogen and progestin combinations, depot injections such as Depo-Provera, the contraceptive implant Implanon, or transdermaslly delivered contraceptives such as Ortho Evra) are allowed
Hormones: thyroid	N	Y	Thyroid hormone replacement is allowed (dosage of thyroid medication should be stable for 3 months before screening).
Hypoglycemic agents	N	Y	Oral and injectable hypoglycemic agents are allowed. Insulin is not allowed
Hypolipidemics	N	Y[b]	Common drugs includes: Ezetimibe (Zetia) is allowed for chronic use.
Hypolipidemics: bile acid sequestrants	N	N	—
Hypolipidemics: fibrates	N	Y[b]	Common drugs include: Gemfibrozil and fenofibrate are allowed for chronic use.
Hypolipidemics: niacin	N	N	Common drugs include: Oral niacin and niacinamide are not allowed
Hypolipidemics: statins	N	Y[b]	Lovastatin, simvastatin, pravastatin, fluvastatin, and rosuvastatin are allowed

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Laxatives	Y	Y[a]	Only fiber-based products and docusate sodium (Colace) are allowed
Lithium	N	Y	Stable in dosing at least four weeks prior to randomization is allowed.
Muscle relaxants	N	N	—
NMDA receptor antagonist	N	N	Common drugs include: Memantine is not allowed.
Opioid agonists/analgesics (e.g., codeine, hydrocodone, methadone, morphine, maperidine, propoxyphene) and antagonists (e.g., naltrexone, naloxone, nalmefene)	Y	N	Intermittent use allowed, if the following criteria are met: • use has been ongoing for at least 4 weeks prior to Screening, • frequency is no more than 4 days/week, and • no dosing within 24 hours of a study visit.
Proton pump inhibitors or H2 receptor blockers	Y	Y	
Sedatives/hypnotics	N	Y	Ongoing, stable hypnotic therapy (e.g., zolpidem, zaleplon, benzodiazepine hypnotics, and low-dose trazodone 50-200mg) will be allowed during the study. Eszopiclone is not allowed.
Steroids/systemic	Y	N	Systemic steroid treatment will be allowed only for medical emergencies, such as severe allergic reactions
Steroids/topical and inhalant	Y	Y	—
Steroids/intra-articular	N	N	—

Drug Name or Class	Episodic Use (PRN)	Chronic Use	Restrictions/Comments
Stimulants	N	Y	Chronic use allowed if taken for at least 12 weeks and at a stable dose for 4 weeks. The use of combinations containing pseudoephedrine or phenylephrine is not allowed.
Supplement and herbals	N	N	The following are the only exclusionary supplements/herbals: St. John's Wort, garlic, chamomile, grapefruit and pineapple juice
Vaccines	Y	NA	—

a If being taken prior to enrolling in the study.

b If being taken for at least 3 months prior to enrolling in the study and the dose has been stable for at least 1 month.

Appendix IV: Amylase and Lipase Normal Ranges_12Jan2024

January 12, 2024

Thank you for contacting me concerning normal values on amylase and lipase across institutions. As mentioned on the call and emails, I reviewed all of the active sites lab reference ranges for lipase and amylase. I reviewed the normal values programmed into the EDC and compared the values to the reference ranges you have on file in the TMF (collected with regulatory documents), and cross-checked both ranges against the ranges listed on recent lab source documents. As stated by you, out of the 14 open sites (113 is not included as they have not randomized a subject), 5 sites were flagged to have inconsistent ranges:

Site	PI	Amylase Reference Range Programmed into EDC (U/L)	Amylase Reference Range listed in Lab Normal Values (U/L)	Lipase Reference Range Programmed into EDC (U/L)	Lipase Reference Range listed in Lab Normal Values (U/L)	Values match most recent subjects lab source?	Comments	Date of Normal Values (or date confirmed on recent source)
101 - UWisc	Sehgal	25-125	25-125	8-78	8-78	No	Lipase range from recent subject visit (13Dec23) **0-60**	10/2021
102 - UCSD	Wallace	28-100	28-100	13-60	13-60	Yes		2023
104 - MGH	Mao	3-100	3-100	13-60	13-60	Yes		9/14/2023
106 - UPN	Asburn	29-103	29-103	11- 82	11- 82	Yes		11/22/2023
107 - UFL	Przkora	28-100	28-100	0-70	0-70	Yes		11/1/2022
108 - UWA	Stacey	27-106	27-106	0-70	0-70	Yes		3/6/2023
109 - MSM	Robinson-Papp	30-110	**31**-110	8 -78	**14-72**	No	Lipase range from recent subject visit (08Nov23) **13-78**	2023
111 - URO	Philip	28-100	28-100	13-60	13-60	Yes		12/4/2023
114 - UTS	Jain	25-125	25-125	0-60	0-60	Yes		12/19/2023
117 - UPT	Wasan	0-65	0-65	15-70	15-70	Yes		11/15/2023
118 - NYU	Samuels	25-125	25-125	7-78	7-78	No	Lipase range from recent subject visit (27Nov23) **8-78**	3/1/2023
119 - UCD	Wise	33-130	**28**-130	13-51	13-**606**	No	Lab values on source match lab normal values in TMF, not in EDC	4/26/2023

130 - MMC	Canaan	23-103	23-103	11-82	11-82	No	Amylase range from recent subject visit (20Nov23): **25-125**	9/15/2023
132 - HRN	Reed	21-101	21-101	7-60	7-60	Yes		10/13/2023

My review and recommendations are as follows. Note all values described below are U/L.

Reference ranges for lab tests are set by each individual laboratory based on the specific method/instrument used (which may differ from lab to lab), normal range data if a formal reference range study was performed, manufacturer provided data, literature reviews, national standards (e.g. HbA1c, LDL) patient characteristics (e.g. age, gender, etc) and potentially many other factors. For most routine chemistry tests the expected normal values across labs are often quite similar albeit minor but clinically irrelevant differences are commonly observed. The key is to differentiate between minor variations of no significance versus variations that are clinically significant. In this specific case I assume you are looking for potential evidence of pancreatitis. Considering this the following observations can be made.

1. The greatest outlier shown on the table above is amylase at UPT (0-65). I spoke with the lab director and this was an historical range set many years ago and they realize it is inappropriate for their method and plan to change it to 31-118 in February of 2023.

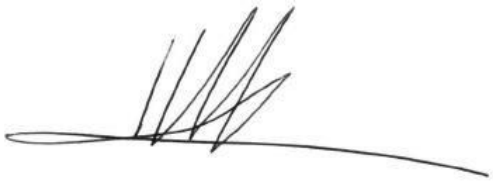
2. On the low end of both amylase and lipase there is no significance in your clinical scenario of hypoamylasemia or hypolipasemia. To avoid unnecessary flagging of “abnormal” low results which are not real or significant, I would suggest setting your multicenter lower range at 0 or 3 for amylase and 0 for lipase.

3. Given the intention of UPT to change their ranges that leaves us with the upper end of normal for amylase and lipase. Given the inherent accuracy and imprecision of these tests and variation in ranges between institutions, there is no meaningful difference between an amylase of 100 versus 130 or a lipase of 60 versus 82. For example, in one guideline “an amylase of greater than 3 times the upper limit of normal supports the diagnosis of acute pancreatitis”. In the case of lipase, “if the sample has 3 to 10 times the normal level of lipase, then it's likely to be acute pancreatitis” according to various sources. Again, to avoid unnecessary flagging of “abnormal” values of no significance I would set the upper range for the study at 130 for amylase and 82 for lipase.

4. Amylase itself is not optimal for the diagnosis of acute pancreatitis. As stated in one guideline “Current guidelines and recommendations indicate that lipase should be preferred over total and pancreatic amylase for the initial diagnosis of acute pancreatitis and that the assessment should not be repeated over time to monitor disease prognosis”. Although it may not be important at this time to eliminate amylase from the study protocol its use is not necessary for detecting pancreatitis.

5. Of note we are currently standardizing normal values for all routine tests across the many hospitals at Mass General Brigham to avoid the confusion over what is normal resulting from minor difference in reference ranges.

Please let me know if you need further information or analysis. Note for regulatory purposes I am the CLIA Medical Director for the laboratories at MGH.



Kent Lewandrowski, MD

Kent Lewandrowski, M.D., FCAP
Associate Chief of Pathology
Director of Clinical Laboratories and
Molecular Medicine
Professor, Harvard Medical School
Editor, Point of Care Journal

Appendix V: Glossary

GLOSSARY:

Abbreviation	Entity
ACR	American College of Rheumatology
AE	Adverse Event
ALK	Alkaline phosphatase level
ALT	Alanine Aminotransferase Test
AST	Aspartate Aminotransferase
AUC	Area Under Curve
BID	Twice daily
BMI	Body mass index
BUN	Blood urea nitrogen
CABG	Coronary Artery Bypass Graft
CBD	Cannabidiol
CBRD	Center for Biospecimen Research and Development (at NYU)
CCC	Clinical Coordinating Center
CCC/PI	Principal Investigator of the Clinical Coordinating Center
CCC/PM	Project Manager of the Clinical Coordinating Center
CCL	Chemokine Ligand
CCR	Chemokine receptor
CDE	Common Data Element
CDER	Center for Drug Evaluation and Research
CFA	Complete Freund's Adjuvant (animal model of inflammatory joint pain)
CFR	Code of Federal Regulations
CI	Confidence interval
cIRB	Central Institutional Review Board
Cmax	Mean maximum plasma concentration
CRA	Clinical Research Associate
CRF	Case Report Form
CXCR	Chemokine receptor
DCC	Data Coordinating Center
DCC/PI	Principal Investigator of the Data Coordinating Center
DCC/PM	Project Manager of the Data Coordinating Center
DDE	Direct Data Entry
DDI	Drug-drug interactions
dL	Deciliters
DSM-IV	Diagnostic and Statistical Manual, Fourth Edition
DSMB	Data Safety and Monitoring Board
ECG	Electrocardiogram

eCRF	Electronic case report form
EDC	Electronic Data Capture
eGFR	Estimated glomerular filtration rate
EPPIC-Net	Early Phase Pain Investigation Clinical Network
ET	Early Termination
FDA	US Food and Drug Administration
GAD-2	Generalized Anxiety Disorder – 2 item scale
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GI	Gastrointestinal
GGT	Gamma-Glutamyl Transferase
GLP	Good Laboratory Practice
HADS	Hospital Anxiety and Depression Scale
HEAL	Helping to End Addiction Long-term
HIPAA	Health Insurance Portability and Accountability Act
hr	Hour
IA	Intra-articular
IB	Investigator's Brochure
ICF	Informed consent form
IND	Investigational New Drug
IP	Investigational Product
IRB	Institutional Review Board
IXRS	Interactive [Web/Phone] Response System
kg	Kilograms
K-L	Kellgren-Lawrence System for Classification of Osteoarthritis
KRI	Key Risk Indicators
m	Meter
MAD	Multi-Ascending Dose
MCH	Mean corpuscular hemoglobin
MCHC	MCH Concentration
MCP	Monocyte Chemoattractant Protein
MCV	Mean corpuscular volume
MDRD	Modification of Diet in Renal Disease
mg	Milligrams
µg	Micrograms
MGH	Massachusetts General Hospital is the Clinical Coordinating Center
MIA	Mono-Iodoacetate (animal model of osteoarthritic pain)
mL	Milliliters
µL	Microliters
MOP	Manual of Procedures
NESS	New England Survey System

NIH	National Institutes of Health
NINDS	National Institute of Neurological Disorders and Stroke
NOAEL	No Observed Adverse Effect Level
NRS	Numeric Rating Scale
NSAID	Nonsteroidal anti-inflammatory drug
NYHA	New York Heart Association
NYU	New York University is the Data Coordinating Center
OA	Osteoarthritis
OUQ	Opioid Use Questionnaire
PD	Pharmacodynamic(s)
PGIC	Patient Global Impression of Change
P-gp	P-glycoprotein
PHI	Protected Health Information
PHQ	Patient Health Questionnaire
PI	Principal Investigator of a clinical site
PK	Pharmacokinetic(s)
PNL	Partial Sciatic Nerve Ligation (animal model of nerve injury pain)
PM	Project Manager
PPI	Protocol Principal Investigator
PRN	As needed
PRO	Patient-Reported Outcome
PROMIS	Patient-Reported Outcomes Measurement Information System
QD	Once a Day
QTcF	QT Correction using Fredericia's Formula
RA	Rheumatoid arthritis
RANTES	T-cell specific protein
RBM	Risk Based Monitoring
RFA	Radiofrequency Ablation
SAD	Single-Ascending Dose
SAE	Serious Adverse Event
SAFER	State, Assessibility, Face and Ecological Validity and the Rule of the 3 Ps [persistent, pervasive, pathological]
SAP	Statistical Analysis Plan
TAPS-1	Tobacco, Alcohol, Prescription medication, and other Substance use Tool
TC	Trial Committee, formerly ENMOC
TEAEs	Treatment emergent adverse events
UA	Urinalysis
ULN	Upper Limit of Normal
V [#]	Visit [Number]
WOCBP	Woman/Women of Childbearing Potential
WOMAC	Western Ontario and McMaster Universities Arthritis Index
XCR	Chemokine receptor