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Lorecivivint, a Novel Intra-articular CLK/DYRK1A Inhibitor and Wnt Pathway Modulator for Treatment of Knee Osteoarthritis: A Phase 2 Randomized Trial

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Running Head: Wnt pathway modulation for the treatment of knee OA

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ABSTRACT

Objective. To assess the safety and efficacy of the novel Wnt pathway modulator lorecivivint (SM04690) for treating pain and inhibiting structural progression in moderate-to-severe symptomatic knee osteoarthritis (OA).

Methods. Subjects in this 52-week, Phase 2a, multicenter, randomized, double-blind, placebo (PBO)-controlled, dose-ranging trial received a single, 2 mL, intra-articular injection of 0.03 mg, 0.07 mg, or 0.23 mg lorecivivint, or PBO. Efficacy was assessed by change in WOMAC Pain [0–100] and WOMAC Function [0–100] subscales and radiographic medial joint space width (mJSW). Baseline-adjusted analysis of covariance with multiple imputation was performed separately to evaluate efficacy. This proof-of-concept study evaluated the intention-to-treat population as well as a prespecified group of subjects with unilateral symptoms (UNI) and an additional post hoc subgroup of unilateral symptomatic subjects without widespread pain (UNI WP-).

Results. Four hundred fifty-five subjects were randomized. The primary endpoint, improvement in WOMAC Pain compared with PBO at Week 13, was not met by any dose group (change from baseline, 0.03 mg, -23.3±2.2; 0.07 mg, -23.5±2.1; 0.23 mg, -21.3±2.2; PBO, -22.1±2.1; all $P>0.05$). All groups (including PBO) demonstrated clinically meaningful (≥20-point) improvements from baseline. The durability of response was evaluated through Week 52. In the prespecified UNI and post hoc UNI WP-groups at Week 52, 0.07 mg lorecivivint significantly improved WOMAC Pain (between-group differences [95% CIs]: UNI, -8.73 [-17.44, -0.03], $P=0.049$; UNI WP-, -11.21 [-20.99, -1.43], $P=0.025$) and WOMAC Function (UNI, -10.26 [-19.82, -0.69], $P=0.036$; UNI WP-, -13.38 [-24.33, -2.43], $P=0.017$) compared with PBO. Compared with baseline, the mean change in mJSW at Week 52 was -0.04 mm in the 0.03 mg cohort, -0.09 mm in the 0.07 mg cohort, -0.16 mm in the 0.23 mg cohort, and -0.14 mm in the PBO cohort; no treatment group achieved a statistically significant change in mJSW compared with PBO at Week 52. In both unilateral subgroups, the 0.07 mg dose significantly increased mJSW compared with PBO at 52 weeks, (UNI, 0.39 mm [0.06, 0.72], $P=0.021$; UNI WP-, 0.42 mm [0.04, 0.80], $P=0.032$). Changes in the 0.03 mg and 0.23 mg dose groups were not statistically different from PBO in any of these measures. Lorecivivint appeared safe and well tolerated.

Conclusion. This Phase 2a, proof-of-concept trial did not meet its primary endpoint; however, it identified a target population in which to evaluate the potential efficacy of lorecivivint.

INTRODUCTION

Knee osteoarthritis (OA) is a common, chronic disorder characterized by cartilage destruction, subchondral bone thickening, and osteophyte formation that are associated with pain, functional limitation, and physical disability (1). Knee OA severity is assessed by a combination of patient-reported outcome measures that include pain and function and objective structural measures such as radiologically assessed joint space narrowing (2). Pharmacologic interventions for knee OA management are symptom-alleviating treatments, including oral and topical nonsteroidal anti-inflammatory drugs (NSAIDs), non-opioid analgesics, and intra-articular (IA) corticosteroid or hyaluronic acid injections (3, 4). However, many of these treatments have limited short-term and long-term efficacy (4–7) and are associated with a high incidence of side effects. There remains a great unmet need for new treatments that provide symptom relief and even more of a need for disease-modifying OA drugs (DMOADs).

The Wnt pathway is integral for tissue homeostasis and regeneration (8, 9) and is a key regulator of progenitor cell differentiation in the knee joint (10). Cartilage homeostasis requires a balance of Wnt pathway activity. While necessary for chondrocyte differentiation and function (11), aberrant Wnt pathway activity in OA directs progenitor cell differentiation in the joint toward osteoblast instead of chondrocyte development (12). Excessive Wnt pathway activation is known to increase OA susceptibility in animals and humans (13–16), whereas excessive inhibition can cause cartilage and bone destruction (17–19). Therefore, a potential Wnt pathway-targeted DMOAD approach would need to maintain signaling within an optimal range.

Lorecivivint (LOR, SM04690) is a small-molecule Wnt pathway modulator currently in development as a potential DMOAD for the treatment of knee OA (20, 21). LOR affects Wnt pathway activity via inhibition of two intranuclear targets, CDC-like kinase 2 (CLK2) and dual-specificity tyrosine phosphorylation-regulated kinase 1A (DYRK1A), through which it acts both

independently and in combination to improve chondrocyte health and function while inhibiting inflammation (22). Preclinical work, including repeat dosing in rats and dogs, has found the no-observed-adverse-effect level to be ~400x the planned human dose (data on file). *In vitro* studies demonstrated that LOR modulated Wnt signaling, reduced release of matrix-degrading enzymes from chondrocytes, demonstrated anabolic activity in chondrocytes, and reduced STAT3 signaling, NF-κB signaling, and inflammatory cytokine production in synoviocytes (20). In an anterior cruciate ligament transection and partial medial meniscectomy knee OA rat model, a single IA injection of LOR protected chondrocytes from catabolic breakdown (20).

In a previous Phase 1, randomized, placebo (PBO)-controlled trial (N=61), a single IA injection of LOR at doses of 0.03 mg, 0.07 mg, or 0.23 mg administered into the target knee joint of subjects with moderately to severely symptomatic knee OA appeared safe and well tolerated and showed no evidence of systemic exposure. While all LOR and placebo groups demonstrated improvements from baseline in Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) Pain and Function scores at Week 24, the 0.07 mg LOR treatment group demonstrated more favorable reductions in both WOMAC indices than placebo. The 0.07 mg treatment group also showed increased radiographic joint space width beyond a minimum detectable difference (MDD) of 0.13 mm (23), thus supporting LOR as a potential DMOAD.

The objective of this Phase 2a trial was to evaluate the safety and efficacy of LOR among subjects with moderately to severely symptomatic knee OA.

SUBJECTS AND METHODS

Study design

This was a 52-week, Phase 2a, multicenter, randomized, double-blind, PBO-controlled, dose-ranging trial of three different dose concentrations of LOR injected into the target (most painful) knee joint of subjects with moderately to severely symptomatic knee OA. This study was conducted at 36 clinical sites in the United States between September 2015 and April 2017. Subjects participated in a screening period of up to 21 days and were periodically observed

during a 52-week follow-up period. Visits were scheduled at screening, treatment visit Day 1, and at follow-up Weeks 4, 13, 26, 39, and 52.

On Day 1, subjects were randomized to receive a single, 2 mL, IA injection of vehicle (phosphate-buffered saline) or 0.03 mg, 0.07 mg, or 0.23 mg LOR. These doses corresponded to the lower, middle, and upper therapeutic ranges that were determined by preclinical studies (data on file). Randomization was accomplished by Medidata Balance (Medidata Solutions, Inc., New York, NY) such that eligible subjects were randomized at a ratio of 1:1:1:1 using a permuted block design with a block size of 8 and stratified by site. An unblinded pharmacist at each site mixed the working dose from a common stock solution bottle, and an unblinded injector performed the injection. Ultrasound guidance and joint aspiration (up to 0.5 cc) were allowed if part of the site's standard IA injection protocol for joint placement. All unblinded site personnel were instructed to minimize any contact with study subjects and were not allowed to perform any study assessments. Study investigators and subjects were blinded to group assignment, and subject blinding was maintained by not allowing them to witness the injection.

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki, the International Council on Harmonization Good Clinical Practice Guidelines, and applicable regulations. The study protocol was approved by each clinic site's independent ethics committee or institutional review board. All subjects provided written informed consent prior to participating in any study-related procedures.

Subjects

Eligible subjects were aged 40–80 years with an established (≥6 months) diagnosis of primary knee OA and fulfilled clinical and radiographic American College of Rheumatology classification criteria (24). Enrolled subjects were required to have Kellgren-Lawrence (KL) radiographic disease stage 2 or 3 in their target knee (defined at screening as the knee with greater pain based upon the subject's evaluation and the investigator's clinical judgement) (25). Subjects were required to have a pain visual analog scale (VAS) score of 30–80 mm (26) and a

WOMAC Total score of 72–192 (of 240) (27) for the target knee at screening. There were no limitations on contralateral knee pain. Subjects were eligible if in good general health and ambulatory; assistive devices (e.g., canes) were allowed if needed <50% of the time, whereas any use of a walker was excluded.

Key exclusion criteria included women who were of childbearing potential, pregnant or lactating, or male subjects with female partners of childbearing potential who refused to use an effective contraceptive method. Further exclusions included body mass index (BMI) >40 kg/m^2, history of partial or complete joint replacement in the target knee, previous exposure to LOR, a major surgery (e.g., interventional arthroscopy) in the target knee within 52 weeks prior to study medication injection, and any planned or elective surgery anywhere in the body during the study period. Additional exclusion criteria included having comorbid conditions that could affect pain assessment of the target knee or a history of malignancy (except for *in situ* cancer or basal or squamous cell skin cancer) <5 years prior to injection. Subjects could not receive IA injection of corticosteroids, hyaluronic acid, or other therapeutic agents into either knee during the study or two, six, or one month(s) prior to randomization, respectively. Electrotherapy or acupuncture for knee OA, chiropractic knee adjustments, or planned or elective surgery (e.g., arthroscopy) were also prohibited. Subjects could not take opioid analgesics or oral glucocorticoids during the study, although a stable background regimen of NSAIDs and acetaminophen was allowed provided that they were not taken within 24 hours prior to study visits.

Data collection

Subject characteristics, medical history, weight, and height were collected at screening. Unilateral or bilateral symptomatic knee OA status was designated by the investigator at baseline based upon history and physical examination. To assess comorbidity-related pain and symptoms, subjects completed the fibromyalgia diagnostic questionnaire, Widespread Pain Index (WPI, total score range: 0–19), and Symptom Severity Scale (total score range: 0–12) assessments at screening (28).

Efficacy assessments

Efficacy assessments administered at all study visits included the WOMAC questionnaire (version NRS 3.1, Pain subscale range: 0–100 [no pain – extreme pain] and Function subscale range: 0–100 [no difficulty with daily activities – extreme difficulty]) and the Patient Global Assessment of Disease Activity (PtGA, VAS score range: 0 mm–100 mm ["doing very well" – "doing very poorly"]). Fixed-flexion, posterior-anterior (PA) radiographs of the tibiofemoral compartments were obtained using the QuAP™ positioner at screening, Week 26, and Week 52. A central imaging lab (Medical Metrics Labs) that was blinded to treatment assignment quality-controlled all radiographs and measured medial joint space width (mJSW) using a landmark-based, fixed-location methodology.

The primary efficacy endpoint was the change from baseline in WOMAC Pain of the target knee at Week 13 between 0.07 mg LOR and PBO. Key secondary endpoints included 1) change from baseline in WOMAC Pain of the target knee at Week 26, 2) change from baseline in WOMAC Function of the target knee at Weeks 13 and 26, 3) change from baseline in PtGA at Weeks 13 and 26, and 4) change from baseline in mJSW of the target knee at Week 26. Exploratory endpoints included 1) change from baseline in WOMAC Pain and Function of the target knee at Weeks 4, 39, and 52; 2) change from baseline in PtGA at Weeks 4, 39, and 52; and 3) change from baseline in mJSW of the target knee at Week 52.

Safety

Safety was assessed by evaluating the incidence, severity, and seriousness of treatment-emergent adverse events (TEAEs) and clinically significant changes in clinical laboratory measures and vital signs; no formal statistical analyses were planned for safety outcomes. Safety measures were summarized for all treatment groups as treated, not as randomized.

Sample size

A sample size of approximately 445 subjects was planned for this trial based upon statistical practice to establish an acceptable level of precision with respect to treatment effect estimation

(29); no formal calculation was used to a priori determine sample size. However, based upon Phase 1b and historical data, a Monte Carlo simulation was conducted to estimate the possible power for WOMAC Pain (estimated power 95.8%) and WOMAC Function (estimated power 78.5%) scores given this sample size. The power estimation is detailed in the statistical analysis plan (Appendix).

Statistical analysis

Statistical analyses were conducted using SAS Version 9.4 (SAS Institute, Cary, NC). Baseline characteristics of treatment groups are presented as means (standard deviations [SD]) for continuous variables and frequencies (proportions) for categorical variables. Efficacy outcome measures were evaluated using analysis of covariance models adjusted for baseline values under the intention-to-treat (ITT) analysis set (all subjects as randomized). Multiple imputation under the missing-at-random assumption was performed for efficacy outcomes with missing values. The least-squares estimate of the difference in change in the outcome from baseline at each time point between each treatment group and PBO, adjusted for baseline value, is reported with 95% confidence intervals (CI). The familywise error rate for the efficacy analyses was controlled in the strong sense using the closed, fixed-sequence testing method (30). Hypothesis tests were evaluated in a prespecified, sequential order that matched clinical inference from prior LOR studies regarding the relative therapeutic benefit of each dose (Supplemental Table 2; the first hypothesis test was change in WOMAC Pain at Week 13 between 0.07 mg LOR and PBO). If a hypothesis test did not meet the critical value of α=0.05, all subsequent tests were considered as exploratory. The fixed sequence is detailed in the statistical analysis plan (Appendix).

In support of the primary and secondary endpoints, interim analyses were conducted after all subjects completed the Week 26 visit and prior to completion of the trial at 52 weeks. An exploratory analysis was prespecified for a clinically relevant subject population (the UNI subgroup), defined by history and physical examination of unilateral symptomatic knee OA. A second exploratory analysis was prespecified based upon the WPI score but did not have sufficient data to explore. A post hoc exploratory analysis was subsequently completed based upon subjects with unilateral symptomatic knee OA without WP, defined as WPI score ≤4 and

Symptom Severity Scale Question 2 score ≤2 (disregarding Question 3) (the UNI WP- subgroup).

A post hoc concordance analysis was conducted to estimate the ability of one outcome to predict another outcome. It employed within-group logistic regression to estimate the likelihood of baseline-adjusted changes in mJSW being associated with positive clinical responses (i.e., achieving both WOMAC Pain and Function improvements of ≥50% relative change and ≥20 [out of 100] points). The area under the curve (AUC) of receiver operator characteristic (ROC) curves represented the concordance between change in mJSW and clinical response. Concordance was defined as "acceptable" when AUC >0.7 and "excellent" when AUC >0.8 (30); an AUC of 0.5 represents concordance no better than statistical chance.

RESULTS

Subject disposition and baseline characteristics

Overall, 1033 subjects were screened and 455 (44.0%) were randomized; 3 subjects terminated the study prior to receiving study drug injection (Figure 1). Cohorts of 112, 117, 109, and 114 subjects were randomized to receive 0.03 mg, 0.07 mg, or 0.23 mg LOR, or PBO, respectively. For UNI subjects (N=164), cohort sizes were 45, 35, 45, and 39 for 0.03 mg, 0.07 mg, 0.23 mg LOR, and PBO, respectively. For UNI WP- subjects (N=128), cohort sizes were 34, 29, 33, and 32 for 0.03 mg, 0.07 mg, 0.23 mg LOR, and PBO, respectively.

Subjects completing the study numbered 103 (92%) in the 0.03 mg cohort, 107 (91.5%) in the 0.07 mg cohort, 95 (86.4%) in the 0.23 mg cohort, and 97 (83.6%) in the PBO cohort.

Mean (± SD) age and body mass index (BMI) at enrollment were 60.3 (± 8.7) years and 29.9 (± 4.6) kg/m^2, respectively. Overall, 268 (58.9%) of enrolled subjects were women, 392 (86.2%) were white, 292 (64.2%) had radiographic disease KL grade 3 in the target knee, and 164 (36.0%) were classified as having unilateral symptomatic disease (Table 1). Among 424 subjects with non-target knees graded by KL, 386 (91%) had equal or worse radiographic disease in the non-target knee. In general, baseline characteristics were balanced between treatment groups.

Clinical outcomes

All subjects: The differences in change from baseline in WOMAC Pain scores between treatment and PBO groups were not statistically significant at Week 13; thus, the primary endpoint was not met, and all analyses are considered exploratory. However, all treatment groups and the PBO group achieved at least a 20-point mean improvement from baseline at Week 13 through Week 52 in WOMAC Pain and Function scales and PtGA at all time points (Figures 2a, 3a, and Supplementary Figure 1a). Treatment with 0.07 mg LOR led to numerically larger improvements in pain and function from baseline compared with PBO than did treatment with either the 0.03 mg or 0.23 mg doses. This was apparent starting at 13 weeks post injection and continuing to 52 weeks.

UNI subjects: Among the unilateral symptomatic subjects, the 0.07 mg dose demonstrated improvements in baseline-adjusted change in WOMAC Pain and Function compared with PBO from Week 13 and continuing to Week 52 (Figures 2b, 3b). At Week 52, the 0.07 mg group had significantly lower (improved) WOMAC Pain (between-group difference, -8.73 [95% CI, -17.44, -0.03], *P*=0.049) and Function (between-group difference, -10.26 [95% CI, -19.82, -0.69], *P*=0.036) scores compared with the PBO group. There were no significant differences in WOMAC Pain or Function between treatment groups for the 0.03 mg or 0.23 mg doses compared with PBO (Figures 2b, 3b) for the UNI subgroup. The 0.03 mg treatment group showed significant improvement in PtGA compared with the PBO group at Weeks 13 and 26 (Supplementary Figure 1b).

UNI WP- subjects: Among the UNI WP- subgroup, the 0.07 mg treatment group demonstrated statistically significant improvements in WOMAC Pain and Function compared with the PBO group at Week 26 (between-group difference, -9.11 [95% CI, -17.75, -0.47], *P*=0.039 and -9.62 [95% CI, -18.14, -1.10], *P*=0.027, respectively), Week 39 (-11.83 [95% CI, -23.23, -0.42], *P*=0.042 and -11.57 [95% CI, -22.31, -0.82], *P*=0.035, respectively), and Week 52 (-11.21 [95% CI, -20.99, -1.43], *P*=0.025 and -13.38 [95% CI, -24.33, -2.43], *P*=0.017, respectively) (Figure

2c, 3c). There were no significant differences in change in pain or function between either the 0.03 mg or 0.23 mg doses and PBO (Figure 2c, 3c). However, the 0.03 mg treatment group showed a significant improvement in PtGA compared with the PBO group at Week 13, and both the 0.03 mg and 0.07 mg treatment groups showed significant improvements in PtGA compared with PBO at Week 26 (Supplementary Figure 1c) in this post hoc analysis.

Radiographic outcomes

All subjects: Compared with baseline, the mean change in mJSW was -0.07 mm at Week 26 and -0.04 mm at Week 52 in the 0.03 mg cohort, -0.11 mm at Week 26 and -0.09 mm at Week 52 in the 0.07 mg cohort, -0.02 mm at Week 26 and -0.16 mm at Week 52 in the 0.23 mg cohort, and -0.20 mm at Week 26 and -0.14 mm at Week 52 in the PBO cohort (Figure 4a). At Week 26, the mean change for the 0.23 mg dose was statistically different from PBO (between-group difference, 0.19 [95% CI, 0.02, 0.36] mm, P=0.032). At Week 52, mean mJSW in the 0.03 mg and 0.07 mg dose groups was similar to that seen at Week 26, whereas that in the 0.23 mg dose and PBO groups had declined (Figure 4a). In the all-subjects analysis, both 0.03 mg LOR (Week-52 change from baseline -0.04 mm) and 0.07 mg LOR (Week-52 change from baseline -0.09 mm), but not 0.23 mg LOR, maintained mJSW when compared with PBO (Week-52 change from baseline -0.14 mm); however, the differences were not statistically significant.

UNI subjects: Subjects in the UNI subgroup treated with 0.07 mg showed improvements in mJSW at Weeks 26 and 52 (mean change from baseline, 0.26 mm and 0.19 mm, respectively), whereas unilateral symptomatic subjects treated with PBO showed worsening of mJSW (mean change from baseline, -0.26 mm and -0.21 mm, respectively) (Figure 4b). The differences between 0.07 mg and PBO were significant at both time points; the mean change in mJSW was 0.52 (95% CI, 0.15, 0.89) mm at Week 26 (P=0.006) and 0.39 (95% CI, 0.06, 0.72) mm at Week 52 (P=0.021). Among unilateral symptomatic subjects, there were no significant differences in change in mJSW when comparing 0.03 mg or 0.23 mg with PBO (Figure 4b).

UNI WP- subjects: In the UNI WP- subgroup, the 0.07 mg treatment group demonstrated improved mJSW at Weeks 26 (mean change from baseline, 0.28 mm) and 52 (mean change from baseline, 0.17 mm), whereas the PBO treatment group had worsening of mJSW (mean change from baseline, -0.26 mm and -0.26 mm, respectively) (Figure 4c). The differences between 0.07 mg and PBO were significant at both time points (between-group difference, 0.53 [95% CI, 0.10, 0.97] mm at Week 26 [*P*=0.016] and 0.42 [95% CI, 0.04, 0.80] mm at Week 52 [*P*=0.032]). UNI WP- subjects in the 0.03 mg or 0.23 treatment groups showed no significant differences in change in mJSW compared with the PBO group (Figure 4c).

Concordance between change in mJSW and clinical response: In the all-subjects analysis, no treatment group achieved AUC >0.7 (Supplementary Figure 2a). For the 0.07 mg dose, concordance was "acceptable" (AUC=0.783) in the UNI subgroup (Supplementary Figure 2b) and "excellent" (AUC=0.825) in the UNI WP- subgroup (Supplementary Figure 2c). No other doses in either subgroup achieved AUC >0.7.

Safety

No clinically significant safety concerns with respect to vital signs, clinical laboratory results, or AEs were observed; rates were comparable between LOR and PBO. No deaths were reported during the study. Fifteen subjects incorrectly received a study injection that diverged from that prescribed by the protocol; these subjects are described as "Other" in the safety analysis.

In total, 547 TEAEs were reported by 237 (52.4%) subjects, with 40 AEs in 32 (7.1%) subjects deemed related to the study drug by the investigator (Table 2). One hundred forty-two TEAEs were reported by 61 (55.0%) subjects in the 0.03 mg cohort, 147 TEAEs by 65 (57.0%) subjects in the 0.07 mg cohort, 107 TEAEs by 47 (45.2%) subjects in the 0.23 mg cohort, 117 TEAEs by 53 (49.1%) subjects in the PBO cohort, and 34 TEAEs by 11 (73.3%) subjects who received an "other" dose. Arthralgia, defined here as an exacerbation (increase in frequency, severity, or specificity) of an existing condition, was the most common AE reported across all study cohorts with 61 AEs in 49 (10.8%) subjects; 38 AEs in 36 (8.0%) subjects were reported in the target

knee. There were 10 AEs in 10 subjects in the non-target knee and 12 AEs in 11 subjects in other (non-knee) joints (7 hip, 4 elbow, 1 wrist).

Twenty-nine serious adverse events (SAEs) were reported by 17 (3.8%) subjects, and all were deemed unrelated to the study drug by the investigator. Seven SAEs were reported by 5 (4.5%) subjects in the 0.03 mg cohort, 12 SAEs by 4 (3.5%) subjects in the 0.07 mg cohort, 5 SAEs by 4 (3.8%) subjects in the 0.23 mg cohort, 3 SAEs by 3 (2.8%) subjects in the PBO cohort, and 2 SAEs by 1 (6.7%) subject who received an unidentified dose. One subject in the 0.07 mg cohort accounts for six of the reported SAEs, all of which were cardiovascular in nature; the other three subjects in the 0.07 mg cohort account for the other half of the reported SAEs within this cohort. The most common SAEs included infections and cardiac disorders (Supplementary Table 1).

DISCUSSION

In this Phase 2a, 52-week, randomized, placebo-controlled, proof-of-concept clinical trial among subjects with moderately to severely symptomatic knee OA, there was no statistically significant difference in improvement in WOMAC Pain scores between treatment groups at Week 13. However, intra-articular injection of LOR generally appeared safe and well tolerated. There were no meaningful differences in the incidence of AEs between treatment arms and PBO. Additionally, no SAEs were deemed related to treatment by the investigators.

Though the primary endpoint of this study was not met, additional preplanned and post hoc analyses of these data suggested that IA injection of LOR could have potential efficacy in the treatment of knee OA. While there were no statistically significant differences (including improvement in WOMAC Pain, the primary endpoint) between PBO and treatment groups in the all-subjects analysis, using a minimal clinically important difference threshold of a 10% (10-point) change in score (32), both LOR and PBO produced clinically meaningful improvements from baseline in WOMAC Pain and Function subscales. The prespecified UNI subgroup analysis showed greater improvements in WOMAC Pain, WOMAC Function, and mJSW for the 0.07 mg cohort compared with PBO. These differences appeared further enhanced in the post hoc analysis

of the 0.07 mg LOR UNI WP- subgroup. Pain reporting by subjects with bilateral symptomatic OA is known to be complicated, not only by contralateral knee pain, but also by other osteoarthritic joints (33, 34), nociceptive biomechanical factors, and other centralized pain conditions (e.g., fibromyalgia) (35). Therefore, improvements compared with PBO observed in the UNI/UNI WP- subgroups versus the all-subjects group after an IA injection of LOR into the target knee may be due to subjects with predominantly unilateral OA symptoms being able to discriminate their target knee pain from other pain sources. These results inform the design of future LOR trials by identifying a target population in which potential symptomatic efficacy could be more clearly delineated.

In addition to symptom improvements, inhibition of structural progression is a key goal of disease modification in OA (36); in fact, the slowing of joint space narrowing (JSN) has been recommended as an appropriate structural endpoint for DMOAD trials (36). In the all-subjects analysis, both 0.03 mg LOR (Week-52 change from baseline -0.04 mm) and 0.07 mg LOR (Week-52 change from baseline -0.09 mm) maintained mJSW at Week 52, numerically, but did not achieve statistical significance when compared with PBO (Week-52 change from baseline -0.14 mm). Unilateral symptomatic subjects (i.e., UNI/UNI WP- subgroups) treated with 0.07 mg LOR showed mean mJSW increases beyond a 0.13 mm MDD (23), whereas PBO subjects showed decreases (narrowing) in mean mJSW from baseline. JSN has also been correlated with clinical outcomes, including an increased risk of total knee replacement with JSN >0.5 mm over two years (an outcome indicative of treatment failure) (36, 37). Knee OA is associated with typical JSN of 0.1–0.3 mm per year (38, 39); though such changes require precise and reproducible measurement methods such as the positioned, fixed-flexion radiography technique employed herein, the accuracy of knee radiograph mJSW measurement can range from 0.04 to 0.5 mm (23, 41, 42). The relative improvement in mJSW in the unilateral symptomatic subject subgroups may also be related to a more favorable local biomechanical environment in individuals with unilateral knee pain (44).

A post hoc analysis of both UNI and UNI WP- subgroups demonstrated that the radiographic (mJSW) and clinical (WOMAC Pain and Function) findings in the 0.07 mg dose group were

concordant (i.e., the change in the former is associated with change in the latter). This suggested a connection between improvement in structural measures and clinical responses. The 2018 FDA draft guidance on OA structural endpoints suggests that additional data are needed to support the relationship between structural measurements and clinical outcomes; this analysis sought to contribute to this growing evidence base.

This Phase 2a study has several limitations, including no formal, preplanned sample size or power calculation and considerable placebo responses for patient-reported outcomes similar to those demonstrated in other OA trials (5, 45). Although trials investigating IA therapies for knee OA commonly use saline as a PBO comparator arm, evidence suggests that IA saline may actually be therapeutic (46). Therefore, further studies of LOR in larger clinical trials with refined inclusion criteria (e.g., unilateral symptomatic subjects, as LOR is administered into the single most painful knee) are needed to disentangle the active treatment and placebo effects. Although radiographic mJSW represents an objective measure for assessing structural progression, the evidence supporting its use is not definitive and other imaging modalities, such as MRI, may also be considered. Larger and longer studies are needed to determine the best methodologies for assessing the disease-modifying abilities of drugs in knee DMOAD trials. Finally, the primary statistical analysis Type 1 error control strategy was not achieved, leading to all statistical results being considered as exploratory. As the (prespecified) UNI and (post hoc) UNI WP- groups were small with respect to the number of subjects, these exploratory analysis results in both groups are considered to be hypothesis generating and thus require validation in a prospective trial.

In summary, although the primary endpoint in all subjects was not met, treatment of subjects with moderately to severely symptomatic knee OA in the UNI and UNI WP- subgroups with IA 0.07 mg LOR resulted in numerical improvements in pain, function, and mJSW compared with PBO. Additionally, this dose demonstrated the greatest improvements in WOMAC Pain and Function and the highest concordance between symptomatic relief and structural changes. This study identified a target group of subjects with unilateral symptomatic knee OA and a potentially

optimal dose of LOR (0.07 mg). The clinical and radiographic outcomes warrant additional studies of the potential of LOR for both analgesia and disease-modifying activity in knee OA.

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Figure 1. Subject disposition and primary reasons for discontinuation

Figure 2. WOMAC Pain average scores over time and ladder plots of baseline-adjusted change-from-baseline comparisons of LOR treatment groups versus placebo over time for A) ITT, B) unilateral symptomatic subjects, and C) unilateral symptomatic subjects without widespread pain

Figure 3. WOMAC Function average scores over time and ladder plots of baseline-adjusted change-from-baseline comparisons of LOR treatment groups versus placebo over time for A) ITT, B) unilateral symptomatic subjects, and C) unilateral symptomatic subjects without widespread pain

Figure 4. Medial joint space width (mJSW) average measurements over time and ladder plots of baseline-adjusted change-from-baseline comparisons of LOR treatment groups versus placebo over time for A) ITT, B) unilateral symptomatic subjects, and C) unilateral symptomatic subjects without widespread pain

Supplementary Figure 1. Patient Global Assessment average scores over time and ladder plots of baseline-adjusted change-from-baseline comparisons of LOR treatment groups versus placebo over time for A) ITT, B) unilateral symptomatic subjects, and C) unilateral symptomatic subjects without widespread pain

Supplementary Figure 2. Concordance between change in medial joint space width (mJSW) and WOMAC Pain and Function response at Week 52 for A) ITT, B) unilateral symptomatic subjects, and C) unilateral symptomatic subjects without widespread pain

Table 1. Demographic and clinical characteristics of eligible subjects at baseline by treatment group

	LOR			
	0.03 mg	**0.07 mg**	**0.23 mg**	**Placebo**
N	112	117	110	116
Age (Years) [mean (SD)]	59.0 (9.0)	60.0 (8.2)	61.3 (8.7)	60.7 (8.9)
Body Mass Index (kg/m^2) [mean (SD)]	29.77 (4.81)	30.81 (4.74)	29.64 (4.45)	29.17 (4.40)
Female [n (%)]	68 (60.7)	60 (51.3)	68 (61.8)	72 (62.1)
Race [n (%)]				
White	92 (82.1)	102 (87.2)	96 (87.3)	102 (87.9)
African American	18 (16.1)	14 (12.0)	12 (10.9)	10 (8.6)

Other	2 (1.8)	1 (0.9)	2 (1.8)	4 (3.4)
Hispanic or Latino Ethnicity [n (%)]	20 (17.9)	23 (19.7)	17 (15.5)	21 (18.1)
Kellgren-Lawrence Grade 3 [n (%)]	74 (66.1)	74 (63.2)	70 (63.6)	74 (63.8)
Unilateral Symptomatic [n (%)]	45 (40.2)	35 (29.9)	45 (40.9)	39 (33.6)
Widespread Pain Index ≤4 and Symptom Severity Scale Question 2 Score ≤2 [n (%)]	73 (65.2)	79 (67.5)	76 (69.1)	75 (64.7)

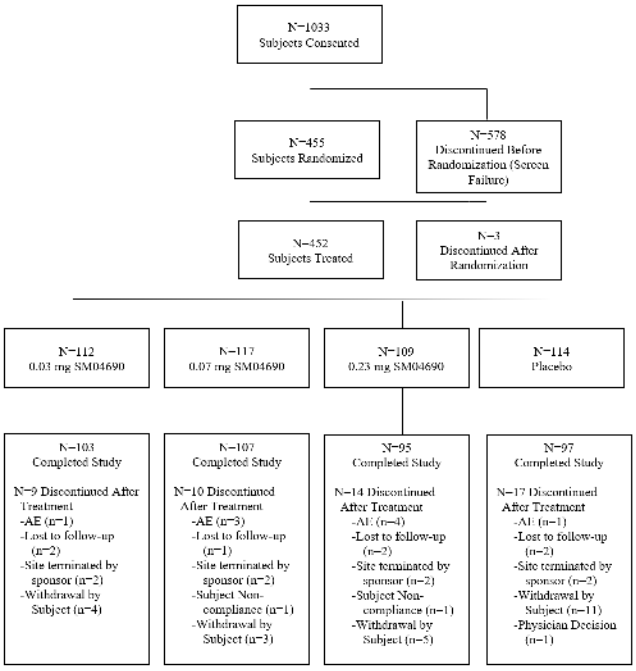
Table 2. Number of treatment-emergent adverse events (#TEAEs) >1% with number (n) and percent (%) of reporting subjects by group

TEAEs Reported [#TEAE / n (%)]	**0.03 mg N=111**	**0.07 mg N=114**	**0.23 mg N=104**	**Placebo N=108**	**All Subje N=452**
Total TEAEs/Unique subjects (%)	**142 / 61 (55.0)**	**147 / 65 (57.0)**	**107 / 47 (45.2)**	**117 / 53 (49.1)**	**547 / 237 (**
Arthralgia	16 / 13 (11.7)	14 / 13 (11.4)	13 / 9 (8.7)	12 / 10 (9.3)	61 / 49 (1
Back pain	0 / 0 (0.0)	2 / 2 (1.8)	1 / 1 (1.0)	2 / 2 (1.9)	5 / 5 (1.
Bronchitis	2 / 2 (1.8)	3 / 3 (2.6)	3 / 2 (1.9)	1 / 1 (0.9)	9 / 8 (1.
Bursitis	2 / 2 (1.8)	3 / 2 (1.8)	2 / 2 (1.9)	0 / 0 (0.0)	7 / 6 (1.
Contusion	1 / 1 (0.9)	2 / 2 (1.8)	3 / 3 (2.9)	2 / 2 (1.9)	8 / 8 (1.
Cystitis	0 / 0 (0.0)	3 / 3 (2.6)	2 / 1 (1.0)	1 / 1 (0.9)	6 / 5 (1.
Fall	2 / 2 (1.8)	2 / 2 (1.8)	0 / 0 (0.0)	1 / 1 (0.9)	5 / 5 (1.
Gastroenteritis	3 / 3 (2.7)	0 / 0 (0.0)	1 / 1 (1.0)	1 / 1 (0.9)	5 / 5 (1.
Headache	0 / 0 (0.0)	6 / 3 (2.6)	2 / 2 (1.9)	4 / 4 (3.7)	13 / 10 (2
Hypertension	0 / 0 (0.0)	4 / 4 (3.5)	4 / 4 (3.8)	3 / 3 (2.8)	11 / 11 (2
Increased AAT[†]	2 / 2 (1.8)	1 / 1 (0.9)	0 / 0 (0.0)	2 / 2 (1.9)	5 / 5 (1.
Influenza	4 / 4 (3.6)	0 / 0 (0.0)	2 / 2 (1.9)	0 / 0 (0.0)	6 / 6 (1.
Joint effusion	5 / 4 (3.6)	2 / 2 (1.8)	1 / 1 (1.0)	2 / 2 (1.9)	10 / 9 (2.
Joint injury	2 / 2 (1.8)	0 / 0 (0.0)	1 / 1 (1.0)	1 / 1 (0.9)	6 / 6 (1.
Joint swelling	5 / 3 (2.7)	4 / 4 (3.5)	2 / 2 (1.9)	6 / 5 (4.6)	17 / 14 (3

Meniscus injury	2 / 2 (1.8)	2 / 2 (1.8)	0 / 0 (0.0)	0 / 0 (0.0)	5 / 5 (1.
Nasopharyngitis	4 / 4 (3.6)	3 / 3 (2.6)	3 / 3 (2.9)	0 / 0 (0.0)	11 / 11 (2
Nausea	2 / 2 (1.8)	1 / 1 (0.9)	2 / 2 (1.9)	1 / 1 (0.9)	6 / 6 (1.
Noncardiac chest pain	1 / 1 (0.9)	2 / 2 (1.8)	1 / 1 (1.0)	1 / 1 (0.9)	6 / 6 (1.
Osteoarthritis	4 / 3 (2.7)	2 / 2 (1.8)	3 / 3 (2.9)	5 / 3 (2.8)	14 / 11 (2
Sinusitis	1 / 1 (0.9)	2 / 2 (1.8)	1 / 1 (1.0)	5 / 5 (4.6)	9 / 9 (2.
Tendonitis	3 / 3 (2.7)	1 / 1 (0.9)	1 / 1 (1.0)	1 / 1 (0.9)	6 / 6 (1.
Upper respiratory tract infection	5 / 5 (4.5)	2 / 2 (1.8)	1 / 1 (1.0)	3 / 3 (2.8)	12 / 12 (2
Urinary tract infection	2 / 2 (1.8)	2 / 2 (1.8)	3 / 2 (1.9)	3 / 3 (2.8)	10 / 9 (2.

*All subjects: Includes those who received a dose of LOR or PBO not specified per protocol (N=15)

†AAT: Aspartate aminotransferase



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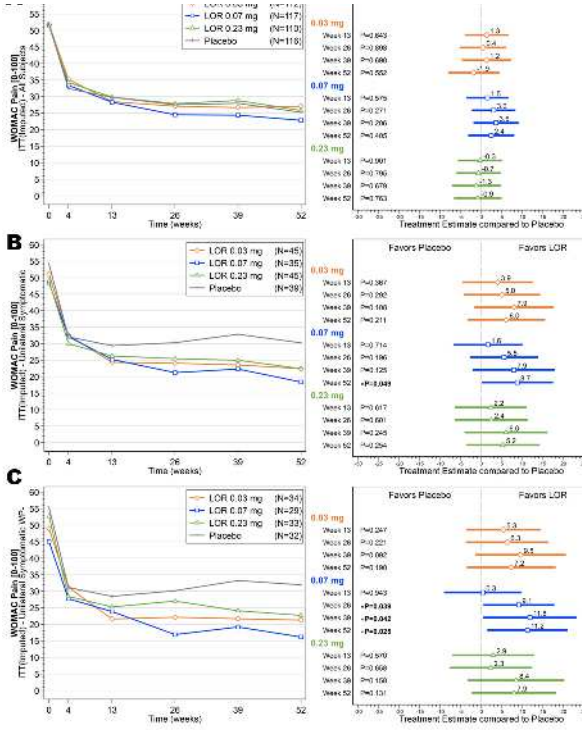

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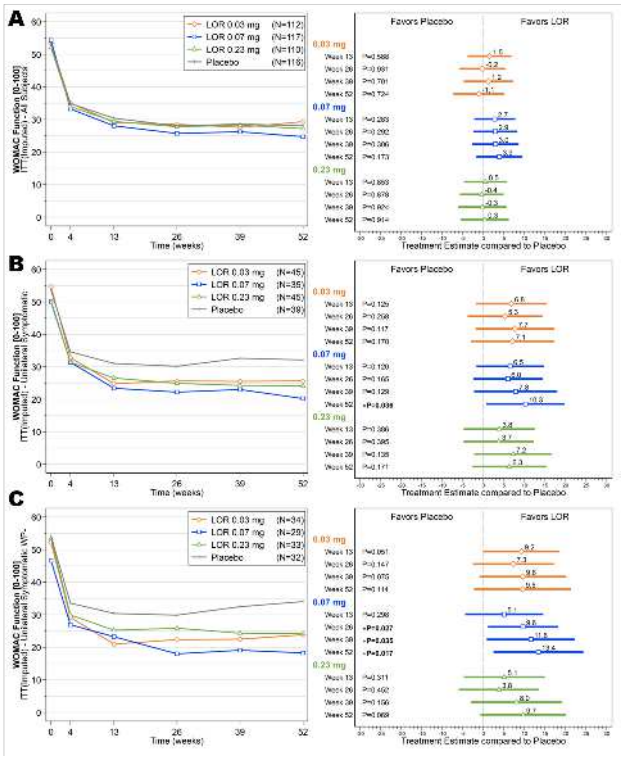

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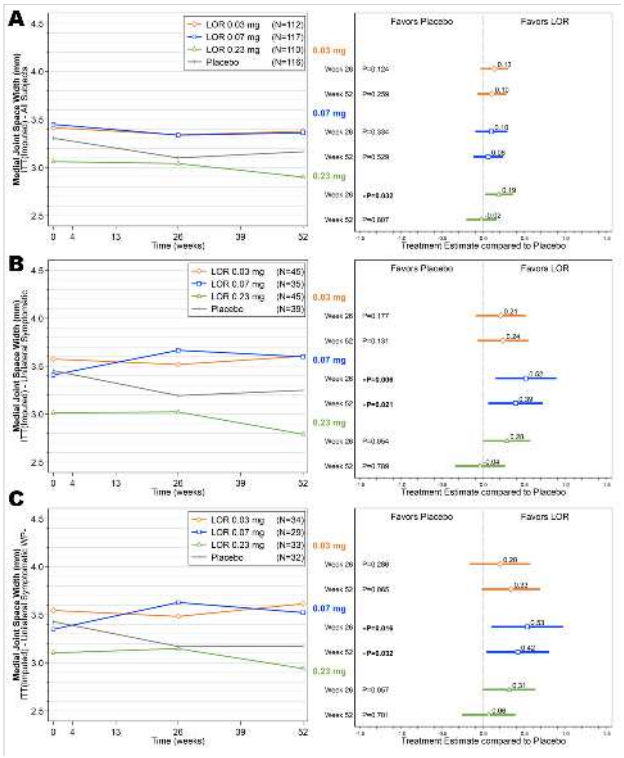

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