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A double-blind, placebo-controlled, multi-crossover trial of treatment with a chemokine antagonist for knee osteoarthritis pain

Robert R. Edwards[a,*], Thaddeus Tarpey[b], Michael Ashburn[c], Caitlin Baer[b], Allison Campbell[a], Robert H. Dworkin[d], Gabrielle Gaspard[b], Martina Flynn[a], Erinn Hade[b], Nitin Jain[e], Heidi Judge[a], Cornelia Kamp[d], Yi Li[b], Sharon Meropol[b], Eva Petkova[b], Annie Philip[d], Rene Przkora[f], James P. Rathmell[a], Jessica Robinson-Papp[g], Jonathan Samuels[b], Nalini Seghal[h], Jackie Sienty[b], Brett Stacey[i], Mark Wallace[j], Ajay D. Wasan[k], Barton Wise[l], Chang Yu[b], Maurizio Fava[a], Andrea B. Troxel[b]

Abstract

Osteoarthritis, especially knee osteoarthritis, is a leading cause of disability and reduced quality of life. The etiology of pain in osteoarthritis is multifactorial, and one promising potential treatment approach involves targeting chemokine systems. The present study was a phase 2, multisite, multiperiod randomized crossover trial of CNTX-6970, a small molecule and selective oral cytokine chemokine receptor type 2 (CCR2) and CCR5 antagonist, in patients with painful knee osteoarthritis (OA). It represents the first trial performed within the National Institutes of Health's Early Phase Pain Investigation Clinical Network. The primary objectives were to evaluate the safety and efficacy of CNTX-6970, relative to placebo, for the treatment of moderate to severe pain related to knee OA. A total of 55 participants were randomized in this multiperiod crossover trial. Linear mixed effects models revealed no significant pain-related benefits of active medication; indeed, trial participants reported slightly higher knee pain intensity when taking the novel chemokine antagonist CNTX-6970 than when taking placebo. In addition, biomarker analysis revealed notably higher level of serum monocyte chemoattractant protein 1 levels when patients were on CNTX-6970 compared to placebo. Overall, although CNTX-6970 was safe and relatively well-tolerated, pharmacologic blockade of specific chemokine receptors with this compound was not effective in reducing moderate-to-severe knee osteoarthritis pain.

Keywords: Pain, Osteoarthritis, Chemokine, CCR2, CCR5, Crossover

1. Introduction

Osteoarthritis (OA) is the most common form of arthritis and a leading cause of disability,[9,31] predominantly because of persistent movement-related pain. The lifetime risk of developing symptomatic, painful knee OA is estimated to be nearly 50%, with major increases in risk/prevalence among older adults as well as persons who are overweight or obese.[3] Given the steady aging of the global population and increasing prevalence of obesity, the impact of OA is expected to rise substantially.[10,31]

The etiology of OA pain is multifactorial, with both intra-articular (eg, synovitis) and extra-articular (eg, central sensitization) risk factors,[7,20,28] which may contribute to the finding that many treatments for OA pain are effective only for a subset of patients.[34] Hence, there is a strong need for new treatments with novel mechanisms of action. Although OA pain was initially conceptualized as because of age-related "wear-and-tear," accumulating evidence has indicated that OA is a systemic musculoskeletal disease involving the activation of innate and adaptive immune systems accompanied by inflammation.[20,37]

One promising area of investigation involves targeting chemokine systems that contribute to inflammation. Chemokines are small chemotactic peptides that control the trafficking of leukocytes to their target tissue.[12] Preclinical data suggest that the CC chemokine receptor type 2 (CCR2) and its endogenous ligands (eg, MIP-1 CCL2) are upregulated in cells responsible for the modulation of pain signals in peripheral nerves, spinal cord, and microglia.[1,15,24] In addition, CCR2 appears to play a role in the signaling of pain at the level of the joint, and CCL2 (the endogenous ligand for CCR2) plays a potentially causal role in the occurrence and development of OA.[37] Conventional nonsteroidal

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R. R. Edwards, T. Tarpey, M. Fava, and A. B. Troxel contributed equally to the development of the manuscript.

[a] Mass General Brigham, Harvard Medical School, Boston, MA, United States, [b] NYU Langone, New York, NY, United States, [c] University of Pennsylvania, Philadelphia, PA, United States, [d] University of Rochester, Rochester, NY, United States, [e] University of Michigan, Ann Arbor, MI, United States, [f] University of Florida, Gainesville, FL, United States, [g] Icahn School of Medicine at Mount Sinai, New York, NY, United States, [h] University of Wisconsin, Madison, WI, United States, [i] University of Washington, Seattle, WA, United States, [j] University of California at San Diego, San Diego, CA, United States, [k] University of Pittsburgh, Pittsburgh, PA, United States, [l] University of California Davis, Sacramento, CA, United States

**Corresponding author. Address: Mass General Brigham, Harvard Medical School, Boston 02115, MA, United States. Tel.: 617-732-9486; E-mail address: rredwards@bwh.harvard.edu (R. R. Edwards).*

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anti-inflammatory drugs (NSAIDs), via their inhibitory actions on cyclooxygenase and prostaglandin pathways, have indirect effects on chemokine levels but do not directly antagonize their function.[1,24]

The present report describes a phase 2, multisite, multiperiod crossover trial of CNTX-6970, a small molecule and selective oral chemokine CCR2 and CCR5 antagonist, in patients with painful knee OA. The rationale for developing CNTX-6970 for the management of painful OA stems from mechanisms directly related to the affected joint, as well as the effects on neural signaling.[24,34,37] CNTX-6970 does not significantly cross the blood–brain barrier, obviating the side effects associated with many analgesics, including abuse and dependence.[30] Chemokine antagonists show effectiveness across multiple animal models of OA, as demonstrated by increased tolerance of weight bearing in an experimentally injured knee or paw.[19,21,29] The trial was performed within the National Institutes of Health's (NIH) Early Phase Pain Investigation Clinical Network (EPPIC-Net), a component of the HEAL Initiative. The network aimed to improve pain treatment by conducting trials on analgesics with minimal abuse potential to identify nonaddictive treatments for pain.[14,17] This study's primary objectives were to evaluate the safety and efficacy of CNTX-6970 for the treatment of moderate to severe knee OA pain.

2. Methods

2.1. Trial oversight

This study was performed in accordance with the Declaration of Helsinki. A Central Institutional Review Board (IRB), Advarra, was used as the IRB of record for all participating clinical sites. Central IRB approval was obtained before participant enrollment and screening, and all subjects signed written informed consent. A National Institute of Neurological Disorders and Stroke Data and Safety Monitoring Board reviewed the progress of the study and monitored participant enrollment, outcomes, adverse events (AEs), and other issues related to participant safety.

2.2. Inclusion/exclusion criteria and eligibility assessment

A total of 22 academic and commercial clinical sites across the United States participated. The trial was preregistered on ClinicalTrials.gov (#NCT05025787, "A Study to Evaluate the Safety and Efficacy of CNTX-6970 in Subjects with Knee Osteoarthritis Pain"), with deidentified data stored on a HEAL registry. The trial was designed to evaluate changes in pain related to primary OA of the knee. Participants aged 40 to 90 years with chronic knee OA (Kellgren–Lawrence [K–L] grade 1-4) were eligible if they had experienced stable moderate to severe pain in the index knee (ie, Western Ontario and McMaster Universities Arthritis Index Part A [WOMAC-A] pain score between 20 and 45 points, with pain variability of <1.2 standard deviations [SD])[5] for at least 6 months before screening and had failed at least 2 prior OA therapies.

Key exclusion criteria included prior knee arthroplasty on the index knee, any other prior surgery on the index knee within 12 months of screening, the presence of painful conditions in the index knee unrelated to OA, or chronic pain in the lower extremities equal to or greater than the index knee pain. Participants were excluded if they were unable to refrain from using certain analgesic medications, including NSAIDs, within 5 days before randomization or during study participation.

Participants were permitted to use acetaminophen, and concomitant analgesic medications were allowed under specific conditions. Concomitant analgesics were permitted if used chronically (at least 12 weeks) at a stable dose (at least 4 weeks) before screening. Participants were excluded if they had used CYP3A4/CYP2C9 inhibitors or inducers P-glycoprotein inhibitors within 7 days of baseline, unless the P-glycoprotein inhibitor had been used continuously for at least 3 months and maintained at a stable dose for 1 month before baseline. Initially, subjects were excluded if a steroid had been injected within 90 days. In an amendment, this period was reduced to 30 days for short-acting steroids but remained at 90 days for injection of a long-acting steroid.

Participants were recruited to the study through advertisements, clinician referrals, and self-referral. After preliminary telephone screening, potential participants attended an in-person screening visit at which written informed consent was provided.

2.3. Trial design and intervention

The EN20-01 Centrexion Knee OA study was a phase 2, randomized, allocation-concealed, multisite, placebo controlled, multiperiod crossover trial designed to evaluate the novel CNTX-6970 as a potential management of chronic knee OA pain. Phase 1 studies indicated that CNTX-6970 is absorbed over 30 to 60 minutes, with a half-life of 8 to 10 hours, reaching steady state after 2 days of twice-daily dosing. Multiperiod crossover designs are ideal for such treatments with rapid onset of action and relatively short half-lives. These designs are highly efficient, generating substantial power with modest sample sizes by removing between subject variability. Conducted under an investigator-initiated investigational new drug application, the study was run independent of Centrexion Therapeutics Corp.

The study was conducted from April 2022 until June 2024. Consenting subjects participated in the study for up to 7 months. Study participation began with a screening period lasting 14 to 28 days before randomization. Eligible participants who were randomized entered a 6-month treatment period, with in-person visits scheduled every 3 weeks ($\pm$4 days). There were 4 treatment periods, each lasting 6 weeks. Participants were randomized to one of 2 sequences: Drug–Placebo–Placebo–Drug (DPPD) or Placebo–Drug–Drug–Placebo (PDDP). Using only these 2 treatment sequences with 2 cross-overs as opposed to sequences with 3 cross-overs mitigates confounding with period effects and leads to increased statistical precision and power. Placebo consisted of inactive tablets that were visually identical to the active treatment tablets. No washout period was included because of the relatively short half-life of the drug, to avoid lengthening the study beyond 6 months, and because washout periods can be associated with increased participant dropout.[22] Study treatment (300 mg twice daily [BID] CNTX-6970 or Placebo BID) was administered BID, with or without food. The first dose of the study medication was administered on site to ensure the participants understood the procedure.

2.3.1. Protocol design change

The original protocol design included 2 additional trial arms alongside the 300 mg BID CNTX-6970 arm: a "lower dose" arm studying 100 mg BID CNTX-6970, and a celecoxib 100 mg BID arm (as an active comparator). These 2 additional trial arms were discontinued early in the study to prioritize completion of the 300 mg BID CNTX-6970 arm, primarily because of delayed

enrollment and constraints related to drug/active comparator expiration and supply. At discontinuation of these 2 arms, 22 participants had been randomized to the lower-dose CNTX-6970 and celecoxib arms. Per protocol, the arms were analyzed separately, and their removal did not impact the primary study objectives.

2.3.2. Design features

Participants attended a total of 10 in-person visits, including Screening, Baseline, and weeks 3, 6, 9, 12, 15, 18, 21, and 24. During the remote screening period, lasting up to 28 days, participants completed periodic assessments, including the WOMAC-A and 0 to 10 Numeric Rating Scale (NRS) to record their daily pain intensity levels. In addition, all subjects completed the SAFER (State, Accessibility, Face and Ecological validity and the Rule of the 3 Ps [persistent, pervasive, pathological]) instrument, which was administered remotely by an MGH psychiatrist or psychologist to confirm pain history, as well as the Hospital Anxiety and Depression Score (HADS).[23,35]

2.4. Randomization and masking

Participants who completed the screening period and met all eligibility criteria were randomized at the Baseline visit. Block randomization was used, stratifying by Kellgren–Lawrence (K–L) grade (ie, "low" grades of 1-2 vs "high" grades of 3-4) to ensure balanced treatment sequences across participants. The study used a multiperiod crossover design consisting of 2 blocks, each with 2 treatment periods (**Fig. 1**). Blinding of the randomization sequence was maintained by using an Interactive Web Response System, with oversight by a designated unblinded statistician.

2.5. Trial endpoints and procedures

The primary objective of the study was to evaluate the safety, tolerability, and efficacy of 300 mg CNTX-6970 BID for the treatment of knee OA pain compared to placebo. The primary safety and tolerability outcome was the assessment of all AEs and Serious Adverse Event (SAEs) reported during study participation. Efficacy was assessed using, as the primary outcome, the WOMAC Part A Pain Subscale,[5] which includes 5 ratings of movement-related knee pain (total scores can range from 0 to 50). Participants completed the WOMAC-A at every in-person visit, and remotely each week between study visits.

The second objective of this study was to evaluate the effect of 300 mg BID CNTX-6970 on general pain-related measures, including physical and psychosocial functioning, as well as biomarkers of pain and inflammation. Secondary endpoints included: NRS ratings of average knee pain intensity (reported daily during the week before each study visit), the WOMAC-C (Function Subscale, with a range of 0-68), Staircase-Evoked Pain Assessment, the HADS, the Patient-Reported Outcomes Measurement Information System (PROMIS) Sleep Disturbance Scale-6A, and the Patient Global Impression of Change scale. In addition, participants completed several measures included in the National Institute of Health Helping End Addiction Long Term Common Data Elements (NIH HEAL CDEs).[2] These assessments were completed at baseline and at the final study visit only. At in-person visits, we collected serum samples to assess circulating levels of serum chemokines/cytokines across visits at weeks 0, 6, 12, 18, and 24. We focus on serum levels of monocyte chemoattractant protein 1 (MCP-1) (CCL2), as this is the endogenous ligand that binds at CCR2, the primary site of CNTX-6970's selective binding inhibition.

2.6. Statistical considerations and sample size

The target sample size was estimated using effect sizes ranging from 0.25 to 0.50 by simulating data from multiperiod crossover models with block, period, and carryover effects, with varying within (W) and between (B) subject variability (B/W = 2/1 and 5/1). With a total sample size of n = 55, using a two-sided type I error rate of $\alpha = 0.05$, the power to detect an effect size of Cohen d = 0.35 exceeds 95% when the B/W ratio = 2/1.

2.6.1. Analysis samples

The primary efficacy analysis was based on an intention-to-treat (ITT) analysis set with modified ITT (mITT) data sets to assess the impact of the different protocol versions. Several additional ITT analyses were conducted, and they are described in the supplemental electronic materials, http://links.lww.com/PAIN/C442. The safety analysis was performed for all participants in all arms, including the 100 mg BID CNTX-6970 and celecoxib arms from the original trial design.

2.6.2. Analysis methods

The primary safety endpoint was the incidence of treatment emergent adverse events (TEAEs), reported between the administration of study drug and completion of the study. There was no hypothesis testing for safety analyses. The primary efficacy endpoint was analyzed using a linear mixed-effects model with repeated measures with a treatment indicator. Sites and patients (nested within sites) were included as random effects in the primary efficacy outcome model. Age, sex, and K–L grade indicator (discretized to high grades 3&4, or low grades 1&2) were used as control variables. An autoregressive of order 1 [AR (1)] structure was used to model the error term in the mixed-effect model. To avoid possible carryover effects for the primary efficacy analysis, only outcomes from the last 2 weeks of each period were used for the primary analysis (exploratory analyses investigated possible carryover effects). The comparison of interest was based on the treatment effect coefficient in the model. Secondary efficacy analyses were conducted on the outcomes (1) WOMAC-C (assessed at baseline and each study visit through week 24), (2) HADS (both the anxiety and depression scales) assessed at in-person visits, (3) Patient Global Impression of Change (PGIC) assessed at week 24, (4) PROMIS Sleep Disturbance Scale-6A assessed at baseline and at in-person visits through week 24, and (5) NRS ratings collected daily for the

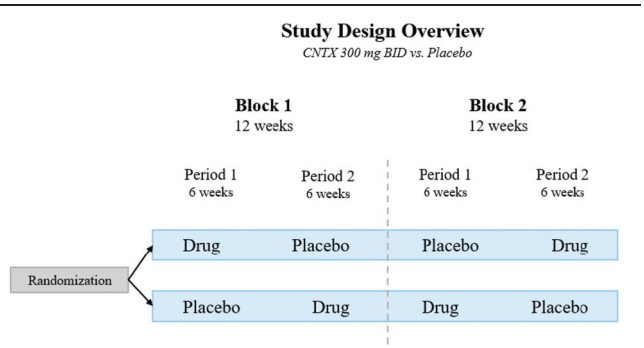

Figure 1. Final study design.

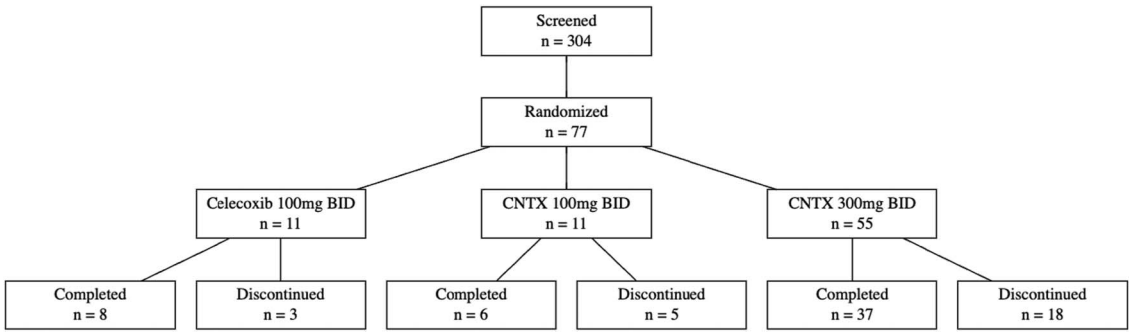

Figure 2. Study flow diagram.

week before each study visit. A linear mixed-effect model was used for outcomes (1), (2), and (4), and (5) using all outcomes with adjustment for possible carryover effects in placebo-treated periods that are preceded by periods with the active drug treatment. The analysis for the ordinal outcome (3) PGIC at week 24 used a proportional odds regression, controlling for treatment, age, sex, and baseline WOMAC-A. For outcomes assessed only at baseline and week 24, generalized linear models were used with the week 24 assessment as the outcome, controlling for age, sex, KL grade, and the baseline value with a treatment indicator (for the treatment in the last period).

The study also assessed the effect of CNTX-6970 compared to placebo with respect to (1) Staircase-Evoked Pain Assessment[36] and (2) serum levels of MCP-1 (CCL2), the endogenous ligand that binds at CCR2. The Staircase-Evoked Pain Assessment involved a standardized repeated stepping task, with assessment of immediate Post-Stepping (0-10) Pain and Maximum Recalled Pain. Collectively, no interim analysis was planned, and no imputation of missing data was implemented for the analyses.

3. Results

3.1. Patient characteristics

Of 304 screened patients, 77 were randomized: 55 to the 300 mg BID CNTX-6970 arm, 11 to the 100 mg BID CNTX-6970 arm, and 11 to the 100 mg BID celecoxib arm. The sample was predominantly female (72.7%) and in their sixties; mean sample BMI was 29.8 (SD = 4.2). Of the 55 patients randomized to the 300 mg BID CNTX-6970 arm, 28 were randomized to the DPPD sequence and 27 to PDDP (**Fig. 2**; and see **Table 1** for demographic and baseline characteristics). All patients had evaluable data for the primary efficacy and safety analysis.

Of the 55 subjects in the 300 mg BID CNTX-6970 arm, 37 (67%) completed the study and 18 (33%) discontinued the study before completion. The most common reasons for

Table 1

Demographic characteristics.

	Celecoxib 100 mg BID N = 11	CNTX-6970 100 mg BID N = 11	CNTX-6970 300 mg BID N = 55	Total N = 77
Age at baseline (y)				
N	11	11	55	77
Mean (SD)	66 (7.9)	64 (5.3)	63 (8.0)	64 (7.7)
Median	67	65	63	64
Min, Max	50, 82	56, 73	50, 85	50, 85
Sex at birth, n (%)				
Male	5 (45.5)	4 (36.4)	12 (21.8)	21 (27.3)
Female	6 (54.5)	7 (63.6)	43 (78.2)	56 (72.7)
Sex identity, n (%)				
Male	5 (45.5)	4 (36.4)	12 (21.8)	21 (27.3)
Female	6 (54.5)	7 (63.6)	43 (78.2)	56 (72.7)
BMI (kg/m^2)				
N	11	11	55	77
Mean (±SD)	29.9 ± 3.5	30.3 ± 4.1	29.7 ± 4.4	29.8 ± 4.2
Median	30.6	30.0	29.5	30.0
Ethnicity, n (%)				
Hispanic or Latino	1 (9.1)	2 (18.2)	14 (25.5)	17 (22.1)
Not Hispanic or Latino	10 (90.9)	9 (81.8)	40 (72.7)	59 (76.6)
Unknown	0 (0.0)	0 (0.0)	1 (1.8)	1 (1.3)
Race, n (%)				
White	8 (72.7)	8 (72.7)	39 (70.9)	55 (71.4)
Black or African American	3 (27.3)	3 (27.3)	13 (23.6)	19 (24.7)
Asian	0 (0.0)	0 (0.0)	1 (1.8)	1 (1.3)
Unknown	0 (0.0)	0 (0.0)	2 (3.6)	2 (2.6)

BID, twice daily; BMI, body mass index.

discontinuation were because of protocol violations (n = 5) and because of an adverse event (n = 4). Discontinuations occurred in each study phase, with the majority occurring in period 1 (n = 7 discontinuations) and period 2 (n = 6 discontinuations). Rates of discontinuations did not differ ($P = 0.815$) between phases of active (n = 10 discontinuations) treatment compared to placebo (n = 8 discontinuations) treatment. Participants were permitted to take acetaminophen as rescue medication. Of the 6 participants in the 300 mg BID CNTX-6970 arm who took acetaminophen, 5 (83.3%) started acetaminophen before enrolling and continued its use during each period of the trial.

3.2. Primary efficacy outcome measure

The coefficient for differentiating CNTX-6970 (300 mg BID) effect from placebo in the primary linear mixed-effects analysis model was estimated as −1.774 (standard error 0.639). The 95% confidence interval for this coefficient is (−3.033, −0.514) indicating that on average, patients had significantly lower WOMAC-A pain scores when on placebo compared to CNTX-6970 300 mg BID. The associated *P*-value is $P = 0.006$ (from testing if the coefficient differs from zero using the Wald test). Similar results were obtained for each mITT data set. **Figure 3** shows patient-specific WOMAC-A trajectories across all weeks for each block/period (indicated by the dashed vertical lines). As a sensitivity analysis, a parallel-arm comparison of CNTX-6970 (300 mg BID) to placebo for the first 6-week period only did not reveal any significant treatment-related differences in WOMAC-A pain scores ($P = 0.594$).

3.3. Secondary outcome measures

We investigated the association of several secondary outcome measures with treatment (300 mg BID CNTX-6970 vs placebo). The results reported here are for the ITT sample. None of the results produced significant findings for secondary outcomes except for the daily NRS pain score. The *P*-values for testing for a significant treatment effect for these secondary outcomes were WOMAC-C ($P = 0.354$), HADS-Anxiety ($P = 0.306$), HADS-depression ($P = 0.504$), PGIC at week 24 ($P = 0.901$), and PROMIS Sleep Disturbance Scale-6A ($P = 0.078$). Mixed-effect modeling revealed a significant treatment effect for daily NRS pain intensity ratings ($P < 0.001$), with 95% confidence interval for the treatment coefficient of (0.264, 0.576) indicating higher average pain when on CNTX-6970 compared to placebo. The NRS model, which included daily pain intensity ratings for the week before each visit during each period, also included a significant linear time effect ($P < 0.001$) and a significant carryover effect ($P < 0.001$) with 95% confidence interval (0.246, 0.705) indicating that the carryover effect corresponds to higher pain during placebo periods preceded by active CNTX-6970 periods. No significant treatment effect was detected for the Staircase-Evoked Pain Assessment for both Post-Stepping Pain Rating ($P = 0.204$) and Maximum Recalled Pain Rating ($P = 0.133$), using a linear mixed-effects model (and controlling for a carryover effect from active to control).

3.4. Biomarker analysis

Limited biomarker data were obtained from 41 of the 55 patients via plasma samples including 54 biomarkers of serum chemokines/cytokines across visits at weeks 0, 6, 12, 18, and 24. The values for many biomarkers fell below the detectable level and were excluded from the modeling analysis. In total, 9 biomarkers had sufficient data for analysis, with values above the minimum detectable threshold.

The results presented here are for MCP-1 (CCL2). A mixed-effects model to investigate the association of WOMAC-A with MCP-1 (log-transformed because of right skewness) did not reveal an association of MCP-1 levels with pain ratings ($P = 0.462$). However, a strong association was demonstrated

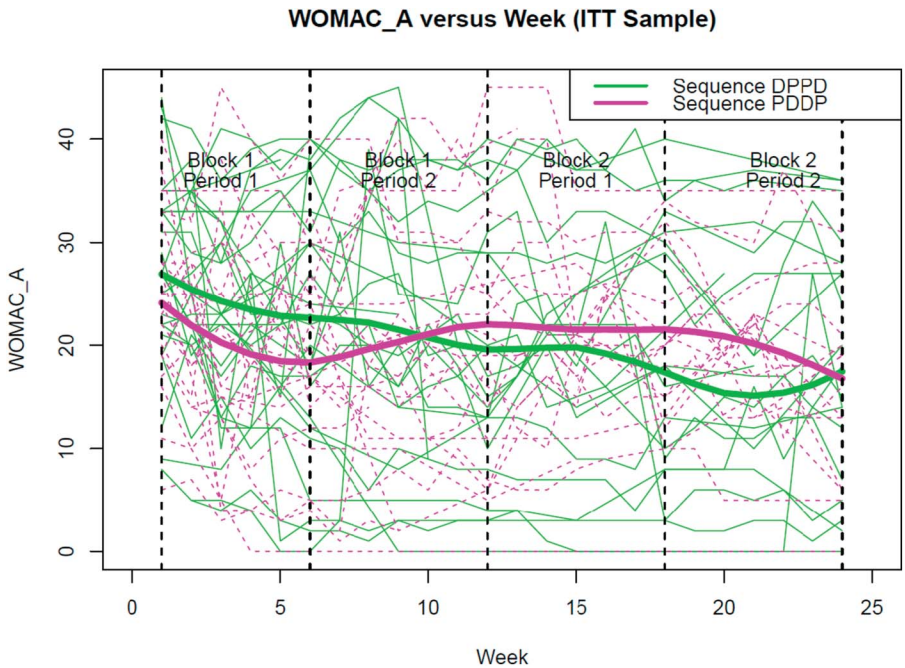

Figure 3. WOMAC-A vs Week (ITT Sample). WOMAC-A trajectories for individual participants (solid green curves are for DPPD and purple for the PDDP treatment sequences). The thick curves show loess-smoothed averages of WOMAC-A across time points. DPPD, drug–placebo–placebo–drug; ITT, intention-to-treat; PDDP, placebo–drug–drug–placebo; WOMAC-A, Western Ontario and McMaster Universities Arthritis Index Part A.

between MCP-1 levels and treatment (CNTX-6970 300 mg BID vs placebo): a mixed-effects model gave an estimated coefficient −1.50 and standard error 0.196 with associated *P*-value $P < 0.001$. A 95% confidence interval for the treatment coefficient is (−1.893, −1.103) with CNTX-6970 as the reference level indicating that log (MCP-1) on average was higher when patients were on CNTX-6970 compared to placebo. **Figure 4** shows a plot of log (MCP-1) vs visit highlighting a strong association of MCP-1 with treatment: visits when patients were on CNTX-6970 300 mg BID (blue dots) had notably higher MCP-1 values compared to visits when patients were on placebo (red dots), which was expected because CNTX-6970 was designed to block CCR2L/MIP-1. None of the other biomarkers with sufficient data showed any association with the WOMAC-A pain outcome.

3.5. Safety and tolerability

There were 52 TEAEs in the 300 mg BID CNTX-6970 arm, with 26 occurring on the active treatment and 26 occurring on the placebo treatment. There were 5 total SAEs reported among 3 participants, including one that was life threatening and one that was potentially life threatening. The life-threatening SAE involved the diagnosis of chronic myeloid leukemia, which occurred for a participant randomized to the 300 mg CNTX-6970 BID vs placebo arm during block 1 treatment period 2 while the participant was taking study medication (300 mg CNTX-6970 BID). The investigator determined that the SAE was definitely not related to study treatment. The potentially life-threatening SAE involved intestinal obstruction, which occurred for a participant randomized to the 300 mg CNTX-6970 BID vs placebo arm during block 1 treatment period 2 while the participant was taking placebo. The investigator determined that the SAE was definitely not related to study treatment.

No deaths were reported in the study. Spontaneously Reported Adverse Events experienced in Block 1 Treatment Period 1, first drug and placebo exposure, are detailed in **Table 2**.

Because of the crossover study, events in **Table 2** are listed according to the treatment column (Active or Placebo) when the event occurred. The AEs reported in the first period remained relatively stable through the remainder of the study. An overall summary of treatment emergent AEs experienced throughout the study is available in the supplemental electronic materials (Supplemental Table 1, http://links.lww.com/PAIN/C442).

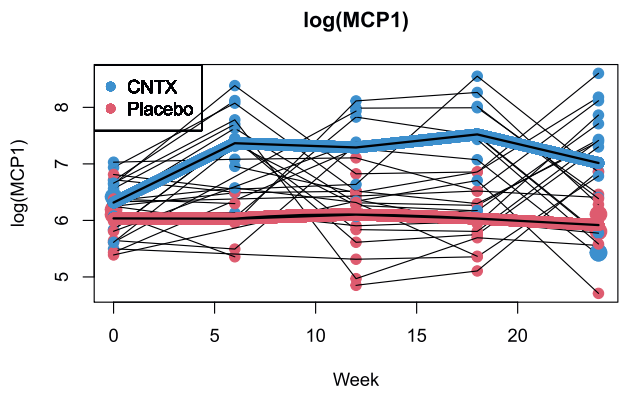

Figure 4. log(MCP-1) concentration vs visit for all patients with available data: blue dots indicate visits on CNTX-6970 300 mg BID and red dots indicate visits on placebo. Thick curves denote weekly averages of log(MCP-1) (blue for CNTX-6970 and red for placebo). BID, twice daily; MCP-1, monocyte chemoattractant protein 1.

3.6. Post-hoc analyses

During the study, the 100 mg BID CNTX-6970 and the celecoxib arms were halted; a post-hoc analysis was performed on these 2 arms for WOMAC-A. The small sample size made fitting the primary efficacy model challenging. Because of the limited data, the post-hoc primary efficacy analysis for these 2 arms was conducted using only the first two 6-week periods. For the 100 mg arm, there was no significant impact of treatment on WOMAC-A pain detected with the estimated coefficient = 2.051 (1.365) and associated *P*-value $P = 0.1493$. For celecoxib, the treatment effect was significant with estimated coefficient 5.650 (1.8874) with $P = 0.006$. The 95% confidence interval for the treatment effect coefficient for the celecoxib arm was (1.752, 9.547) indicating that on average, WOMAC-A pain was estimated to be 1.752 to 9.547 units higher on placebo compared to celecoxib. This result provides evidence of assay sensitivity for the network, confirming celecoxib's known efficacy for OA knee pain treatment, despite the small sample size. There was a significant period effect for both celecoxib and 100 mg CNTX-6970 for these post-hoc analyses.

Overall, we observed a substantial discontinuation rate, which was expected given the lengthy duration of the study. A post-hoc analysis shows evidence that dropouts (average age = 66.9) were older than completers (average age 61.5). For race, the discontinuation rate for White participants (16/39 or 41%) was higher than for Black participants (1/12 or 8%). The efficacy analysis for the primary outcome using only data from study completers gives a conclusion consistent with the ITT analysis results (ie, significantly lower WOMAC-A pain scores when on placebo compared to CNTX-6970 300 mg BID).

3.7. Efficacy summary

The only outcomes for which there appeared to be a statistically meaningful signal distinguishing the active CNTX-6970 and placebo treatments were the primary efficacy outcome, WOMAC-A, and daily NRS pain ratings. The primary conclusion from the efficacy analysis confidence interval was that average patient-reported pain was slightly higher when participants were on CNTX-6970 compared to placebo. For all other outcomes, the analyses showed that 300 mg BID CNTX-6970 did not have an effect that differed from placebo (all *P*-values > 0.05).

4. Discussion

In this phase 2b, multisite, multiperiod crossover randomized trial, patients with moderate to severe knee pain associated with OA did not appear to experience pain-related benefits while taking the novel CCR2 antagonist CNTX-6970 compared to placebo. Participant allocation to treatment groups was similar, suggesting adequate randomization, and demographic characteristics were typical of OA studies, with a mean age of 64 ± 7.7 years and a female predominance (72.7%).[25]

Interestingly, analysis of the primary outcome measure, patient-reported WOMAC-A knee pain,[5,6] demonstrated pain ratings that were slightly lower with placebo than CNTX-6970. The WOMAC has shown sensitivity to improvements related to effective pharmacologic treatment,[13,26] physical modalities,[16] and surgical intervention.[33] The Minimal Within Patient Change (MWPC) deemed clinically meaningful on the WOMAC-A Pain Subscale has been estimated at 13.5% to 15.9% on this subscale, corresponding to 6.8 to 8.0 points of the 50-point total.[8] Median WOMAC pain scores during period 1 of block 1 (the first treatment period) in the ITT group were 22.5 Interquartile

Table 2

Treatment-emergent adverse events by system organ class, preferred term, and treatment period—safety population block 1 period 1.

MedDRA system organ class Preferred term	Celecoxib 100 mg BID		CNTX-6970 100 mg BID		CNTX-6970 300 mg BID	
	DPPD active N = 6	PDDP placebo N = 5	DPPD active N = 5	PDDP placebo N = 6	DPPD active N = 28	PDDP placebo N = 27
Subjects with at least one TEAE	2 (33.3)	1 (20.0)	5 (100.0)	3 (50.0)	14 (50.0)	14 (51.9)
Nervous system symptoms	1 (16.7)	1 (20.0)	2 (40.0)	1 (16.7)	3 (10.7)	3 (11.1)
Gastrointestinal symptoms	1 (16.7)	0 (0.0)	2 (40.0)	0 (0.0)	2 (7.1)	4 (14.8)
Musculoskeletal and connective tissue symptoms	1 (16.7)	0 (0.0)	0 (0.0)	1 (16.7)	4 (14.3)	3 (11.1)
General symptoms and administration site conditions	1 (16.7)	0 (0.0)	2 (40.0)	1 (16.7)	2 (7.1)	0 (0.0)
Respiratory, thoracic, and mediastinal symptoms	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.1)	3 (11.1)
Skin and subcutaneous tissue symptoms	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	3 (10.7)	1 (3.7)
Infections and infestations*	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (3.6)	2 (7.4)
Investigations	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (7.1)	1 (3.7)
Metabolism and nutrition symptoms	0 (0.0)	0 (0.0)	1 (20.0)	0 (0.0)	1 (3.6)	1 (3.7)
Psychiatric symptoms	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)
Eye symptoms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Injury, poisoning, and procedural complications	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Renal and urinary symptoms	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)
Surgical and medical procedures	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.6)	0 (0.0)
Vascular symptoms	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Numbers in parentheses are percentages based on number of safety subjects in each group at each treatment period.
A subject who experienced multiple events within a system organ class (SOC) was counted once.
A subject who experienced multiple events within a preferred term was counted once for that preferred term.
Adverse events were coded with MedDRA Dictionary Version 25.0.
* Infestations reflect blood phosphorus abnormal, lipase abnormal, lipase increased.
BID, twice daily; DPPD, drug–placebo–placebo–drug; PDDP, placebo–drug–drug–placebo; TEAEs, treatment emergent adverse events.

Range (IQR) 13.8; 32.5) for patients receiving CNTX-6970 and 19.0 (IQR 13.0; 25.0) in patients receiving placebo; this 3.5-point difference between active and placebo phases is unlikely to be clinically significant.

The secondary outcome of NRS daily pain also suggested that CNTX-6970 did not provide pain-related reductions relative to placebo. Indeed, similar to the WOMAC findings, participants reported slightly higher daily average pain when on the CNTX-6970 compared to placebo. The analysis of the NRS pain scores suggest that there was a significant carryover effect corresponding to higher reported pain during placebo periods preceded by active CNTX-6970 periods. Other secondary outcome measures, including WOMAC-C (composite scores assessing pain, stiffness, and mobility) and HADS subscales, were typical of patients with symptomatic OA at baseline[4,5,27,36] and generally did not differ significantly between the 2 treatment groups. The PGIC assessment (conducted at week 24) showed no significant difference between groups, supporting the lack of significant treatment effect during the study.

Analysis of biomarkers included measurement of cytokines, chemokines, and growth factors related to inflammation. For the 9 biomarkers with sufficient data, evaluable levels were similar in both treatment groups and consistent with levels previously reported on the same assay in patients with OA.[11] However, the biomarker analysis did demonstrate a strong association between treatment and serum MCP-1 levels, with notably higher MCP-1 values measured at visits when patients were on CNTX-6970. Monocyte chemoattractant protein 1 (also known as chemokine ligand 2 [CCL2]) is an endogenous proinflammatory chemoattractant protein that recruits monocytes and macrophages to sites of inflammation and is important in the progression of inflammatory arthritis.[32] Monocyte chemoattractant protein 1 (CCL2) binds at the CCR2 receptor, the primary site of antagonism by CNTX-6970. The elevation of MCP-1 blood levels during CNTX-6970 treatment presumably results from displacement of MCP-1 at the CCR2 receptor. One potential explanation for the slight worsening of pain during CNTX-6970 treatment phases involves MCP-1 binding to a receptor other than CCR2. As one possibility, there is some evidence suggesting that MCP-1 binds CCR4, which has been associated with arthritic pain in mice.[18] Alternately, antagonism by CNTX-6970 at the CCR2 receptor might result in greater MCP-1 ligand release through an as-yet-unidentified feedback mechanism activated by the presence of the antagonist.

CNTX-6970 was safe and relatively well tolerated, with adverse effects that were mild to moderate in severity and similar in type and frequency to those observed with placebo. The most common adverse effects were nervous system symptoms (10.7% in CNTX-6970 group and 11.1% in the placebo group with headache and dizziness most common), gastrointestinal disorders (7.1% in CNTX-6970 group and 14.8% in the placebo group with gastroesophageal reflux and nausea most common), and musculoskeletal disorders (14.3% in CNTX-6970 group and 11.1% in the placebo group with arthralgias and muscle spasm most common). There were no serious adverse effects that were judged to be related to the study drug.

This study included 3 treatment arms: 100 mg BID and 300 mg BID doses of CNTX-6970, as well as celecoxib, each compared to placebo in separate crossover arms. The 100 mg and celecoxib arms were halted to assure study completion within the allotted timeframe. Post-hoc analysis provided evidence of assay sensitivity for the multisite network by demonstrating that celecoxib, a treatment known to be efficacious for treating OA pain,[26] was more effective than placebo, even in a small sample of participants. For the lower-dose CNTX-6970 arm (100 mg BID), there was no significant benefit for WOMAC pain, similar to the higher-dose results, although the small sample size for the lower dose precludes definitive conclusions.

Although the multiperiod crossover design used in the present study likely provided robust power under some study conditions (as evidenced by the significant benefit of celecoxib relative to placebo even in a sample of 11 participants), it has inherent limitations as well. A post-hoc analysis showed that approximately 80% of the patients who showed a response (a 30% or greater reduction in WOMAC pain scores) in the last 2 weeks of block 1, period 1 (the first treatment period) maintained such response in the last 2 weeks of block 1, period 2 (compared to the block 1, period 1 baseline), regardless of treatment assignment. These findings suggest that treatment periods of 6 weeks (with no washout) may not eliminate a significant carryover effect even in the last 2 weeks of treatment, which may lessen the sensitivity of the analyses. Importantly, the PK of the active molecule is short, and thus, the biological washout is assured. In addition, the treatment design of the study is symmetrical, and residual bias is thought to mitigate any low-level carryover effects. These carryover effects were confirmed in analyses of WOMAC and NRS pain scores, corresponding to higher reported pain during placebo periods preceded by active CNTX-6970 periods. Such results challenge the view that crossover designs that use longer treatment periods may minimize carryover effects and indicate that caution is warranted when considering the strengths and limitations of specific crossover designs in clinical trials of analgesic treatments. Additional study limitations include the small sample size in the lower-dose study arm, a phenotypically heterogeneous sample (eg, we did not selectively recruit individuals with a particular grade of OA, or with active synovitis), as well as a significant dropout rate (which did not differ between active treatment and placebo periods).

Despite these limitations, this multisite, phase 2 crossover trial established that when patients with moderate to severe knee pain associated with OA were treated with CNTX-6970 300 mg twice daily, they did not experience reductions in knee OA pain relative to periods of placebo treatment. Indeed, average pain was slightly, although not clinically significantly, lower in intensity during placebo treatment. CNTX-6970 300 mg twice daily was safe with generally mild adverse effects (eg, headache, dizziness) experienced by a minority of patients. Interestingly, treatment with CNTX-6970 resulted in a significant increase in the blood levels of MCP-1, the endogenous ligand for the CCR2 receptor, which may have contributed to the lack of benefit of CNTX-6970. Collectively, we conclude that pharmacologic blockade of CCR2 receptors with this specific compound was not effective in reducing moderate-to-severe knee osteoarthritis pain.

Conflict of interest statement

The authors have no conflict of interest to declare.

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