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Version: Accepted Version

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Journal:	*Arthritis & Rheumatology*
Manuscript ID	ar-18-0665.R2
Wiley - Manuscript type:	Full Length
Date Submitted by the Author:	n/a
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Keywords:	Inflammation, Cytokines, DMOADs (biologic), knee osteoarthritis, interleukin-1 inhibition
<B>Disease Category: Please select the category from the list below that best describes the content of your	Osteoarthritis

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A Phase 2 Trial of Lutikizumab, an Anti–Interleukin 1α/β Dual Variable Domain Immunoglobulin, in Knee Osteoarthritis Patients With Synovitis

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Running Head: Lutikizumab efficacy and safety in knee OA

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Funding Source:

AbbVie Inc. funded this study.

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Abstract

Objective: To assess the efficacy and safety of the anti-interleukin (IL)-1α/β dual variable domain immunoglobulin lutikizumab (ABT-981) in subjects with knee osteoarthritis (OA) and evidence of synovitis.

Methods: Subjects (N=350; 347 analyzed) with Kellgren-Lawrence grade 2–3 knee OA and synovitis (determined by magnetic resonance imaging [MRI] or ultrasound) were randomized to placebo or lutikizumab 25, 100 or 200 mg subcutaneously every 2 weeks for 50 weeks. The co-primary endpoints were change from baseline in Western Ontario and McMaster Universities Osteoarthritis (WOMAC) pain index at week 16 and change from baseline in MRI synovitis at week 26.

Results: WOMAC pain at week 16 improved significantly versus placebo with lutikizumab 100 mg (P=0.050) but not 25 or 200 mg. Beyond week 16, WOMAC pain was reduced in all groups but was not significantly different for lutikizumab and placebo. Changes from baseline in MRI synovitis at week 26 and other key symptom- and most structure-related endpoints at weeks 26 and 52 were not significantly different for lutikizumab and placebo. Injection site reactions, neutropenia, and discontinuations due to neutropenia were more frequent with lutikizumab versus placebo. Neutrophil and high-sensitivity C-reactive protein reductions plateaued at lutikizumab 100 mg. Immunogenicity to lutikizumab did not meaningfully affect systemic lutikizumab concentrations.

Conclusion: The limited improvement of WOMAC pain and the lack of synovitis improvement with lutikizumab, together with published trial results for other IL-1 inhibitors, suggest that IL-1 inhibition is not an effective analgesic/anti-inflammatory therapy in most patients with knee OA and associated synovitis.

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INTRODUCTION

Osteoarthritis (OA) is the most common arthritis,(1) and main cause of disability among US adults.(2) OA is commonly considered a non-inflammatory arthritis but at least half of patients with knee OA have ultrasound or magnetic resonance imaging (MRI) evidence of synovitis.(3, 4) Synovitis is associated with a risk of developing radiographic knee OA,(5, 6) greater knee pain,(7, 8) and total joint replacement.(9)

Interleukin (IL)–1α and IL-1β are pro-inflammatory cytokines and pain mediators that are thought to be involved in the pathogenesis of OA.(10, 11) IL-1β is secreted by innate immune cells after cleavage by caspase-1.(10) In contrast, IL-1α is stored intracellularly or membrane bound,(10) is released in an active form upon cell damage,(11) and can induce IL-β activation and production of other cytokines important in the pathogenesis of OA.(10) IL-1α and IL-1β bind to the same receptor, IL-1R1, resulting in inflammatory and pain responses.(10, 12, 13)

IL-1α and IL-1β are expressed in the cartilage and synovial membrane, and are elevated in sera and synovial fluid in patients with OA.(14-16) Synovial macrophages are an important source of IL-1 in patients with knee OA.(17) IL-1 may promote structural damage associated with OA, because IL-1 activates enzymes involved in cartilage destruction,(18) inhibits collagen synthesis,(19) and promotes osteoclastogenesis.(10, 20) The IL-1 pathway may mediate OA pain through pathways in the peripheral and central nervous systems.(12, 13) In some, but not all animal models of OA, blocking the IL-1 pathway improves OA manifestations.(21) However, in clinical trials in subjects with knee OA not selected for synovitis, an IL-1 receptor antagonist

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(anakinra)(22) or an antibody to the IL-1R1 (AMG 108)(23) did not meet the primary symptom-based study endpoints.

Lutikizumab (ABT-981) is a novel human dual variable domain immunoglobulin (DVD-Ig) that has been shown to bind and inhibit IL-1α and IL-1β.(24) In mouse OA models, a mouse anti–IL-1α/β DVD-Ig increased the threshold for pain and reduced cartilage degeneration to a greater extent than inhibition of either IL-1α or IL-1β alone.(25) In phase 1 studies of ≤8 weeks in healthy subjects and subjects with knee OA, single and multiple doses of lutikizumab were well tolerated; the most frequently reported adverse events (AEs) were injection site reactions and headache.(26, 27) Among the knee OA subjects in a phase 1 study, lutikizumab therapy was associated with reductions in serum inflammatory biomarkers.(27)

The current trial enrolled subjects with knee OA and MRI and/or ultrasound evidence of synovitis, a population presumed to be at high risk of progression, to test the hypothesis that dual inhibition of IL-1α and IL-1β would demonstrate efficacy and safety in knee OA with inflammation.

METHODS

Study Design

The objective was to determine the efficacy and safety of lutikizumab in subjects with knee OA and synovitis. The study was conducted in accordance with International Conference on Harmonization guidelines and the Declaration of Helsinki. This phase 2, randomized, double-blind, placebo-controlled, parallel-group study (NCT02087904; ILLUSTRATE-K) was approved

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by institutional review boards, and signed informed consent was obtained from all subjects. After

screening and washout periods totaling approximately 45 days **(Supplemental Figure 1)**,

eligible subjects were randomized (1:1:1:1) to double-blind lutikizumab 25, 100, or 200 mg or

matching placebo subcutaneously every 2 weeks for 52 weeks (last dose of study medication,

week 50).

Rescue medication included acetaminophen (maximum, 3000 mg/d) during the washout period

through week 26, and ibuprofen (maximum, 1200 mg/d), with or without acetaminophen, during

weeks 16 to 26 for breakthrough knee pain, although analgesics were stopped ≥48 hours before

the first dose of study drug and 24 hours before each pain assessment. From weeks 26 to 52, oral

standard-of-care (SOC) medications for knee OA including non-steroidal anti-inflammatory

drugs (NSAIDs), non-opioid analgesics, and nutraceuticals (e.g., glucosamine, chondroitin

sulfate, shark cartilage, diacerein, soy extract) were permitted; SOC medications for knee OA

were stopped ≥24 hours before each pain assessment.

Subjects

Adult subjects (35–74 years old) with radiographic evidence of knee OA in the medial

compartment of the index knee with Kellgren-Lawrence (KL) grade 2 or 3(28) were eligible if

meeting other inclusion criteria including signs and symptoms of active inflammation (e.g.,

localized pain, stiffness, swelling, or effusion) in the index knee; presence of synovitis in the

index knee by either ultrasound (local reader, per an ultrasound guide) or MRI (central reader);

pain score ≥4 and ≤8 (11-point numeric rating scale [NRS-11], 0–10 representing no pain to

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worst possible pain)(29) in the index knee for ≥14 days over the past 30 days; and patient global

assessment of arthritis status ≥4 (NRS-11, 0–10 representing best to worst disease status).(30)

Key exclusion criteria included other inflammatory arthritis (e.g., rheumatoid arthritis, psoriatic

arthritis, or gout) or a painful myofascial syndrome such as fibromyalgia **(Supplemental**

Methods).

Subjects must have discontinued use of all analgesics, NSAIDs, and nutraceuticals for ≥5 half-

lives of the longest-acting therapy or 48 hours, whichever was longer, before the first dose of

study drug. Subjects receiving concomitant medications for indications other than OA (if

allowed by the protocol) had to be on stable doses for ≥1 month before the first dose of study

drug.

Efficacy

The co-primary endpoints were (1) change from baseline in Western Ontario and McMaster

Universities Osteoarthritis index (WOMAC) pain score (0–50 scale; NRS-11 subscales)(31) at

week 16 and (2) change in MRI synovitis from baseline in the index knee at week 26. To meet

the latter co-primary endpoint , we required reductions in all 3 of the following measurements:

(a) quantitative synovial membrane thickness,(32) (b) quantitative synovial fluid volume,(33)

and (c) semi-quantitative synovitis/effusion score measured on a scale of 0 to 3 using the Whole-

Organ Magnetic Resonance Imaging Score (WORMS).(34)

Secondary endpoints included changes from baseline in WOMAC pain scores at weeks 26 and

52(31); Intermittent and Constant Pain (ICOAP) score(35) at weeks 16, 26 and 52; and 3 types of

pain intensity measures using NRS-11 scales **(Supplemental Methods)**.

Exploratory endpoints included Outcome Measures in Rheumatology Clinical

Trials/Osteoarthritis Research Society International (OMERACT/OARSI) response(36) at weeks

16, 26 and 52; radiographic medial and lateral joint space narrowing (JSN; centralized

measurement of the minimum joint space width compared with baseline in the index knee at

week 52; and changes from baseline to week 26 in synovitis as assessed by dynamic contrast-

enhanced MRI (DCE-MRI).(37)

Imaging

Patients were screened for presence of synovitis using MRI or musculoskeletal ultrasound

(Supplemental Methods), which has shown good to excellent inter- and intra-reader agreement

in detecting knee synovitis.(38, 39) Posteroanterior weight-bearing radiographs of the target knee

were acquired at screening and at week 52. MRI of the target knee was performed using 1.5- or

3.0-T whole-body scanners and commercial knee coils at screening, week 26, and week 52.

Given the limitations associated with non–contrast-enhanced MRI for assessment of synovitis,(8)

dynamic contrast-enhanced MRI (DCE-MRI) was performed as an exploratory substudy (n=39),

as described previously.(37)

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Pharmacokinetics

Blood samples were collected throughout the 52 weeks to assess concentrations of lutikizumab

and anti-drug antibody (ADA) responses in serum as previously described.(26)

Pharmacodynamics

Blood neutrophil counts and serum high-sensitivity C-reactive protein (hsCRP) levels (ICON,

ARCHITECT platforms C8000 or C16000, Abbott Laboratories, Abbott Park, IL, United States)

were measured repeatedly from baseline to week 52; other biomarkers were measured at baseline

and weeks 16, 26 and 52. Serum concentrations of free IL-1α and IL-1β were determined using

the Singulex Erenna (MilliporeSigma, Billerica, MA, United States) and SIMOA platforms

(Quanterix, Lexington, MA, United States), respectively, using AbbVie proprietary capture and

detection antibodies. Other biomarkers were measured by BioClinica Molecular Marker Lab

(Lyon, France) using validated enzyme-linked immunosorbent assays (from Nordic Bioscience

[Herlev, Denmark; metalloproteinase-degraded collagen types I and III and matrix

metalloproteinase-generated fragment of CRP], Corgenix [Broomfield, CO, United States;

hyaluronic acid], EMD Millipore [Darmstadt, Germany; N-propeptide of collagen IIA], and

Roche Diagnostics [Indianapolis, IN, United States; C-terminal telopeptide fragments of type II

collagen]), adhering to standard operating procedures from regulatory guidance for clinical

studies.

Safety

Adverse events, vital signs, physical examinations, and laboratory data were assessed throughout

the study. AEs were coded using the *Medical Dictionary for Regulatory Activities*, *version 19.0*,

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preferred term, and system organ class. AE severity was classified according to the Common

Terminology Criteria for Adverse Events, version 4.03.(40)

Statistics and Analyses

The co-primary and secondary efficacy outcomes were analyzed in the modified intent-to-treat

population, comprising randomized patients who received ≥1 dose of study drug. Continuous

efficacy assessments were analyzed with analysis of covariance (ANCOVA) with main factors of

treatment, age group, and KL grade and covariates of baseline values, except analysis of daily

rescue medication use, which was analyzed with analysis of variance (ANOVA). Categorical

variables analysis used the Cochran-Mantel-Haenszel test with age group and KL grade as

stratification factors. Last-observation-carried-forward (LOCF) imputation of missing values was

used for non-imaging co-primary and selected secondary endpoints. The non-imaging co-

primary endpoint was also assessed using multiple imputation (MI) for missing values in a post

hoc analysis. In this phase 2 study, there was no adjustment for multiplicity of assessments. The

safety analysis set included subjects who received ≥1 dose of study drug. To identify factors

associated with the development of neutropenia (defined as at least one episode where absolute

neutrophil count [ANC] <1,500 cells/µL), explanatory variables significant at the $P<0.1$ level in

univariate analyses were tested in a multiple logistic regression model.

A sample size of approximately 80 subjects per treatment group was estimated to provide ≥80%

power to detect a significant difference between lutikizumab and placebo, based on a

significance level of 0.05, for each of the two co-primary endpoints (WOMAC pain and synovial

inflammation based on synovial membrane thickness by MRI).

RESULTS

Subjects

Of 1571 subjects screened, 350 were eligible and were randomized; 347 received ≥1 dose of study drug (June 2014 to November 2016) and were analyzed for efficacy and safety **(Figure 1)**. The most common reasons for screen failure were clinical history related to entry criteria, absence of KL-2 or KL-3 radiographic evidence of knee OA, lack of synovitis on ultrasound or by non–contrast-enhanced MRI, and severe knee malalignment.(41) Most subjects (229; 65.4%) were enrolled based on ultrasound evidence of knee synovitis; 118 (33.7%) were enrolled based on MRI evidence **(Supplemental Table 1)**. Among the subjects enrolled based solely on screening ultrasound, 185/220 (84.1%) had baseline MRI evidence of synovitis per WORMS scoring conducted at the end of the study. Demographics, baseline disease characteristics, and use of medications were generally well matched across treatment groups **(Table 1)**. Of the randomized subjects, 60/85 (70.6%) who received placebo and 202/265 (76.2%) who received lutikizumab completed the 52-week study **(Figure 1)**. The major reasons for discontinuation included AEs, lack of efficacy, and withdrawal of consent.

Efficacy

Co-primary Endpoints

The co-primary endpoint of WOMAC pain at week 16 (and at most early time points) improved significantly, compared with placebo, for the lutikizumab 100 mg dose group (*P*=0.050) but not for the lutikizumab 25 mg (*P*=0.834) and 200 mg (*P*=0.415) dose groups **(Figure 2A, Table 2, Supplemental Figure 2A)**. Post hoc analysis for WOMAC pain using MI yielded results

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consistent with those of LOCF **(Supplemental Figure 3)**. WOMAC pain reduction in all

lutikizumab groups, as well as placebo, was sustained from weeks 16 to 52, but differences

between lutikizumab and placebo for WOMAC pain were not significant between weeks 16 and

52 **(Figure 3A)**.

The other co-primary endpoint, change in synovitis (as measured by synovial membrane

thickness, synovial fluid volume, and WORMS synovitis/effusion score) from baseline to week

26, did not differ between the lutikizumab and placebo groups **(Table 2)**.

Post hoc analyses were performed to determine efficacy including only subjects with a baseline

WORMS synovitis/effusion score >0, indicating MRI evidence of synovitis. Results in this post

hoc population were not substantially different from those in the original, prospective population

enrolled on the basis of positive MRI or ultrasound (**Supplemental Figure 4, Supplemental**

Table 2).

Other Signs and Symptoms Endpoints

WOMAC function **(Figure 2B, Supplemental Figure 2B)** and OMERACT/OARSI response

were numerically better but not significantly different between the placebo and lutikizumab

treatment groups **(Table 2)** at 16, 26 and 52 weeks. Change from baseline in WOMAC function

was significantly different ($P \leq 0.01$) from placebo with lutikizumab 100 mg at weeks 4 and 8

(Figure 2B). OMERACT/OARSI placebo responses were high, e.g., 60%–71%, at weeks 26 and

52, respectively **(Table 2, Figure 2)**.

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Other Structural Endpoints

Among secondary and exploratory endpoints, other than medial (*P*=0.017) and lateral (*P*=0.005) JSN with lutikizumab 25 mg at week 52 **(Table 2),** there were no structural endpoints statistically significantly different between placebo and the lutikizumab dose groups. MRI assessments of cartilage volume, thickness, and WORMS scores were nearly identical in all treatment groups at baseline, week 26 and week 52. Synovitis, as assessed by DCE-MRI, also demonstrated no differences between subjects treated with placebo versus each dose of lutikizumab **(Supplemental Table 3)**.

Rescue Medication Use

The proportion of subjects receiving concomitant pain medication (acetaminophen, ibuprofen) during the study was generally similar among treatment groups **(Supplemental Table 4)**. The least squares mean daily dose of rescue acetaminophen up to week 16 was similar in the placebo (511 mg, n=58) and lutikizumab 25 mg (500 mg, n=53), 100 mg (413 mg, n=63) and 200 mg (426 mg, n=67) dose groups. Similarly, the least squares mean daily dose of rescue ibuprofen between weeks 16 and 26 was not significantly different between the placebo (200 mg, n=18) and lutikizumab 25 mg (104 mg, n=18), 100 mg (241 mg, n=19), and 200 mg (155 mg, n=24) dose groups.

Pre-planned Efficacy Subgroup Analyses

In pre-planned subgroup analyses, there were no meaningful differences in WOMAC pain scores at weeks 16, 26, and 52 based on age, gender, race, or body weight. In the 100 mg dose group, there were statistically significantly greater decreases in WOMAC pain scores compared with

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placebo, among subjects with an index knee KL grade of 3 (but not KL grade of 2) (week 26, P=0.029; week 52, 0.016) and among subjects who did not use concomitant medications for index knee pain through week 26 (week 16, P=0.027; week 26, 0.045). This was not noted in the 25 mg or 200 mg dose group.

Pharmacokinetics

Lutikizumab trough concentrations were stable between weeks 6 and 52 and consistent with assessments of steady-state in previous studies **(Supplemental Figure 5A)**.(26, 42) A greater ADA incidence was observed for the lutikizumab 25 mg dose group (46%) compared with the 100 mg and 200 mg dose groups (32% and 23%, respectively). Lutikizumab serum concentrations were generally similar among subjects with and without ADAs for each dose group **(Supplemental Figure 5B)**; thus, the immunogenic response did not appear to have a meaningful impact on lutikizumab pharmacokinetics.

Pharmacodynamics

Mean blood neutrophil counts (ANC) decreased with lutikizumab 100 mg and 200 mg treatment at all time points and with lutikizumab 25 mg at most time points relative to baseline and placebo **(Figure 3A)**; neutrophil counts were similar in the 100 mg and 200 mg dose groups throughout the study. There was an exposure-response relationship between ANCs and lutikizumab blood levels **(Supplemental Figure 6)**. In a similar way, hsCRP levels were reduced at most time points in the lutikizumab groups compared with baseline and placebo **(Figure 3B)**, reaching statistical significance at several time points for the 25 mg and 100 mg doses but with high variability.

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Serum levels of IL-1α and IL-1β at baseline were low and most were below the lower limits of

quantification. In subjects with detectable levels at baseline, IL-1α and IL-1β levels were reduced

within 2 weeks of treatment initiation to a greater extent in subjects receiving lutikizumab

compared with subjects receiving placebo **(Supplemental Figure 7, Supplemental Table 5)**.

Changes in other biomarkers are shown in **Supplemental Figure 8**. With lutikizumab treatment,

compared with placebo, there were reductions in metalloproteinase-degraded collagen type I

(C1M), metalloproteinase-degraded collagen type III (C3M), matrix metalloproteinase-generated

fragment of CRP (CRPM), IL-6, and alkaline phosphatase.

Safety

Similar proportions of subjects receiving placebo or lutikizumab experienced a treatment-

emergent AE or serious AE during the study **(Table 3)**. Serious infections were infrequent and

had similar incidences among treatment groups, including placebo. A greater proportion of

subjects in the lutikizumab total treatment groups compared with the placebo group had injection

site reactions (25.2% vs 15.3%) and neutropenia (27.5% vs 2.4%). The incidence of both events

increased in a dose-dependent manner. All reported neutropenia laboratory abnormalities were

grade 3 or less; there was no grade 4 neutropenia. One subject who received lutikizumab 25 mg

and 6 subjects who received lutikizumab 200 mg discontinued study treatment because of

neutropenia.

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Treatment-emergent AEs of malignancy were reported in 5 subjects (1.9%; 2.2 events per 100 patient-years) in the total lutikizumab group and in no subjects in the placebo group. Basal cell carcinoma was reported in 3 of these 5 subjects.

DISCUSSION

This study assessed the efficacy and safety of blocking IL-1α and IL-1β with lutikizumab in subjects with knee OA and associated synovitis. To our knowledge, this is the first study to test the hypothesis that a systemic anti-inflammatory, anti-cytokine therapy may be effective in knee OA patients with synovitis. Lutikizumab met the co-primary clinical endpoint of reduction in WOMAC pain compared with placebo at week 16 in only the 100 mg dose group, but not in the 25 mg and 200 mg dose groups, and the differences compared with placebo were not sustained past week 16 for any dose. In the subgroup of patients treated with lutikizumab 100 mg with KL grade 3 knee OA and among the subgroup of subjects not using rescue or off-protocol pain medications, compared with placebo, the change from baseline at weeks 26 and 52 in WOMAC pain was significantly greater; the reason why this occurred only in the 100 mg dose group and only at these time points is unclear. The co-primary endpoint, change from baseline in synovitis as measured by synovial membrane thickness, synovial effusion volume, and semiquantitative MRI synovitis/effusion (WORMS) at week 26, was also not significantly different for lutikizumab versus placebo. Furthermore, when compared with placebo, lutikizumab was not associated with improvements or slowing in the rates of JSN and MRI cartilage thickness changes. These results indicate that lutikizumab had no significant impact on structural endpoints that were assessed. The analytical assay used to quantify lutikizumab serum concentrations required at least one free binding site for each molecule of IL-1α and IL-1β.(26) The modest

impact of ADAs on trough concentrations **(Supplemental Figure 5B)** suggested that development of ADAs did not interfere with the biological activity of lutikizumab, and thus they were unlikely to have impacted efficacy.

The unexpected lack of an effect of IL-1 inhibition on synovitis may signify that, although the synovium is an important source of IL-1,(43) IL-1 by itself may not be required to sustain synovitis. Other factors, such as cartilage degradation products and adipokines may have a greater role in the development and maintenance of synovitis.(44) Overall, these observations are consistent with a recent study using a medial menisectomy animal model that found that IL-1 may not play a role in the structural progression of OA.(21) A phase 2 study of lutikizumab in erosive hand OA also was negative.(42)

The reason(s) for the failure to demonstrate a sustained beneficial analgesic effect of lutikizumab compared with placebo in this study is not clear. Lutikizumab serum concentrations were stable throughout the duration of the study, unaffected by development of ADAs and at exposures consistent with a previous phase 1 study.(27) The probability of achieving positive results might have been improved by using a flare study design, although a difference in effect size has not been shown to be statistically significant versus a non-flare study design.(45) Although serum IL-1α and IL-1β levels could not be reliably quantified, similar reductions in absolute neutrophil count and hsCRP between the lutikizumab 100 mg and 200 mg dose groups suggested that both dose groups achieved maximum suppression of IL-1α and IL-1β. However, it is possible that these systemic pharmacodynamic endpoints are not reflective of target engagement within the joint.

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As there was no positive control treatment in this study, it is not clear whether lutikizumab did have a positive effect masked by a placebo response. The use of SOC therapies following week 26 may have masked any lutikizumab treatment effects and could account for the lack of sustained WOMAC pain differences between the lutikizumab 100 mg treatment group and the placebo group, but would not explain the inefficacy of the 25 mg and 200 mg doses. The pre-planned subset analyses suggested that the use of rescue medication affected the WOMAC pain co-primary endpoint results; however, meta-analysis has concluded that acetaminophen has little if any analgesic effect in OA.(46) Another pre-planned subset analysis suggested that subjects with KL grade 3 knee OA (in contrast to subjects with KL grade 2 knee OA) had statistically significant WOMAC pain treatment effects, implying that knee OA subjects with greater amounts of structural disease may benefit from IL-1 inhibition. The hypothesis that the concomitant use of pain medication and the degree of radiographic damage could predict response to lutikizumab would have to be proven in a well-designed prospective study.

The lack of a clear lutikizumab treatment effect may have also been due to a strong, sustained placebo effect **(Figure 3A)**. Placebo response rates are high in knee OA trials (e.g., 40%–50% using OMERACT-OARSI criteria)(47); placebo response rates in this trial were 60%, 62% and 71% at weeks 16, 26 and 52 **(Table 2)**. High placebo responses have been reported in studies of injectable drugs.(48)

There were no unexpected AEs compared with other IL-1 inhibitors,(23) except that there were 5 malignancies in the combined lutikizumab treatment groups versus no malignancies in the

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placebo group. While immunosuppression is associated with a risk of malignancy, two published studies of IL-1 inhibitors (CANTOS and RESURGE) have not identified an increased risk of malignancies.(49, 50) The CANTOS study of subjects treated with the IL-1β inhibitor canakinumab identified a significantly lower risk of incident lung cancer.(49) Given the CANTOS and RESURGE study data and the low numbers of malignancies observed in our study, it is currently unclear how best to interpret the greater number of malignancies in the combined lutikizumab treatment groups compared with the placebo group. Any future studies of lutikizumab should carefully monitor the incidence of malignancy to determine whether the findings observed in the present study are replicated.

Strengths of this study include the measurement of efficacy and pharmacodynamic endpoints in the same subjects. Plateauing of neutrophil decreases from baseline between the lutikizumab 100 mg and 200 mg dose groups suggested that systemic IL-1 was maximally suppressed; similar reductions in neutrophil levels were achieved with the IL-1R inhibitor AMG 108.(23) Another strength was enrichment for subjects with evidence of inflammation using ultrasound, MRI, and clinical characteristics, with the assumption that this increased the likelihood of treating subjects with elevated joint levels of IL-1. Multiple signs and symptoms and structural endpoints were evaluated over 52 weeks, which maximized the opportunity to demonstrate an effect of lutikizumab. Confounding by other potentially disease-modifying agents was minimal. Further investigation could probe whether subgroups of patients with knee OA have a significant clinical response to lutikizumab.

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Several limitations of this study should be noted. Importantly, levels of lutikizumab, IL-1α, and IL-1β in the synovial fluid of the knee joint could not be assessed. In addition, approximately two-thirds of subjects had relatively mild radiographic changes (KL-2, 62.8%) and none had severe disease (KL-4, 0%). Studies of tanezumab, a monoclonal antibody that targets nerve growth factor, enrolled a higher percentage of subjects with KL-3 and KL-4 disease (38.6%–46.9% and 14.1%–27.7%, respectively),(47, 51) suggesting that patients with more severe disease may be more responsive to treatment, consistent with the pre-planned sub-analysis of subjects with KL grade 3 in the present study. This suggests that, by enrolling subjects with a lower average KL grade, the likelihood of observing significant pain improvements may have been reduced in the current study. As noted previously, a robust placebo response was found, possibly enriched by the allowance of rescue pain medication and the frequent study visits with subcutaneous drug administration. Another potential limitation of our study was highlighted by the discrepancy between ultrasound and MRI grading of synovitis. Of the limited number of cases (n=6) where an ultrasound and MRI were both submitted to screen for synovitis prior to enrollment, all 6 subjects were positive by ultrasound and only 3 of the 6 were positive by non–contrast-enhanced MRI, supporting the idea that the lack of contrast-enhancement may have limited our ability to detect changes in synovitis with lutikizumab therapy.

In conclusion, although the 100 mg dose of lutikizumab met the co-primary endpoint of reduction in WOMAC pain at week 16 compared with placebo, neither the 25 mg nor the 200 mg dose did so, and the difference from placebo was not sustained after week 16. Measures of synovitis, cartilage thickness, and other structural endpoints were similar between lutikizumab and placebo at all time points and with all 3 doses evaluated. The safety profile of lutikizumab

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was consistent with the AEs reported for other IL-1 inhibitors, including neutropenia and injection site reactions. The results of this study suggest that IL-1 inhibition is not an effective disease-modifying therapy in patients with knee OA. Whether subgroups of knee OA patients might have symptomatic benefit from IL-1 inhibition remains an open question.

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ACKNOWLEDGMENTS

Data requests can be submitted at any time and the data will be accessible for 12 months, with possible extensions considered. For more information on the process, or to submit a request, visit the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-information-sharing/data-and-information-sharing-with-qualified-researchers.html.

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Figure Legends

Figure 1. Patient disposition. Q2W, every 2 weeks; SC, subcutaneous. *Major protocol violations: (1) actual treatment is not according to randomization assignment, (2) premature unblinding, (3) use of a narcotic or non-steroidal anti-inflammatory drug for >50% of the days before week 16, (4) baseline index knee pain intensity <4 (11-point numeric rating scale), and (5) absence of synovitis in the index knee by ultrasound or magnetic resonance imaging during screening. [†]Study completion at 52 weeks.

Figure 2. LS mean change from baseline over time for WOMAC pain **(A)** and WOMAC function **(B)**. LS, least squares; Q2W, every 2 weeks; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index. *$P \leq 0.05$ vs placebo; [†]$P \leq 0.01$ vs placebo.

Figure 3. Time course of mean total neutrophils **(A)** and mean hsCRP levels **(B)** from baseline (week 0) to week 52. hsCRP, high-sensitivity C-reactive protein; Q2W, every 2 weeks. *$P < 0.05$ or a higher level of significance compared with placebo.

A Phase 2 Trial of Lutikizumab, an Anti–Interleukin 1α/β Dual Variable Domain Immunoglobulin, in Knee Osteoarthritis Patients With Synovitis

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Running Head: Lutikizumab efficacy and safety in knee OA

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AbbVie Inc. funded this study.

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Abstract

Objective: To assess the efficacy and safety of the anti-interleukin (IL)-1α/β dual variable domain immunoglobulin lutikizumab (ABT-981) in subjects with knee osteoarthritis (OA) and evidence of synovitis.

Methods: Subjects (N=350; 347 analyzed) with Kellgren-Lawrence grade 2–3 knee OA and synovitis (determined by magnetic resonance imaging [MRI] or ultrasound) were randomized to placebo or lutikizumab 25, 100 or 200 mg subcutaneously every 2 weeks for 50 weeks. The co-primary endpoints were change from baseline in Western Ontario and McMaster Universities Osteoarthritis (WOMAC) pain index at week 16 and change from baseline in MRI synovitis at week 26.

Results: WOMAC pain at week 16 improved significantly versus placebo with lutikizumab 100 mg (*P*=0.050) but not 25 or 200 mg. Beyond week 16, WOMAC pain was reduced in all groups but was ~~similar~~ not significantly different for lutikizumab and placebo. Changes from baseline in MRI synovitis at week 26 and other key symptom- and most structure-related endpoints at weeks 26 and 52 were not significantly different~~similar~~ for lutikizumab and placebo. Injection site reactions, neutropenia, and discontinuations due to neutropenia were more frequent with lutikizumab versus placebo. Neutrophil and high-sensitivity C-reactive protein reductions plateaued at lutikizumab 100 mg. Immunogenicity to lutikizumab did not meaningfully affect systemic lutikizumab concentrations.

Conclusion: The limited improvement of WOMAC pain and the lack of synovitis improvement with lutikizumab, together with published trial results for other IL-1 inhibitors, suggest that IL-1 inhibition is not an effective analgesic/anti-inflammatory therapy in most patients with knee OA and associated synovitis.

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INTRODUCTION

Osteoarthritis (OA) is the most common arthritis,(1) and main cause of disability among US

adults.(2) OA is commonly considered a non-inflammatory arthritis but at least half of patients

with knee OA have ultrasound or magnetic resonance imaging (MRI) evidence of synovitis.(3, 4)

Synovitis is associated with a risk of developing radiographic knee OA,(5, 6) greater knee

pain,(7, 8) and total joint replacement.(9)

Interleukin (IL)–1α and IL-1β are pro-inflammatory cytokines and pain mediators that are

thought to be involved in the pathogenesis of OA.(10, 11) IL-1β is secreted by innate immune

cells after cleavage by caspase-1.(10) In contrast, IL-1α is stored intracellularly or membrane

bound,(10) is released in an active form upon cell damage,(11) and can induce IL-β activation

and production of other cytokines important in the pathogenesis of OA.(10) IL-1α and IL-1β

bind to the same receptor, IL-1R1, resulting in inflammatory and pain responses.(10, 12, 13)

IL-1α and IL-1β are expressed in the cartilage and synovial membrane, and are elevated in sera

and synovial fluid in patients with OA.(14-16) Synovial macrophages are an important source of

IL-1 in patients with knee OA.(17) IL-1 may promote structural damage associated with OA,

because IL-1 activates enzymes involved in cartilage destruction,(18) inhibits collagen

synthesis,(19) and promotes osteoclastogenesis.(10, 20) The IL-1 pathway may mediate OA pain

through pathways in the peripheral and central nervous systems.(12, 13) In some, but not all

animal models of OA, blocking the IL-1 pathway improves OA manifestations.(21) However, in

clinical trials in subjects with knee OA not selected for synovitis, an IL-1 receptor antagonist

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(anakinra)(22) or an antibody to the IL-1R1 (AMG 108)(23) did not meet the primary symptom-based study endpoints.

Lutikizumab (ABT-981) is a novel human dual variable domain immunoglobulin (DVD-Ig) that has been shown to bind and inhibit IL-1α and IL-1β.(24) In mouse OA models, a mouse anti–IL-1α/β DVD-Ig increased the threshold for pain and reduced cartilage degeneration to a greater extent than inhibition of either IL-1α or IL-1β alone.(25) In phase 1 studies of ≤8 weeks in healthy subjects and subjects with knee OA, single and multiple doses of lutikizumab were well tolerated; the most frequently reported adverse events (AEs) were injection site reactions and headache.(26, 27) Among the knee OA subjects in a phase 1 study, lutikizumab therapy was associated with reductions in serum inflammatory biomarkers.(27)

The current trial enrolled subjects with knee OA and MRI and/or ultrasound evidence of synovitis, a population presumed to be at high risk of progression, to test the hypothesis that dual inhibition of IL-1α and IL-1β would demonstrate efficacy and safety in knee OA with inflammation.

METHODS

Study Design

The objective was to determine the efficacy and safety of lutikizumab in subjects with knee OA and synovitis. The study was conducted in accordance with the International Conference on Harmonization guidelines and the Declaration of Helsinki. This phase 2, randomized, double-blind, placebo-controlled, parallel-group study (NCT02087904; ILLUSTRATE-K) was approved

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by institutional review boards, and signed informed consent was obtained from all subjects. After

screening and washout periods totaling approximately 45 days **(Supplemental Figure 1)**,

eligible subjects were randomized (1:1:1:1) to double-blind lutikizumab 25, 100, or 200 mg or

matching placebo subcutaneously every 2 weeks for 52 weeks (last dose of study medication,

week 50).

Rescue medication included acetaminophen (maximum, 3000 mg/d) during the washout period

through week 26, and ibuprofen (maximum, 1200 mg/d), with or without acetaminophen, during

weeks 16 to 26 for breakthrough knee pain, although analgesics were stopped ≥48 hours before

the first dose of study drug and 24 hours before each pain assessment. From weeks 26 to 52, oral

standard-of-care (SOC) medications for knee OA including non-steroidal anti-inflammatory

drugs (NSAIDs), non-opioid analgesics, and nutraceuticals (e.g., glucosamine, chondroitin

sulfate, shark cartilage, diacerein, soy extract) were permitted; SOC medications for knee OA

were stopped ≥24 hours before each pain assessment.

Subjects

Adult subjects (35–74 years old) with radiographic evidence of knee OA in the medial

compartment of the index knee with Kellgren-Lawrence (KL) grade 2 or 3(28) were eligible if

meeting other inclusion criteria including signs and symptoms of active inflammation (e.g.,

localized pain, stiffness, swelling, or effusion) in the index knee; presence of synovitis in the

index knee by either ultrasound (local reader, per an ultrasound guide) or MRI (central reader);

pain score ≥4 and ≤8 (11-point numeric rating scale [NRS-11], 0–10 representing no pain to

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worst possible pain)(29) in the index knee for ≥14 days over the past 30 days; and patient global

assessment of arthritis status ≥4 (NRS-11, 0–10 representing best to worst disease status).(30)

Key exclusion criteria included other inflammatory arthritis (e.g., rheumatoid arthritis, psoriatic

arthritis, or gout) or a painful myofascial syndrome such as fibromyalgia **(Supplemental**

Methods).

Subjects must have discontinued use of all analgesics, NSAIDs, and nutraceuticals for ≥5 half-

lives of the longest-acting therapy or 48 hours, whichever was longer, before the first dose of

study drug. Subjects receiving concomitant medications for indications other than OA (if

allowed by the protocol) had to be on stable doses for ≥1 month before the first dose of study

drug.

Efficacy

The co-primary endpoints were (1) change from baseline in Western Ontario and McMaster

Universities Osteoarthritis index (WOMAC) pain score (0–50 scale; NRS-11 subscales)(31) at

week 16 and (2) change in MRI synovitis from baseline in the index knee at week 26. To meet

the latter co-primary endpoint , we required reductions in all 3 of the following measurements:

(a) quantitative synovial membrane thickness,(32) (b) quantitative synovial fluid volume,(33)

and (c) semi-quantitative synovitis/effusion score measured on a scale of 0 to 3 using the Whole-

Organ Magnetic Resonance Imaging Score (WORMS).(34)

Secondary endpoints included changes from baseline in WOMAC pain scores at weeks 26 and 52(31); Intermittent and Constant Pain (ICOAP) score(35) at weeks 16, 26 and 52; and 3 types of pain intensity measures using NRS-11 scales **(Supplemental Methods)**.

Exploratory endpoints included Outcome Measures in Rheumatology Clinical Trials/Osteoarthritis Research Society International (OMERACT/OARSI) response(36) at weeks 16, 26 and 52; radiographic medial and lateral joint space narrowing (JSN; centralized measurement of the minimum joint space width compared with baseline in the index knee at week 52; and changes from baseline to week 26 in synovitis as assessed by dynamic contrast-enhanced MRI (DCE-MRI).(37)

Imaging

Patients were screened for presence of synovitis using MRI or musculoskeletal ultrasound **(Supplemental Methods)**, which has shown good to excellent inter- and intra-reader agreement in detecting knee synovitis.(38, 39) Posteroanterior weight-bearing radiographs of the target knee were acquired at screening and at week 52. MRI of the target knee was performed using 1.5- or 3.0-T whole-body scanners and commercial knee coils at screening, week 26, and week 52. Given the limitations associated with non–contrast-enhanced MRI for assessment of synovitis,(8) dynamic contrast-enhanced MRI (DCE-MRI) was performed as an exploratory substudy (n=39), as described previously.(37)

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Pharmacokinetics

Blood samples were collected throughout the 52 weeks to assess concentrations of lutikizumab

and anti-drug antibody (ADA) responses in serum as previously described.(26)

Pharmacodynamics

Blood neutrophil counts and serum high-sensitivity C-reactive protein (hsCRP) levels (ICON,

ARCHITECT platforms C8000 or C16000, Abbott Laboratories, Abbott Park, IL, United States)

were measured repeatedly from baseline to week 52; other biomarkers were measured at baseline

and weeks 16, 26 and 52. Serum concentrations of free IL-1α and IL-1β were determined using

the Singulex Erenna (MilliporeSigma, Billerica, MA, United States) and SIMOA platforms

(Quanterix, Lexington, MA, United States), respectively, using AbbVie proprietary capture and

detection antibodies. Other biomarkers were measured by BioClinica Molecular Marker Lab

(Lyon, France) using validated enzyme-linked immunosorbent assays (from Nordic Bioscience

[Herlev, Denmark; metalloproteinase-degraded collagen types I and III and matrix

metalloproteinase-generated fragment of CRP], Corgenix [Broomfield, CO, United States;

hyaluronic acid], EMD Millipore [Darmstadt, Germany; N-propeptide of collagen IIA], and

Roche Diagnostics [Indianapolis, IN, United States; C-terminal telopeptide fragments of type II

collagen]), adhering to standard operating procedures from regulatory guidance for clinical

studies.

Safety

Adverse events, vital signs, physical examinations, and laboratory data were assessed throughout

the study. AEs were coded using the *Medical Dictionary for Regulatory Activities*, *version 19.0*,

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preferred term, and system organ class. AE severity was classified according to the Common

Terminology Criteria for Adverse Events, version 4.03.(40)

Statistics and Analyses

The co-primary and secondary efficacy outcomes were analyzed in the modified intent-to-treat

population, comprising randomized patients who received ≥1 dose of study drug. Continuous

efficacy assessments were analyzed with analysis of covariance (ANCOVA) with main factors of

treatment, age group, and KL grade and covariates of baseline values, except analysis of daily

rescue medication use, which was analyzed with analysis of variance (ANOVA). Categorical

variables analysis used the Cochran-Mantel-Haenszel test with age group and KL grade as

stratification factors. Last-observation-carried-forward (LOCF) imputation of missing values was

used for non-imaging co-primary and selected secondary endpoints. The non-imaging co-

primary endpoint was also assessed using multiple imputation (MI) for missing values in a post

hoc analysis. In this phase 2 study, there was no adjustment for multiplicity of assessments. The

safety analysis set included subjects who received ≥1 dose of study drug. To identify factors

associated with the development of neutropenia (defined as at least one episode where absolute

neutrophil count [ANC] <1,500 cells/µL), explanatory variables significant at the $P<0.1$ level in

univariate analyses were tested in a multiple logistic regression model.

A sample size of approximately 80 subjects per treatment group was estimated to provide ≥80%

power to detect a significant difference between lutikizumab and placebo, based on a

significance level of 0.05, for each of the two co-primary endpoints (WOMAC pain and synovial

inflammation based on synovial membrane thickness by MRI).

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RESULTS

Subjects

Of 1571 subjects screened, 350 were eligible and were randomized; 347 received ≥1 dose of study drug (June 2014 to November 2016) and were analyzed for efficacy and safety **(Figure 1)**. The most common reasons for screen failure were clinical history related to entry criteria, absence of KL-2 or KL-3 radiographic evidence of knee OA, lack of synovitis on ultrasound or by non–contrast-enhanced MRI, and severe knee malalignment.(41) Most subjects (229; 65.4%) were enrolled based on ultrasound evidence of knee synovitis; 118 (33.7%) were enrolled based on MRI evidence **(Supplemental Table 1)**. Among the subjects enrolled based solely on screening ultrasound, 185/220 (84.1%) had baseline MRI evidence of synovitis per WORMS scoring conducted at the end of the study. Demographics, baseline disease characteristics, and use of medications were generally well matched across treatment groups **(Table 1)**. Of the randomized subjects, 60/85 (70.6%) who received placebo and 202/265 (76.2%) who received lutikizumab completed the 52-week study **(Figure 1)**. The major reasons for discontinuation included AEs, lack of efficacy, and withdrawal of consent.

Efficacy

Co-primary Endpoints

The co-primary endpoint of WOMAC pain at week 16 (and at most early time points) improved significantly, compared with placebo, for the lutikizumab 100 mg dose group (*P*=0.050) but not for the lutikizumab 25 mg (*P*=0.834) and 200 mg (*P*=0.415) dose groups **(Figure 2A, Table 2, Supplemental Figure 2A)**. Post hoc analysis for WOMAC pain using MI yielded results

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consistent with those of LOCF **(Supplemental Figure 3**). WOMAC pain reduction in all

lutikizumab groups, as well as placebo, was sustained from weeks 16 to 52, but differences

between lutikizumab and placebo for WOMAC pain were not significant between weeks 16 and

52 **(Figure 3A)**.

The other co-primary endpoint, change in synovitis (as measured by synovial membrane

thickness, synovial fluid volume, and WORMS synovitis/effusion score) from baseline to week

26, did not differ between the lutikizumab and placebo groups **(Table 2)**.

Post hoc analyses were performed to determine efficacy including only subjects with a baseline

WORMS synovitis/effusion score >0, indicating MRI evidence of synovitis. Results in this post

hoc population were not substantially different from those in the original, prospective population

enrolled on the basis of positive MRI or ultrasound **(Supplemental Figure 4, Supplemental**

Table 2).

Other Signs and Symptoms Endpoints

WOMAC function **(Figure 2B, Supplemental Figure 2B)** and OMERACT/OARSI response

were numerically better but not significantly different between the placebo and lutikizumab

treatment groups **(Table 2)** at 16, 26 and 52 weeks. Change from baseline in WOMAC function

was significantly different ($P \leq 0.01$) from placebo with lutikizumab 100 mg at weeks 4 and 8

(Figure 2B). OMERACT/OARSI placebo responses were high, e.g., 60%–71%, at weeks 26 and

52, respectively **(Table 2, Figure 2)**.

Other Structural Endpoints

Among secondary and exploratory endpoints, other than medial (*P*=0.017) and lateral (*P*=0.005) JSN with lutikizumab 25 mg at week 52 **(Table 2),** there were no structural endpoints statistically significantly different between placebo and the lutikizumab dose groups. MRI assessments of cartilage volume, thickness, and WORMS scores were nearly identical in all treatment groups at baseline, week 26 and week 52. Synovitis, as assessed by DCE-MRI, also demonstrated no differences between subjects treated with placebo versus each dose of lutikizumab **(Supplemental Table 32)**.

Rescue Medication Use

The proportion of subjects receiving concomitant pain medication (acetaminophen, ibuprofen) during the study was generally similar among treatment groups **(Supplemental Table 43)**. The least squares mean daily dose of rescue acetaminophen up to week 16 was similar in the placebo (511 mg, n=58) and lutikizumab 25 mg (500 mg, n=53), 100 mg (413 mg, n=63) and 200 mg (426 mg, n=67) dose groups. Similarly, the least squares mean daily dose of rescue ibuprofen between weeks 16 and 26 was not significantly different between the placebo (200 mg, n=18) and lutikizumab 25 mg (104 mg, n=18), 100 mg (241 mg, n=19), and 200 mg (155 mg, n=24) dose groups.

Pre-planned Efficacy Subgroup Analyses

In pre-planned subgroup analyses, there were no meaningful differences in WOMAC pain scores at weeks 16, 26, and 52 based on age, gender, race, or body weight. In the 100 mg dose group, there were statistically significantly greater decreases in WOMAC pain scores compared with

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placebo, among subjects with an index knee KL grade of 3 (but not KL grade of 2) (week 26, *P*=0.029; week 52, 0.016) and among subjects who did not use concomitant medications for index knee pain through week 26 (week 16, *P*=0.027; week 26, 0.045). This was not noted in the 25 mg or 200 mg dose group.

Pharmacokinetics

Lutikizumab trough concentrations were stable between weeks 6 and 52 and consistent with assessments of steady-state in previous studies **(Supplemental Figure 5A3A)**.(26, 42) A greater ~~anti-drug antibody~~ADA incidence was observed for the lutikizumab 25 mg dose group (46%) compared with the 100 mg and 200 mg dose groups (32% and 23%, respectively). Lutikizumab serum concentrations were generally similar among subjects with and without ~~anti-drug antibodies~~ADAs for each dose group **(Supplemental Figure 5B3B)**; thus, the immunogenic response did not appear to have a meaningful impact on lutikizumab pharmacokinetics.

Pharmacodynamics

Mean blood neutrophil counts (ANC) decreased with lutikizumab 100 mg and 200 mg treatment at all time points and with lutikizumab 25 mg at most time points relative to baseline and placebo **(Figure 3A)**; neutrophil counts were similar in the 100 mg and 200 mg dose groups throughout the study. There was an exposure-response relationship between ANCs and lutikizumab blood levels **(Supplemental Figure 64)**. In a similar way, hsCRP levels were reduced at most time points in the lutikizumab groups compared with baseline and placebo **(Figure 3B)**, reaching statistical significance at several time points for the 25 mg and 100 mg doses but with high variability.

Serum levels of IL-1α and IL-1β at baseline were low and most were below the lower limits of quantification. In subjects with detectable levels at baseline, IL-1α and IL-1β levels were reduced within 2 weeks of treatment initiation to a greater extent in subjects receiving lutikizumab compared with subjects receiving placebo **(Supplemental Figure 75, Supplemental Table 45)**. Changes in other biomarkers are shown in **Supplemental Figure 86**. With lutikizumab treatment, compared with placebo, there were reductions in metalloproteinase-degraded collagen type I (C1M), metalloproteinase-degraded collagen type III (C3M), matrix metalloproteinase-generated fragment of CRP (CRPM), IL-6, and alkaline phosphatase.

Safety

Similar proportions of subjects receiving placebo or lutikizumab experienced a treatment-emergent AE or serious AE during the study **(Table 3)**. Serious infections were infrequent and had similar incidences among treatment groups, including placebo. A greater proportion of subjects in the lutikizumab total treatment groups compared with the placebo group had injection site reactions (25.2% vs 15.3%) and neutropenia (27.5% vs 2.4%). The incidence of both events increased in a dose-dependent manner. All reported neutropenia laboratory abnormalities were grade 3 or less; there was no grade 4 neutropenia. One subject who received lutikizumab 25 mg and 6 subjects who received lutikizumab 200 mg discontinued study treatment because of neutropenia.

A multiple logistic regression model was used to identify factors associated with the development of neutropenia. In addition to lutikizumab dose ($P<0.0001$), baseline neutrophil

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~~count (odds ratio [OR], 0.19; 95% CI, 0.12–0.30; *P*<0.0001), age <62 (OR, 2.11; 95% CI, 1.09–~~

~~4.09; *P*=0.027), and female sex (OR, 2.86; 95% CI, 1.34–6.10; *P*=0.007) were significantly~~

~~associated with the development of neutropenia **(Supplemental Table 5)**. Grade 3 or 4~~

~~lymphopenia and hypertriglyceridemia occurred in a few subjects, without clear patterns; there~~

~~was no grade 3 or 4 thrombocytopenia or hypercholesterolemia.~~

Treatment-emergent AEs of malignancy were reported in 5 subjects (1.9%; 2.2 events per 100

patient-years) in the total lutikizumab group and in no subjects in the placebo group. Basal cell

carcinoma was reported in 3 of these 5 subjects.

DISCUSSION

This study assessed the efficacy and safety of blocking IL-1α and IL-1β with lutikizumab in

subjects with knee OA and associated synovitis. To our knowledge, this is the first study to test

the hypothesis that a systemic anti-inflammatory, anti-cytokine therapy may be effective in knee

OA patients with synovitis. Lutikizumab met the co-primary clinical endpoint of reduction in

WOMAC pain compared with placebo at week 16 in only the 100 mg dose group, but not in the

25 mg and 200 mg dose groups, and the differences compared with placebo were not sustained

past week 16 for any dose. In the subgroup of patients treated with lutikizumab 100 mg with KL

grade 3 knee OA and among the subgroup of subjects not using rescue or off-protocol pain

medications, compared with placebo, the change from baseline at weeks 26 and 52 in WOMAC

pain was significantly greater; the reason why this occurred only in the 100 mg dose group and

only at these time points is unclear. The co-primary endpoint, change from baseline in synovitis

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as measured by synovial membrane thickness, synovial effusion volume, and semiquantitative

MRI synovitis/effusion (WORMS) at week 26, was also not significantly different for

lutikizumab versus placebo. Furthermore, when compared with placebo, lutikizumab was not

associated with improvements or slowing in the rates of JSN and MRI cartilage thickness

changes. These results indicate that lutikizumab had no significant impact on structural endpoints

that were assessed. The analytical assay used to quantify lutikizumab serum concentrations

required at least one free binding site for each molecule of IL-1α and IL-1β.(26) The modest

impact of ADAs on trough concentrations **(Supplemental Figure 5B3B)** suggested that

development of ADAs did not interfere with the biological activity of lutikizumab, and thus they

were unlikely to have impacted efficacy.

The unexpected lack of an effect of IL-1 inhibition on synovitis may signify that, although the

synovium is an important source of IL-1,(43) IL-1 by itself may not be required to sustain

synovitis. Other factors, such as cartilage degradation products and adipokines may have a

greater role in the development and maintenance of synovitis.(44) Overall, these observations are

consistent with a recent study using a medial menisectomy animal model that found that IL-1

may not play a role in the structural progression of OA.(21) A phase 2 study of lutikizumab in

erosive hand OA also was negative.(42)

The reason(s) for the failure to demonstrate a sustained beneficial analgesic effect of lutikizumab

compared with placebo in this study is not clear. Lutikizumab serum concentrations were stable

throughout the duration of the study, unaffected by development of anti-drug antibodiesADAs

and at exposures consistent with a previous phase 1 study.(27) The probability of achieving

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positive results might have been improved by using a flare study design, although a difference in effect size has not been shown to be statistically significant versus a non-flare study design.(45) Although serum IL-1α and IL-1β levels could not be reliably quantified, similar reductions in absolute neutrophil count and hsCRP between the lutikizumab 100 mg and 200 mg dose groups suggested that both dose groups achieved maximum suppression of IL-1α and IL-1β. However, it is possible that these systemic pharmacodynamic endpoints are not reflective of target engagement within the joint.

As there was no positive control treatment in this study, it is not clear whether lutikizumab did have a positive effect masked by a placebo response. The use of SOC therapies following week 26 may have masked any lutikizumab treatment effects and could account for the lack of sustained WOMAC pain differences between the lutikizumab 100 mg treatment group and the placebo group, but would not explain the inefficacy of the 25 mg and 200 mg doses. The pre-planned subset analyses suggested that the use of rescue medication affected the WOMAC pain co-primary endpoint results; however, meta-analysis has concluded that acetaminophen has little if any analgesic effect in OA.(46) Another pre-planned subset analysis suggested that subjects with KL grade 3 knee OA (in contrast to subjects with KL grade 2 knee OA) had statistically significant WOMAC pain treatment effects, implying that knee OA subjects with greater amounts of structural disease may benefit from IL-1 inhibition. The hypothesis that the concomitant use of pain medication and the degree of radiographic damage could predict response to lutikizumab would have to be proven in a well-designed prospective study.

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The lack of a clear lutikizumab treatment effect may have also been due to a strong, sustained placebo effect **(Figure 3A)**. Placebo response rates are high in knee OA trials (e.g., 40%–50% using OMERACT-OARSI criteria)(47); placebo response rates in this trial were 60%, 62% and 71% at weeks 16, 26 and 52 **(Table 2)**. High placebo responses have been reported in studies of injectable drugs.(48)

There were no unexpected AEs compared with other IL-1 inhibitors,(23) except that there were 5 malignancies in the combined lutikizumab treatment groups versus no malignancies in the placebo group. While immunosuppression is associated with a risk of malignancy, two published studies of IL-1 inhibitors (CANTOS and RESURGE) have not identified an increased risk of malignancies.(49, 50) The CANTOS study of subjects treated with the IL-1β inhibitor canakinumab identified a significantly lower risk of incident lung cancer.(49) Given the CANTOS and RESURGE study data and the low numbers of malignancies observed in our study, it is currently unclear how best to interpret the greater number of malignancies in the combined lutikizumab treatment groups compared with the placebo group. Any future studies of lutikizumab should carefully monitor the incidence of malignancy to determine whether the findings observed in the present study are replicated.

Strengths of this study include the measurement of efficacy and pharmacodynamic endpoints in the same subjects. Plateauing of neutrophil decreases from baseline between the lutikizumab 100 mg and 200 mg dose groups suggested that systemic IL-1 was maximally suppressed; similar reductions in neutrophil levels were achieved with the IL-1R inhibitor AMG 108.(23) Another strength was enrichment for subjects with evidence of inflammation using ultrasound, MRI, and

clinical characteristics, with the assumption that this increased the likelihood of treating subjects with elevated joint levels of IL-1. Multiple signs and symptoms and structural endpoints were evaluated over 52 weeks, which maximized the opportunity to demonstrate an effect of lutikizumab. Confounding by other potentially disease-modifying agents was minimal. Further investigation could probe whether subgroups of patients with knee OA have a significant clinical response to lutikizumab.

Several limitations of this study should be noted. Importantly, levels of lutikizumab, IL-1α, and IL-1β in the synovial fluid of the knee joint could not be assessed. In addition, approximately two-thirds of subjects had relatively mild radiographic changes (KL-2, 62.8%) and none had severe disease (KL-4, 0%). Studies of tanezumab, a monoclonal antibody that targets nerve growth factor, enrolled a higher percentage of subjects with KL-3 and KL-4 disease (38.6%–46.9% and 14.1%–27.7%, respectively),(47, 51) suggesting that patients with more severe disease may be more responsive to treatment, consistent with the pre-planned sub-analysis of subjects with KL grade 3 in the present study. This suggests that, by enrolling subjects with a lower average KL grade, the likelihood of observing significant pain improvements may have been reduced in the current study. As noted previously, a robust placebo response was found, possibly enriched by the allowance of rescue pain medication and the frequent study visits with subcutaneous drug administration. Another potential limitation of our study was highlighted by the discrepancy between ultrasound and MRI grading of synovitis. Of the limited number of cases (n=6) where an ultrasound and MRI were both submitted to screen for synovitis prior to enrollment, all 6 subjects were positive by ultrasound and only 3 of the 6 were positive by non–

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contrast-enhanced MRI, supporting the idea that the lack of contrast-enhancement may have limited our ability to detect changes in synovitis with lutikizumab therapy.

In conclusion, although the 100 mg dose of lutikizumab met the co-primary endpoint of reduction in WOMAC pain at week 16 compared with placebo, neither the 25 mg nor the 200 mg dose did so, and the difference from placebo was not sustained after week 16. Measures of synovitis, cartilage thickness, and other structural endpoints were similar between lutikizumab and placebo at all time points and with all 3 doses evaluated. The safety profile of lutikizumab was consistent with the AEs reported for other IL-1 inhibitors, including neutropenia and injection site reactions. The results of this study suggest that IL-1 inhibition is not an effective disease-modifying therapy in patients with knee OA. Whether subgroups of knee OA patients might have symptomatic benefit from IL-1 inhibition remains an open question.

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ACKNOWLEDGMENTS

Data requests can be submitted at any time and the data will be accessible for 12 months, with

possible extensions considered. For more information on the process, or to submit a request, visit

the following link: https://www.abbvie.com/our-science/clinical-trials/clinical-trials-data-and-

information-sharing/data-and-information-sharing-with-qualified-researchers.html.

AUTHORSHIP

All authors contributed to the development of the content, all authors and AbbVie reviewed and

approved the final manuscript, and the authors maintained control over the final content.

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Figure Legends

Figure 1. Patient disposition. Q2W, every 2 weeks; SC, subcutaneous. *Major protocol

violations: (1) actual treatment is not according to randomization assignment, (2) premature

unblinding, (3) use of a narcotic or non-steroidal anti-inflammatory drug for >50% of the days

before week 16, (4) baseline index knee pain intensity <4 (11-point numeric rating scale), and (5)

absence of synovitis in the index knee by ultrasound or magnetic resonance imaging during

screening. †Study completion at 52 weeks.

Figure 2. LS mean change from baseline over time for WOMAC pain **(A)** and WOMAC

function **(B)**. LS, least squares; Q2W, every 2 weeks; WOMAC, Western Ontario and McMaster

Universities Osteoarthritis index. *$P \leq 0.05$ vs placebo; †$P \leq 0.01$ vs placebo.

Figure 3. Time course of mean total neutrophils **(A)** and mean hsCRP levels **(B)** from baseline

(week 0) to week 52. hsCRP, high-sensitivity C-reactive protein; Q2W, every 2 weeks. *$P<0.05$

or a higher level of significance compared with placebo.

Table 1. Demographics, Baseline Disease Characteristics, and Prior Medications

Characteristic	Placebo (n=85)	Lutikizumab SC Q2W, mg		
		25 (n=89)	100 (n=85)	200 (n=88)
Age, y	59.5±8.9	61.6±7.5	60.2±8.2	59.1±10.3
Women, n (%)	52 (61.2)	63 (70.8)	53 (62.4)	57 (64.8)
Race, n (%)				
White	78 (91.8)	78 (87.6)	70 (82.4)	79 (89.8)
Black	4 (4.7)	8 (9.0)	10 (11.8)	7 (8.0)
Other	3 (3.5)	3 (3.4)	5 (5.9)	2 (2.3)
BMI, kg/m^2	28.6±3.6	28.7±3.8	29.0±3.5	28.7±3.5
OA duration, y	7.9±8.0	7.6±9.0	7.9±8.7	8.7±8.6
KL grade,* n (%)				
2	53 (62.4)	57 (64.0)	52 (61.2)	56 (63.6)
3	32 (37.6)	32 (36.0)	33 (38.8)	32 (36.4)
WOMAC total (scale 0–240; higher scores indicate worse condition)	128.7±40.3	132.7±43.5	134.7±36.0[†]	132.3±39.2[†]
WOMAC pain (scale 0–50)	26.2±8.0	27.0±9.3	28.4±8.4[†]	27.1±8.6
WOMAC function (scale 0–170)	90.8±31.2	93.7±31.8	94.7±26.4[†]	93.1±29.1[†]
WOMAC stiffness (scale 0–20)	11.7±3.8	12.0±4.1	11.7±3.8[†]	11.9±4.2
Synovial membrane thickness, mm	0.88±0.28[‡]	0.84±0.28[§]	0.89±0.31[‡]	0.84±0.26[‡]
Synovial fluid volume, mL	16.4±15.0[∥]	15.5±11.6[¶]	17.3±19.8[#]	15.2±15.0**
WORMS semiquantitative synovitis/effusion (scale 0–3)	1.2±0.8	1.3±0.7	1.1±0.8[††]	1.2±0.7[†]
WORMS total (scale 0–400; higher scores indicate worse condition)	108.8±73.3[‡‡]	109.1±70.4[‡‡]	108.1±76.9[§§]	103.4±69.0[∥]
WORMS total BML (scale 0–45)	3.6±2.8[†]	3.0±2.6[†]	2.9±2.7[‡‡]	2.5±2.1[#]
WORMS total osteophytes (scale	13.6±13.5[†]	13.6±11.9[#]	14.2±13.8[¶]	13.9±12.7[†]

0–112)

ANC, × 10^9/L (normal, 1.80–7.00 × 10^9/L)	3.9±1.2	3.7±1.3	3.8±1.6	3.6±1.2
hsCRP, mg/L	3.0±2.7	4.3±6.4	4.0±7.9	2.9±3.1
Prior knee OA therapy, n (%)[a][‖]				
NSAID	38 (44.7)	31 (34.8)	32 (37.6)	40 (45.5)
Intra-articular corticosteroid	1 (1.2)	1 (1.2)	0	0
Narcotic[¶¶]	4 (4.7)	3 (3.4)	2 (2.4)	3 (3.4)
Concomitant knee and systemic pain medication at baseline	79 (92.9)	70 (78.7)	71 (83.5)	78 (88.6)

Data are mean ± SD except where noted.

ANC, absolute neutrophil count; BMI, body mass index; BML, bone marrow lesions; hsCRP, high-sensitivity C-reactive protein; KL, Kellgren-Lawrence; NSAID, non-steroidal anti-inflammatory drug; OA, osteoarthritis; Q2W, every 2 weeks; SC, subcutaneous; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

*KL grade 2: definite osteophytes and possible joint space narrowing on anteroposterior weight-bearing radiograph. KL grade 3: multiple osteophytes, definite joint space narrowing, sclerosis, possible bony deformity.

[†]1, [‡]9, [§]10, [‖]7, [¶]6, [#]2, [**]5, [††]3, [‡‡]4, and [§§]8 subjects with missing data.

[‖‖]Prior or concomitant therapy with intra-articular hyaluronan was not excluded, and 1 subject reported prior hyaluronan therapy and 2 patients reported intra-articular hyaluronan therapy after the study started.

[¶¶]Subjects could have taken more than 1 kind of narcotic previously. Hydrocodone with acetaminophen (n=7), oxycodone (n=2), oxycodone with acetaminophen (n=2), codeine with acetaminophen (n=1), hydrocodone (n=1), and oxycodone with naloxone (n=1).

Table 2. Changes From Baseline in Efficacy Endpoints

Signs and Symptoms Endpoints (LOCF Data)

Endpoint (Full Scale)	PBO (n=85)	Week 16 Lutikizumab SC Q2W, mg 25 (n=89)	100 (n=85)	200 (n=88)	PBO (n=85)	Week 52 Lutikizumab SC Q2W, mg 25 (n=89)	100 (n=85)	200 (n=88)
Co-primary endpoint								
WOMAC pain (0–50; higher scores indicate worse condition)	−8.9 (−11.0, −6.9)	−9.2 (−11.2, −7.2)	−11.8 (−13.8, −9.8)	−10.1 (−12.1, −8.1)	−10.0 (−12.2, 7.7)	−11.0 (−13.3, −8.8)	−12.1 (−14.4, −9.8)	−12.2 (−14.5, −10.0)
Difference vs PBO		−0.3 (−3.13, 2.53)	−2.9 (−5.73, 0.01)	−1.2 (−4.00, 1.66)		−1.1 (−4.26, 2.08)	−2.2 (−5.39, 1.05)	−2.3 (−5.46, 0.88)
P value vs PBO		0.834	**0.050***	0.415		0.500	0.186	0.157
Secondary endpoint								
WOMAC function (0–170; higher scores indicate worse condition)	−28.7 (−35.3, −22.2)	−29.8 (−36.3, −23.3)	−36.3 (−42.9, −29.7)	−32.1 (−38.6, −25.6)	−32.9 (−40.7, −25.1)	−36.1 (−43.8, −28.5)	−38.7 (−46.5, −30.9)	−39.7 (−47.4, −32.0)
Difference vs PBO		−1.1 (−10.22, 8.08)	−7.6 (−16.83, 1.69)	−3.4 (−12.58, 5.76)		−3.2 (−14.03, 7.59)	−5.8 (−16.77, 5.11)	−6.8 (−17.63, 4.04)
P value vs PBO		0.818	0.109	0.465		0.558	0.295	0.218
Exploratory endpoint								
OMERACT/OARSI response, %[†]	60.0 (49.6, 70.4)	67.0 (57.2, 76.9)	72.6 (63.1, 82.2)	65.5 (55.5, 75.5)	70.6 (60.9, 80.3)	69.3 (59.7, 79.0)	71.4 (61.8, 81.1)	72.7 (63.4, 82.0)
Difference vs PBO		7.0 (−7.3, 21.4)	12.6 (−1.5, 26.7)	5.5 (−8.9, 19.9)		−1.3 (−14.9, 12.4)	0.8 (−12.8, 14.5)	2.1 (−11.3, 15.6)
P value vs PBO		0.311	0.080	0.435		0.824	0.964	0.763

Structural Endpoints (Observed Data)

Endpoint	PBO	Week 26 Lutikizumab SC Q2W, mg 25	100	200	PBO	Week 52 Lutikizumab SC Q2W, mg 25	100	200
Co-primary endpoint								
Synovial membrane thickness, mm	−0.05 (−0.11, 0.01)	0.01 (−0.05, 0.07)	−0.08 (−0.13, −0.02)	0.01 (−0.05, 0.07)	−0.07 (−0.14, −0.01)	−0.04 (−0.10, 0.02)	−0.05 (−0.11, 0.01)	−0.02 (−0.08, 0.05)
n	59	65	59	63	50	59	55	53
Difference vs PBO		0.06 (−0.02, 0.14)	−0.03 (−0.11, 0.06)	0.06 (−0.02, 0.14)		0.03 (−0.06, 0.12)	0.02 (−0.07, 0.11)	0.06 (−0.03, 0.14)
P value vs PBO		0.145	0.520	0.159		0.474	0.637	0.221
Synovial fluid volume,	0.03	0.26	−1.04	−1.49	−1.90	1.17	−0.67	−1.83

mL	(−2.50, 2.56)	(−2.11, 2.62)	(−3.42, 1.35)	(−3.87, 0.90)	(−5.03, 1.23)	(−1.78, 4.12)	(3.59, 2.25)	(−4.82, 1.16)
n	60	69	67	68	53	60	60	58
Difference vs PBO		0.22 (−3.19, 3.64)	−1.07 (−4.52, 2.38)	−1.52 (−4.95, 1.92)		3.08 (−1.16, 7.32)	1.23 (−3.02, 5.49)	0.07 (−4.21, 4.35)
P value vs PBO		0.897	0.542	0.385		0.154	0.569	0.973
WORMS semiquantitative synovitis/effusion volume	0.07 (−0.06, 0.19)	−0.01 (−0.13, 0.11)	−0.08 (−0.21, 0.04)	−0.07 (−0.20, 0.05)	−0.05 (−0.18, 0.09)	−0.05 (−0.18, −0.08)	−0.06 (−0.19, 0.08)	−0.01 (−0.14, 0.12)
n	70	76	70	75	59	66	62	66
Difference vs PBO		−0.08 (−0.25, 0.10)	−0.15 (−0.32, 0.03)	−0.14 (−0.31, 0.03)		0.00 (−0.19, 0.18)	−0.01 (−0.20, 0.18)	0.04 (−0.15, 0.22)
P value vs PBO		0.384	0.095	0.106		0.967	0.923	0.692
Secondary endpoint Cartilage volume, mm^3								
n	58	65	53	66	49	57	50	56
Total	−326.0 (−400.8, −251.1)	−325.5 (−397.1, −253.8)	−322.4 (−400.2, −244.6)	−359.0 (−429.7, −288.4)	−557.0 (−659.9, −454.1)	−598.7 (−694.7, −502.8)	−554.3 (−654.9, −453.6)	−583.1 (−678.9, −487.2)
Difference vs PBO		0.5 (−101.6, 102.6)	3.6 (−104.0, 111.1)	−33.1 (−134.8, 68.7)		−41.7 (−180.5, 97.0)	2.7 (−140.9, 146.3)	−26.1 (−165.5, 113.3)
P value vs PBO		0.992	0.948	0.523		0.554	0.970	0.713
Medial	−166.9 (−209.7, −124.1)	−159.5 (−200.6, −118.5)	−163.1 (−207.7, −118.6)	−153.7 (−194.1, −113.3)	−286.8 (−353.3, −220.3)	−317.3 (−379.4, −255.3)	−266.7 (−331.8, −201.6)	−310.9 (−372.8, −249.0)
Difference vs PBO		7.4 (−51.1, 65.8)	3.8 (−57.8, 65.3)	13.2 (−45.0, 71.4)		−30.5 (−120.2, 59.1)	20.1 (−72.8, 112.9)	−24.0 (−114.0, 65.9)
P value vs PBO		0.804	0.904	0.655		0.503	0.670	0.599
Lateral	−159.6 (−214.2, −104.9)	−166.3 (−218.5, −114.0)	−158.0 (−214.8, −101.3)	−204.1 (−255.7, −152.4)	−270.7 (−336.3, −205.1)	−280.9 (−342.1, −219.7)	−287.9 (−352.0, −223.7)	−271.1 (−332.3, −210.0)
Difference vs PBO		−6.7 (−81.2, 67.9)	1.6 (−76.9, 80.0)	−44.5 (−118.7, 29.8)		−10.2 (−98.7, 78.3)	−17.2 (−108.7, 74.3)	−0.4 (−89.4, 88.5)
P value vs PBO		0.860	0.969	0.239		0.820	0.711	0.992
Exploratory endpoint JSN, mm								
n	−	−	−	−	58	70	64	68
Medial	−	−	−	−	0.00	−0.18	−0.11	−0.06

					(−0.110, 0.119)	(−0.287, −0.079)	(−0.218, −0.003)	(−0.165, 0.047)
Difference vs PBO						−0.19 (−0.341, −0.034)	−0.12 (−0.272, 0.041)	−0.06 (−0.218, 0.090)
P value vs PBO	−	−	−	−		0.017*	0.148	0.415
Lateral	−	−	−	−	−0.16 (−0.310, −0.005)	0.14 (−0.003, 0.275)	−0.07 (−0.216, 0.073)	0.04 (−0.106, 0.177)
Difference vs PBO						0.29 (0.089, 0.498)	0.09 (−0.123, 0.295)	0.19 (−0.012, 0.399)
P value vs PBO	−	−	−	−		0.005*	0.418	0.065

Data are least squares means (95% CI).

JSN, joint space narrowing; KL, Kellgren-Lawrence; LOCF, last observation carried forward; OMERACT/OARSI, Outcome Measures in Rheumatology Clinical Trials/Osteoarthritis Research Society International; PBO, placebo; Q2W, every 2 weeks; SC, subcutaneous; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

*$P \leq 0.05$ vs PBO. Continuous variables compared with analysis of covariance with treatment, age, and KL grade as main factors and baseline as covariate. Categorical variables compared with Cochran-Mantel-Haenszel test with age group and KL grade as stratification factors.

†OMERACT/OARSI response defined as either (1) ≥50% relative and ≥20% absolute improvement from baseline in WOMAC pain or function or (2) ≥20% relative and ≥10% from baseline in at least 2 of 3 measures (WOMAC pain, WOMAC function, and patient global assessment).

Table 3. Safety Results

AE, n (%)	Placebo (n=85)	Lutikizumab SC Q2W, mg		
		25 (n=89)	100 (n=85)	200 (n=88)
Any AE	78 (91.8)	78 (87.6)	77 (90.6)	81 (92.0)
Discontinuation of study drug due to AE	10 (11.8)	5 (5.6)	5 (5.9)	12 (13.6)
Deaths	0	0	0	0
Serious AE	8 (9.4)	11 (12.4)	8 (9.4)	4 (4.5)
Serious AE occurring in ≥2 subjects/arm				
Infection*	2 (2.4)	2 (2.2)	1 (1.2)	0
Fracture and injury*	4 (4.7)	3 (3.4)	3 (3.5)	1 (1.1)
Malignancy	0	3 (3.4)	1 (1.2)	2 (2.3)
Basal cell carcinoma	0	3 (3.4)	0	0
Invasive ductal breast carcinoma	0	0	0	1 (1.1)
Lung cancer metastatic	0	0	0	1 (1.1)
PSA increased[†]	0	0	1 (1.2)	0
Any infection	39 (45.9)	34 (38.2)	42 (49.4)	49 (55.7)
Serious infection	2 (2.4)	2 (2.2)	1 (1.2)	0
Appendicitis	0	1 (1.1)	0	0
Diverticulitis	1 (1.2)	0	0	0
Peritonitis	0	1 (1.1)	0	0
Pneumonia	0	0	1 (1.2)	0
Urinary tract infection	1 (1.2)	0	0	0
Injection site reaction	13 (15.3)	16 (18.0)	21 (24.7)	29 (33.0)[¶]
Laboratory Abnormality, n (%)				
Neutropenia (grade 2, 3, or 4)	3 (3.5)	18 (20.5)[‖,¶]	26 (30.6)[¶]	33 (37.5)[¶]
Neutropenia (grade 2; 1000–<1500/mm^3)	3 (3.5)	17 (19.3)[‖,¶]	25 (29.4)[¶]	28 (31.8)[¶]
Neutropenia (grade 3; 500–<1000/mm^3)	0	1 (1.1)[‖]	1 (1.2)	5 (5.7)
Neutropenia (grade 4; <500/mm^3)	0	0[‖]	0	0
Neutropenia leading to discontinuation of study drug	0	1 (1.1)	0	6 (6.8)[¶]
Lymphopenia (grade 3 or 4[‡])	1 (1.2)	1 (1.2)[‖]	1 (1.2)	2 (2.3)
Hypertriglyceridemia (grade 3 or 4[§])	0	3 (3.4)[‖]	1 (1.2)	2 (2.3)

AE, adverse event; Q2W, every 2 weeks; PSA, prostatic specific antigen; SC, subcutaneous.

*A patient who reported 2 or more different preferred terms which are in the same system organ class was counted only once in the total.

[†]The PSA was described by the site investigator as "slightly elevated." A prostate biopsy was performed and described as "normal." For coding purposes, the elevated PSA is listed under

malignancies in this table, but the total number of malignancies is reported as 5, rather than 6, elsewhere in the article.

‡Grade 3, 200–<500 /mm^3; grade 4, <200/mm^3.

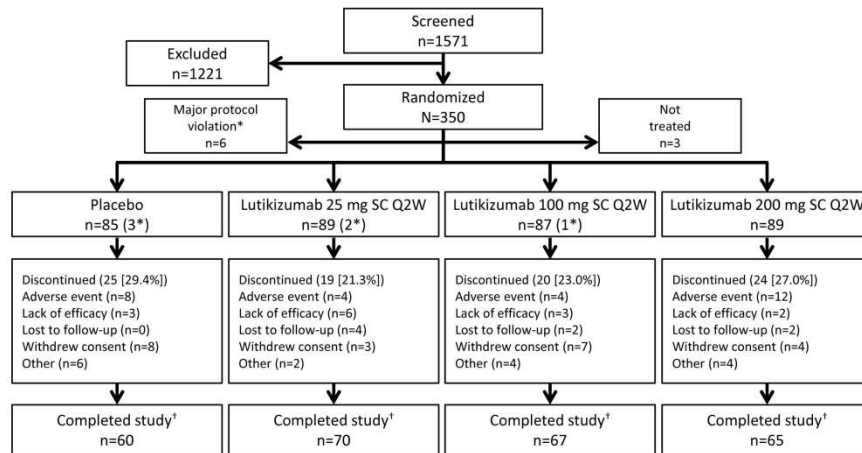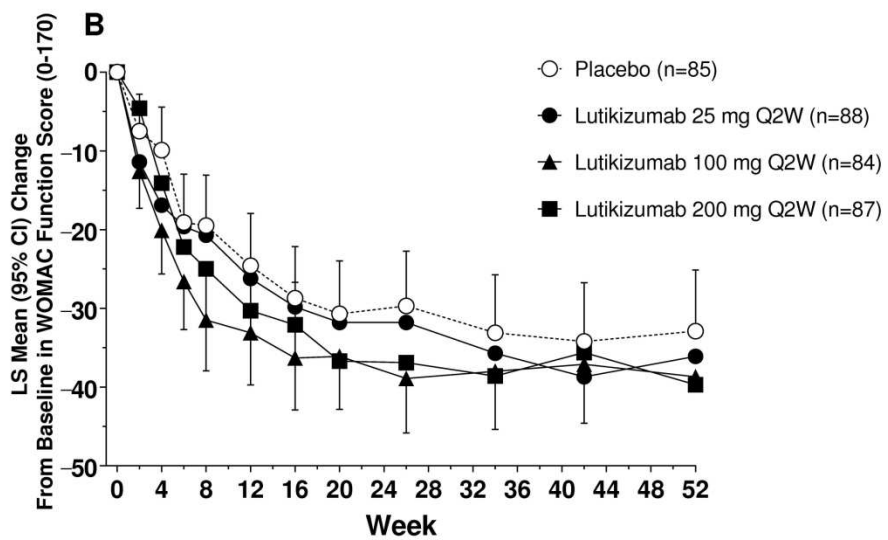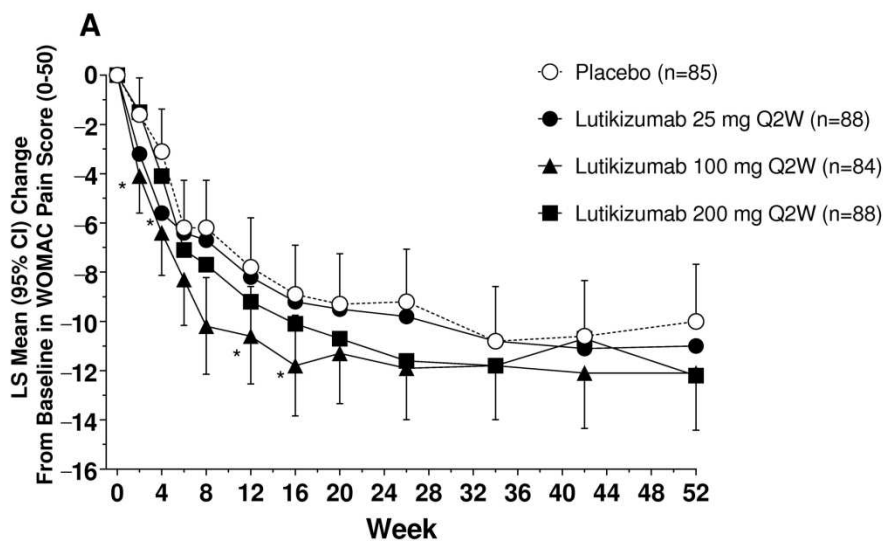§Grade 3, >500–1000 mg/dL; grade 4, >1000 mg/dL.

‖Data missing for 1 patient.

¶P<0.05 for comparison with placebo, Fisher exact test.

Grades for laboratory abnormalities were defined by Common Terminology Criteria for Adverse Events, version 4.03.(40)
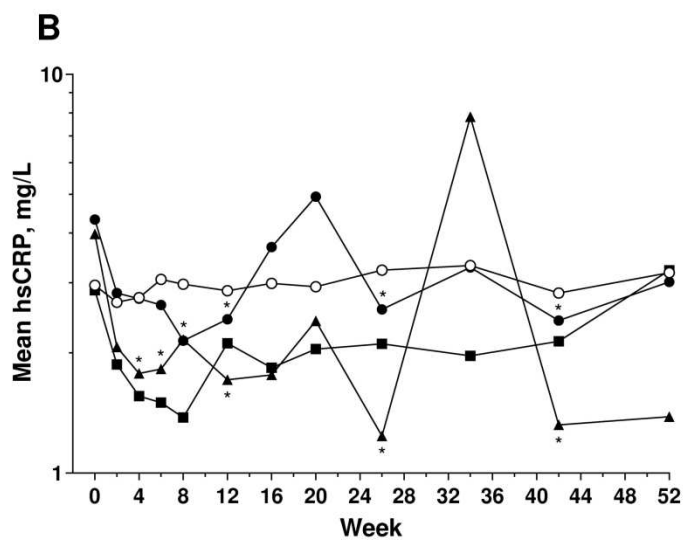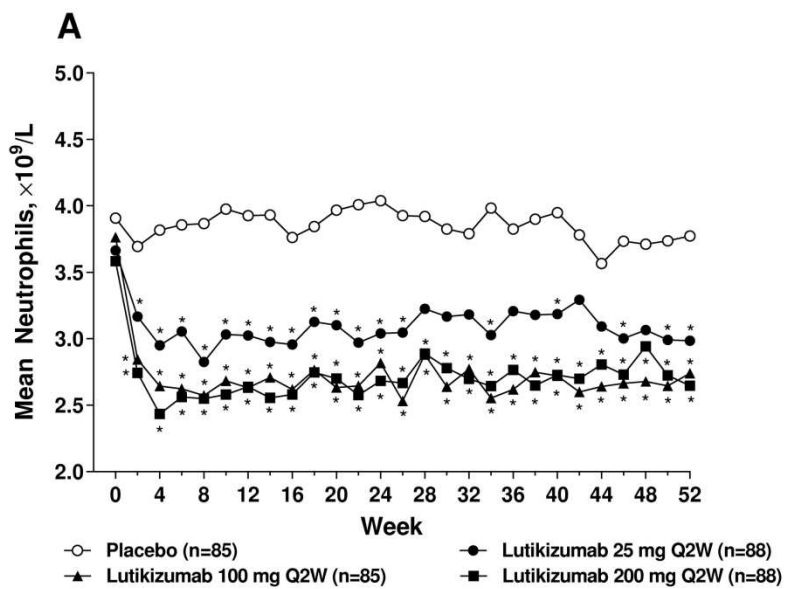
253x142mm (300 x 300 DPI)

200x256mm (300 x 300 DPI)

198x282mm (300 x 300 DPI)

Supplemental Methods

Subjects

Key exclusion criteria included significant trauma or surgery to the index knee within the last year or arthroscopy of the index knee within 6 months of the initial screening visit; severe knee malalignment, either >4.0° in varus or >8.0° in valgus angulation in the index knee; previous exposure to anti–IL-1 treatment; intra-articular corticosteroids within 3 months or via another route within 1 month before screening; and use of any investigational or immunosuppressive therapy within 1 month or 5 half-lives, whichever was longer, before the first dose of study drug.

Subject disposition was calculated for the intent-to-treat population, comprising all randomized patients.

Efficacy

Secondary endpoints included 3 types of pain intensity measures using 11-point numeric rating scales (NRS-11's; patient-rated pain from 0 ["no pain"] to 10 ["worst possible pain"]) in which subjects were asked about the average pain intensity during the past week (7-day recall period), worst pain during activity over the past 24 hours (activity pain), and pain intensity before and after a 40-meter walk (performance pain) at weeks 16, 26, and 52; Western Ontario and McMaster Universities Osteoarthritis (WOMAC) physical function scores (0–170 scale, NRS-11 subscales)(1) at weeks 16, 26, and 52; patient global assessment of arthritis at weeks 16, 26, and 52; Whole-Organ Magnetic Resonance Imaging Score (WORMS) bone marrow lesion(s)(2) at

1

weeks 26 and 52; and magnetic resonance imaging (MRI) cartilage volume and thickness at weeks 26 and 52.

Ultrasound

Patients meeting criteria for B-mode synovial hypertrophy or power Doppler were selected for further screening. For selection and quality assurance, sonographers were asked to submit test scans for B-mode and power Doppler images. B-mode frequency started at 12 MhZ and could be reduced for penetration. Gain was set at approximately 50%. Using the index fingertip, Doppler settings were optimized for respective equipment to show vascularity in at least one third of the finger pulp. The pulse repetition frequency was set in a range between 400 to 600 Hz, with Doppler box to cover recess and superficial tissue, and gain just above noise. Doppler frequency was adjusted for the highest sensitivity. The knee was placed in 30° flexion, and the B-mode synovial hypertrophy was measured at the suprapatellar recess and parapatellar recesses.(3, 4) The following cutoffs were used to select patients for further screening: distension of midline suprapatellar recess ≥3.6 mm, medial parapatellar recess ≥3.0 mm, lateral parapatellar recess ≥3.3 mm. Since the suprapatellar recess is insensitive to Doppler inspection for synovitis, the medial and lateral parapatellar recesses were chosen. Scans were acquired using a gel standoff assuring minimal pressure on the recess. Semiquantitative scoring was used: grade 0, no signal; grade 1, ≤3 single vessels; grade 2, >3 single or confluent blood vessels in less than half the synovial area; grade 3, vessel signals in more than half the synovial area. In addition to meeting the B-mode criteria for inclusion, a power Doppler score of ≥2 at the parapatellar recesses was one of the independent cutoffs for further screening.

2

Posteroanterior weight-bearing radiographs of the target knee were acquired at screening and at week 52, using the fixed-flexion technique with 10° caudal beam angulation using a positioning frame and calibration phantom. Knee joint space width (JSW) measurements were made using a computer-assisted algorithm (KneeAnalyzer, Optasia Medical, Manchester, United Kingdom) that identified the contours of the femoral condyles and tibia plateau. A single radiologist (O.V.) reviewed computer-generated contours and adjusted them, if necessary. Software then computed the minimum JSW in the weight-bearing region of the medial and lateral compartments. The method was highly reproducible, with intraclass correlation coefficient (ICC) of 0.98 and root mean square coefficient of variation of 0.18 mm.

Magnetic Resonance Imaging

Pulse sequences included sagittal 2-dimensional (2D) proton-density weighted (PD-w) fast spin echo (FSE) with spectral fat saturation (FS), axial 2D in/out-of-phase T1-weighted gradient echo (GRE), sagittal 3-dimensional T1-weighted GRE with FS or selective water excitation, coronal 2D PD-w FSE with FS, and axial 2D PD-w FSE with FS. The same scanners were used at baseline and follow-up.

All WORMS assessments were performed with multiple time points viewed simultaneously by a single central radiologist who was blinded to time point order. WORMS inter-reader ICCs have previously been shown to range from 0.61 to 0.99, depending on the feature.(2)

3

The extent of synovitis was assessed by measuring its thickness in mm in 4 regions of interest: the medial and lateral recess and the medial and lateral border of the suprapatellar bursa.(5) The intra-reader and inter-reader correlation coefficients were r=0.91 and r=0.82, respectively (*P*<0.0001).

The synovial fluid volume was assessed using a fully automated system as described (6) with a correlation coefficient with manual quantification of r=0.98 (*P*<0.0001) and direct aspiration r=0.88 (*P*=0.0008).

Semi-automated cartilage volume/thickness was measured as previously described.(7-9). The change in knee cartilage volume/thickness was obtained by subtracting the initial (baseline) volume/thickness from follow-up volume/thickness and calculated compared with initial (baseline) volume/thickness in percentage values. Between-reader agreement of measurement had ICCs ranging from 0.958 to 0.997 (*P*<0.0001) for global knee cartilage. Test-retest reliability of within-reader measurements had Pearson correlation coefficients ranging from 0.978 to 0.999 (*P*<0.0001).(8) Automated cartilage volume/thickness was assessed as described and validated (10, 11) with a test-retest measurement error of 0.3%±1.6% for the global knee (10).

Dynamic Contrast Enhanced Magnetic Resonance Imaging

The following parameters were defined for quantitative analysis of the dynamic changes in synovial fluid using dynamic contrast enhanced MRI (DCE-MRI):

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- Initial rate of enhancement (IRE): slope of the enhancement versus time curve from initial onset of enhancement to the time of plateau or until the end of acquisition if a plateau pattern is not observed. IRE is computed for each voxel in the image stack and the mean IRE is calculated for all voxels within the measurement volume of interest

- Maximum enhancement (ME): maximum increase in enhancement from precontrast images to the time point of maximum enhancement. ME is computed for each voxel in the image stack and the mean ME is calculated for all voxels within the measurement volume of interest

- Enhancing volume (E_VOL): volume of tissue (in mL) demonstrating plateau or washout characteristics. E_VOL is equal to the product of the number of enhancing voxels within the volume of interest, plateau, or washout) and the volume of an individual voxel, determined by the area of a voxel in-plane and the slice separation

    o E_VOL with persistent uptake: E_VOL × number of pixels with persistent uptake/classified pixels

    o E_VOL with plateau uptake: E_VOL × number of pixels with plateau uptake/classified pixels

    o E_VOL with washout uptake: E_VOL × number of pixels with washout uptake/classified pixels

- IAUGC60: initial area under the gadolinium curve at time 60 seconds

- DEMRIQ ME Score (DEMRIQ_ME): mean ME × E_VOL

- DEMRIQ IRE score (DEMRIQ_IRE): mean IRE × E_VOL

- Ktrans: rate contrast for the diffusion of contrast between locally defined blood and tissue regions of interest

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- VE: proportion (0–1) of extravascular extracellular space present in the tissue region of interest

The analysis of the DCE MRI scans used 2 independent readers and a third reader for adjudication of discrepancies. For each single measurement, if there was only 1 raw read, it was used as the measurement value; if there were 2 reads, they were averaged; if there were 2 raw reads and 1 adjudicator read, the adjudicator read was averaged with the closest raw read.

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Supplemental Table 1. Qualification for Study Entry Based on Evidence of Knee Synovitis

Evidence Source, n	Placebo	Lutikizumab SC Q2W, mg			Untreated	Total
		25	100	200		
Ultrasound or MRI	84	89	84	87	3	347
Ultrasound	53	56	55	63	2	229
Without MRI	48	56	54	60	2	220
With positive MRI	3	0	1	2	0	6
With negative MRI	2	0	0	1	0	3
MRI	31	33	29	24	1	118
None	0	0	1*	0	0	1*
Missing	1	0	1*	0	0	2*

MRI, magnetic resonance imaging; Q2W, every 2 weeks; SC, subcutaneous.

*Inadvertently enrolled despite the lack of evidence of synovitis; 1 of these 3 subjects had synovitis at baseline based on the Whole-Organ Magnetic Resonance Imaging Score assessment.

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Supplemental Table 2. Changes From Baseline in Efficacy Endpoints in Subjects With Positive WORMS Synovitis/Effusion at Baseline

Signs and Symptoms Endpoints (LOCF Data)

Endpoint (Full Scale)	Week 16				Week 52			
		Lutikizumab SC Q2W, mg				Lutikizumab SC Q2W, mg		
	PBO (n=72)	25 (n=78)	100 (n=65)	200 (n=79)	PBO (n=72)	25 (n=78)	100 (n=65)	200 (n=79)
Co-primary endpoint								
WOMAC pain (0–50; higher scores indicate worse condition)	−8.1 (−10.44, −5.79)	−10.3 (−12.58, −8.08)	−11.7 (−14.17, −9.26)	−11.9 (−14.11, −9.66)	−9.1 (−11.60, −6.57)	−12.1 (−14.48, −9.62)	−11.9 (−14.58, −9.27)	−12.5 (−14.88, −10.07)
Difference vs PBO		−2.2 (−5.43, 1.00)	−3.6 (−6.98, −0.22)	−3.8 (−6.97, −0.56)		−3.0 (−6.44, 0.51)	−2.8 (−6.50, 0.81)	−3.4 (−6.86, 0.07)
P value vs PBO		0.176	**0.037***	**0.021***		0.094	0.127	0.055
Secondary endpoint								
WOMAC function (0–170; higher scores indicate worse condition)	−29.1 (−36.46, −21.82)	−31.4 (−38.53, −24.34)	−36.3 (−43.99, −28.53)	−32.6 (−39.61, −25.52)	−31.0 (−39.62, −22.36)	−39.2 (−47.59, −30.87)	−39.0 (−48.13, −29.91)	−40.4 (48.65, −32.05)
Difference vs PBO		−2.3 (−12.44, 7.84)	−7.1 (−17.76, 3.52)	−3.4 (−13.53, 6.68)		−8.2 (−20.18, 3.70)	−8.0 (−20.57, 4.50)	−9.4 (−21.27, −2.55)
P value vs PBO		0.656	0.189	0.506		0.176	0.208	0.123
Exploratory endpoint								
OMERACT/OARSI response, %[†]	59.7 (48.4, 71.1)	68.8 (58.5, 79.2)	71.9 (60.9, 82.9)	65.4 (54.8, 75.9)	68.1 (57.3, 78.8)	74.0 (64.2, 83.8)	73.4 (62.6, 84.3)	73.4 (63.7, 83.2)
Difference vs PBO		9.1 (−6.2, 24.5)	12.2 (−3.6, 28.0)	5.7 (−9.8, 21.1)		6.0 (−8.6, 20.5)	5.4 (−9.9, 20.6)	5.4 (−9.2, 19.9)
P value vs PBO		0.253	0.123	0.457		0.448	0.577	0.484

Structural Endpoints (Observed Data)

Endpoint	Week 26				Week 52			
		Lutikizumab SC Q2W, mg				Lutikizumab SC Q2W, mg		
	PBO	25	100	200	PBO	25	100	200
Co-primary endpoint								
Synovial membrane thickness, mm	−0.06 (−0.12, 0.01)	0.03 (−0.03, 0.09)	−0.07 (−0.13, −0.003)	−0.00 (−0.06, 0.06)	−0.08 (−0.15, −0.01)	−0.03 (−0.09, 0.03)	−0.05 (−0.12, 0.02)	−0.02 (−0.09, 0.04)
n	49	56	49	59	44	52	46	50
Difference vs PBO		0.08 (−0.01, 0.17)	−0.01 (−0.10, 0.08)	0.05 (−0.03, 0.14)		0.05 (−0.04, 0.14)	0.03 (−0.07, 0.12)	0.05 (−0.04, 0.15)
P value vs PBO		0.064	0.819	0.219		0.291	0.554	0.247

Synovial fluid volume, mL	−0.01 (−2.99, 2.96)	0.07 (−2.58, 2.73)	−0.75 (−3.60, 2.10)	−2.15 (−4.82, 0.52)	−2.38 (−5.94, 1.18)	0.88 (−2.38, 4.13)	−0.45 (−3.92, 3.02)	−2.33 (−5.64, 0.98)
n	49	62	53	61	46	55	48	53
Difference vs PBO		0.09 (−3.87, 4.04)	−0.74 (−4.84, 3.37)	−2.13 (−6.11, 1.84)		3.26 (−1.52, 8.04)	1.93 (−3.04, 6.90)	0.05 (−4.78, 4.88)
P value vs PBO		0.966	0.724	0.291		0.181	0.444	0.984
WORMS semiquantitative synovitis/effusion volume	0.09 (−0.05, 0.23)	−0.02 (−0.16, 0.11)	−0.08 (−0.23, 0.06)	−0.12 (−0.25, 0.02)	−0.06 (−0.20, 0.09)	−0.07 (−0.21, 0.07)	−0.08 (−0.23, 0.07)	−0.06 (−0.20, 0.07)
n	58	67	57	68	51	58	51	61
Difference vs PBO		−0.11 (−0.31, 0.08)	−0.17 (−0.37, 0.03)	−0.20 (−0.40, −0.01)		−0.02 (−0.22, 0.19)	−0.02 (−0.23, 0.18)	−0.01 (−0.20, 0.19)
P value vs PBO		0.255	0.098	0.039		0.880	0.813	0.955

Data are least squares means (95% CI).

KL, Kellgren-Lawrence; LOCF, last observation carried forward; OMERACT/OARSI, Outcome Measures in Rheumatology Clinical Trials/Osteoarthritis Research Society International; PBO, placebo; Q2W, every 2 weeks; SC, subcutaneous; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

*$P \leq 0.05$ vs PBO. Continuous variables compared with analysis of covariance with treatment, age, and KL grade as main factors and baseline as covariate. Categorical variables compared with Cochran-Mantel-Haenszel test with age group and KL grade as stratification factors.

[†]OMERACT/OARSI response defined as either (1) ≥50% relative and ≥20% absolute improvement from baseline in WOMAC pain or function or (2) ≥20% relative and ≥10% from baseline in at least 2 of 3 measures (WOMAC pain, WOMAC function, and patient global assessment).

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Supplemental Table 3. Change From Baseline in DCE-MRI Parameters in Synovial Membrane at Week 26

Parameter, LS Mean (%) Change	Placebo (n=7)	Lutikizumab SC Q2W, mg		
		25 (n=10)	100 (n=10)	200 (n=12)
IRE*	−0.002 (−25.0)	−0.002 (−5.5)	0.002 (46.3‡)	−0.003 (−7.9)
ME*	−0.040 (−3.2)	0.030 (2.4)	0.119 (9.5)	−0.096 (−3.3)
E_VOL, mL*	0.337 (67.7)	0.711 (273.9)	7.001 (28.6)	−6.950 (15.3)
Persistent uptake*	0.049 (7.1)	0.129 (113.0)	−0.136 (27.1)	−0.023 (59.2)
Plateau uptake*	−0.363 (87.3)	0.672 (379.2)	7.254 (37.6)	−5.091 (18.1)
Washout uptake*	0.872 (106.3)	−0.214 (244.5)	−0.240 (16.4)	−1.519§ (37.1)
IAUGC60, mmol•s†	−0.519 (−20.4)	0.191 (0.8)	1.241 (61.6)	−1.134 (7.2)
DEMRIQ ME score, mL*	−0.140 (67.2)	4.025 (318.6)	20.505 (35.1)	−18.734 (34.0)
DEMRIQ IRE score, mL*	−0.134 (35.9)	−0.091 (342.9)	0.188 (56.1)	−0.242 (49.8)
Ktrans, min^{-1}†	−0.058 (−51.5)	−0.003 (25.5)	0.052 (329.9)	−0.045 (319.1)
VE†	−0.391 (23.6)	0.169 (20.9)	−0.515 (5.5)	−0.545 (38.3)

DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; DEMRIQ, DCE-MRI quantification; E_VOL, enhancing volume; IAUGC60, initial area under the gadolinium concentration-time curve over 60 s; IRE, initial rate of enhancement; KL, Kellgren-Lawrence; Ktrans, volume transfer coefficient; LS, least squares; ME, maximal enhancement; Q2W, every 2 weeks; SC, subcutaneous; VE, fractional extracellular extravascular space.

*n=5, 8, 10, and 10 and †n= 5, 6, 10, and 10 for placebo and lutikizumab 25 mg, 100 mg, and 200 mg, respectively.

†P=0.016 and §P=0.043 vs placebo from analysis of covariance with treatment, age, and KL grade as main factors and baseline as covariate.

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Supplemental Table 4. Concomitant Medication Use During the Treatment Period*

n (%)	Placebo (n=85)	Lutikizumab SC Q2W, mg		
		25 (n=89)	100 (n=85)	200 (n=88)
Any concomitant medication, n (%)	84 (98.8)	87 (97.8)	82 (96.5)	87 (98.9)
Ibuprofen	37 (43.5)	26 (29.2)	29 (34.1)	39 (44.3)
Acetaminophen	75 (88.2)	66 (74.2)	68 (80.0)	78 (88.6)

Q2W, every 2 weeks; SC, subcutaneous.

*First study drug dose through 14 days after the final study drug dose.

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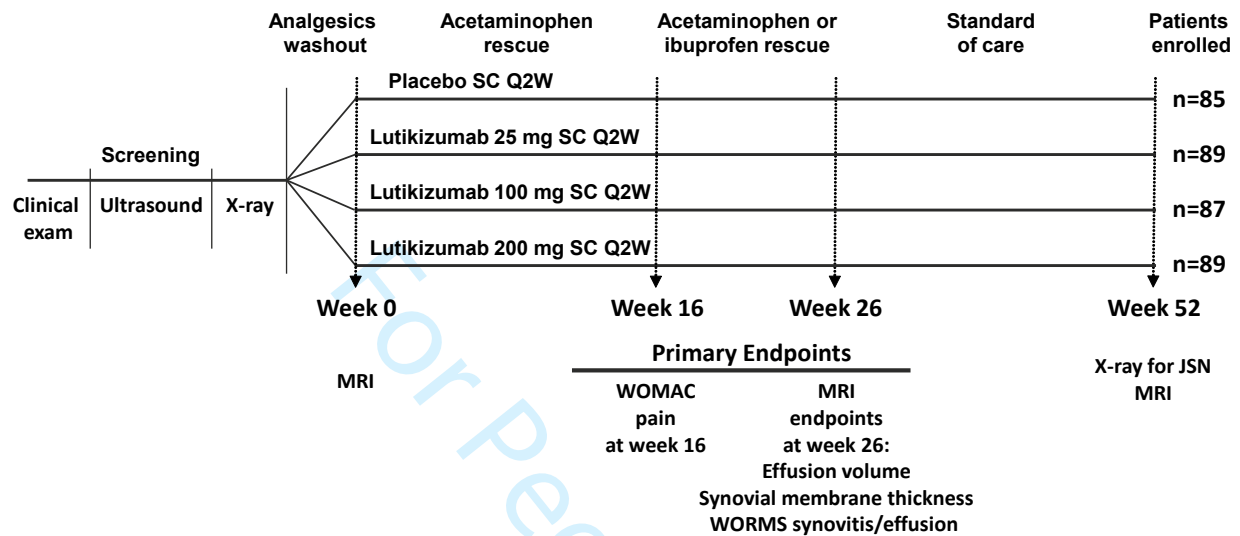
Supplemental Table 5. Proportion of Subjects With Decreases From Baseline in IL-1β 2 Weeks After Initiating Treatment With Placebo or Lutikizumab

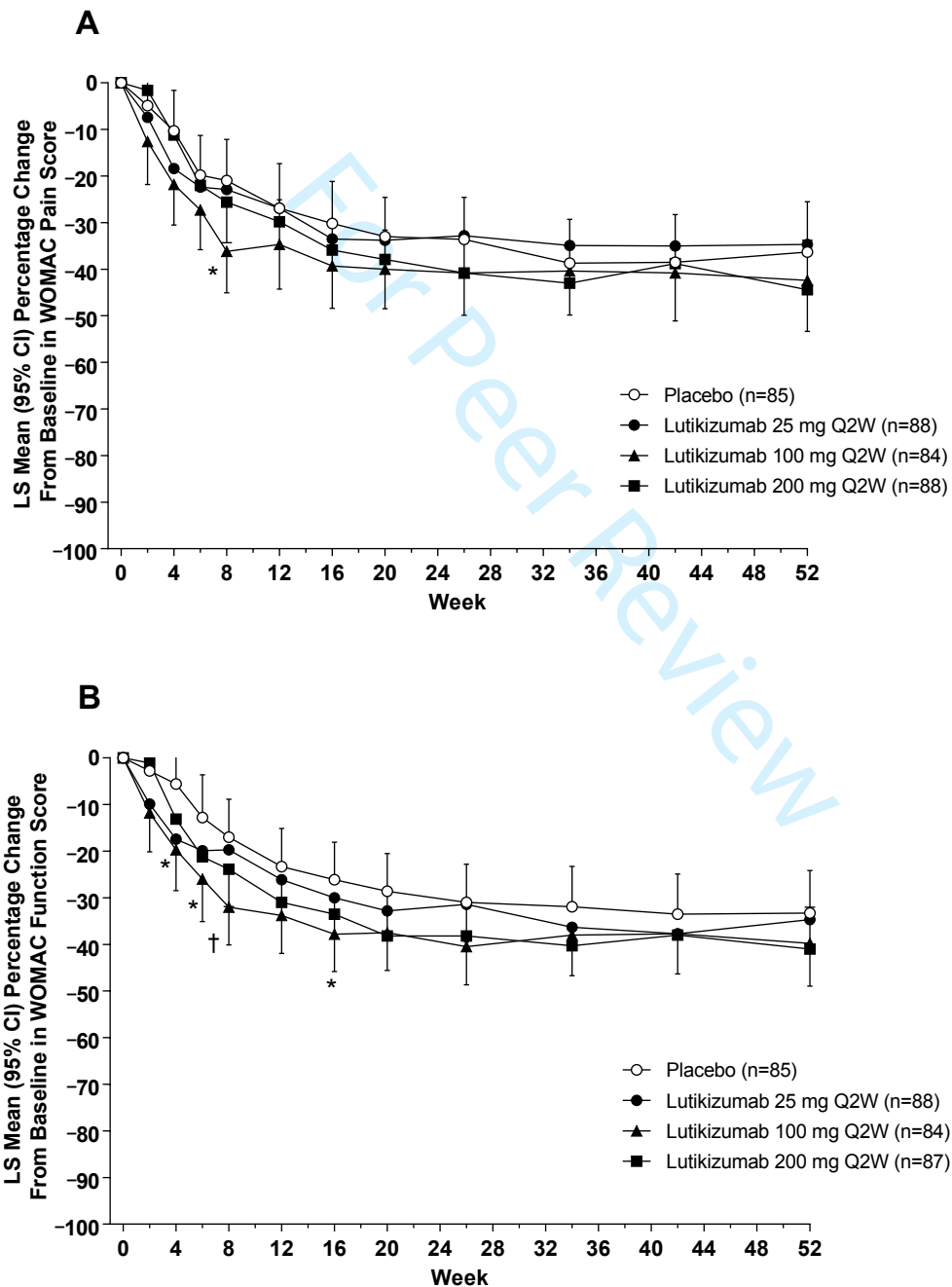| Treatment	Subjects, n/N (%)
Placebo	4/40 (10.0)
Lutikizumab 25 mg SC Q2W	17/37 (45.9)
Lutikizumab 100 mg SC Q2W	10/34 (29.4)
Lutikizumab 200 mg SC Q2W	11/40 (27.5)

IL-1β=interleukin-1β; Q2W, every 2 weeks; SC, subcutaneous.

Supplemental Figure 1. Study design. JSN, joint space narrowing; MRI, magnetic resonance imaging; Q2W, every 2 weeks; SC, subcutaneous; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.
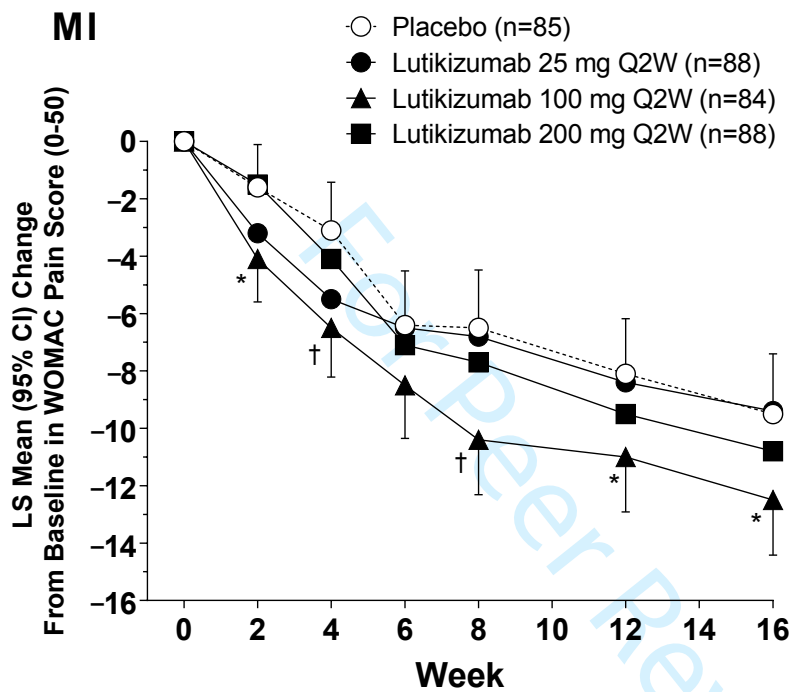
Supplemental Figure 2. LS mean percentage change from baseline over time for WOMAC pain **(A)** and WOMAC function **(B)**. LS, least squares; Q2W, every 2 weeks; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index. *$P \leq 0.05$ vs placebo; †$P \leq 0.01$ vs placebo.

Supplemental Figure 3. LS mean change from baseline over time for WOMAC pain using MI. LS, least squares; MI, multiple imputation; Q2W, every 2 weeks; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index. *$P \leq 0.05$ vs placebo; †$P \leq 0.01$ vs placebo.
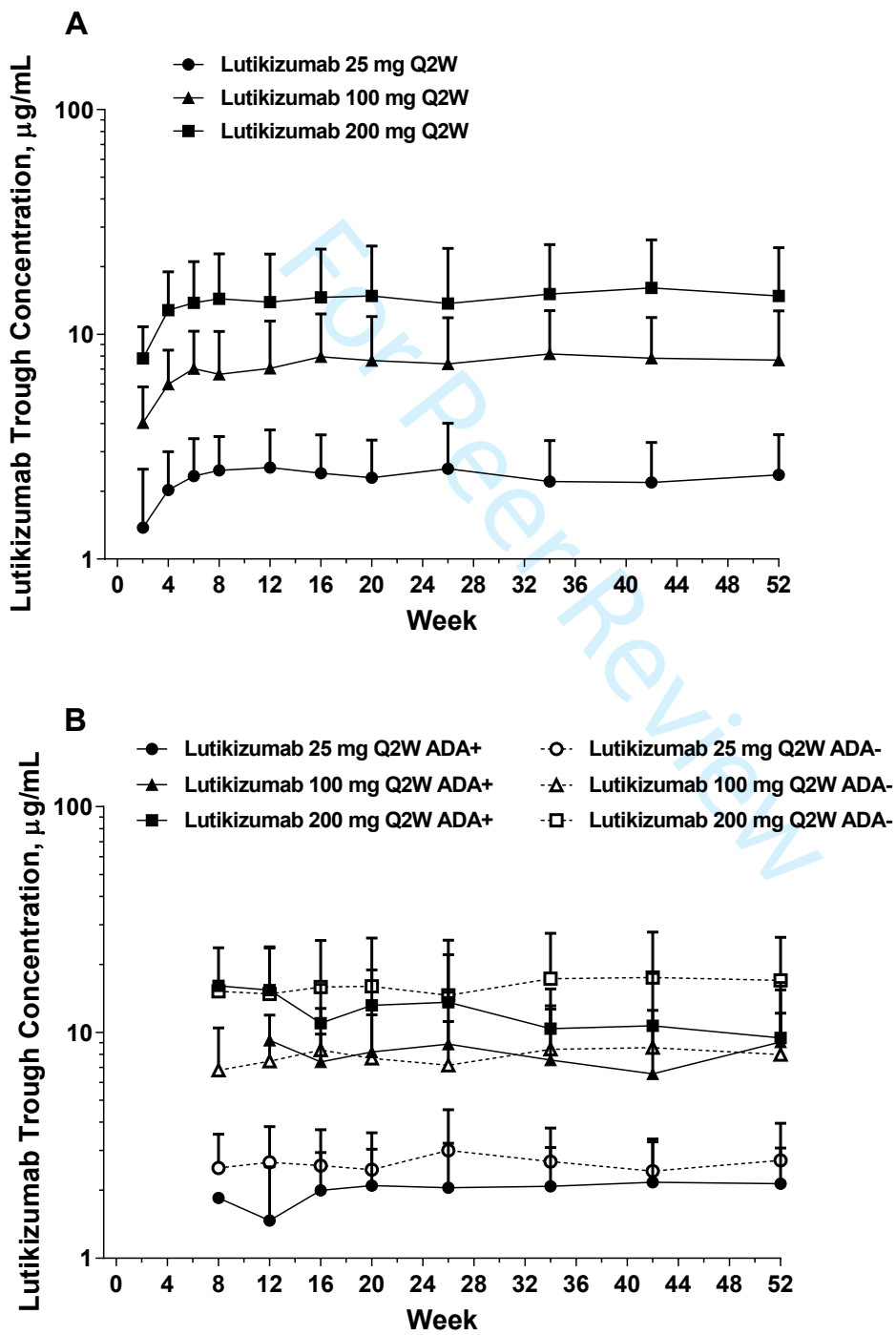
Supplemental Figure 4. LS mean change from baseline over time for WOMAC pain in subjects

with positive WORMS synovitis/effusion at baseline. LS, least squares; Q2W, every 2 weeks;

WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; WORMS, Whole-

Organ Magnetic Resonance Imaging Score. *$P \leq 0.05$ vs placebo; †$P \leq 0.01$ vs placebo.
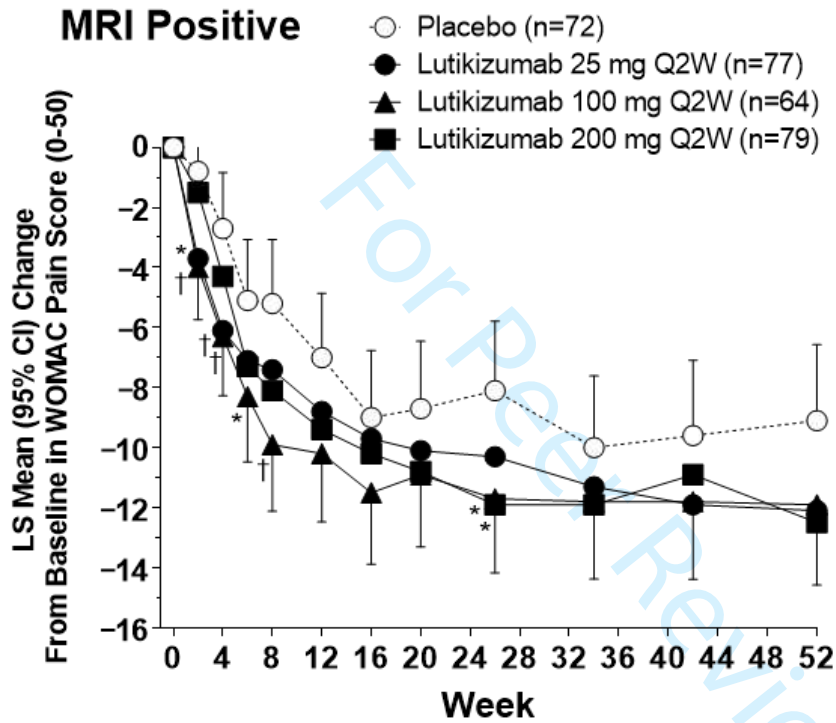
Supplemental Figure 5. Mean (SD) trough serum concentrations of lutikizumab over time for all subjects **(A)** and separated by presence of ADAs **(B)**. ADA, anti-drug antibody; Q2W, every 2 weeks.
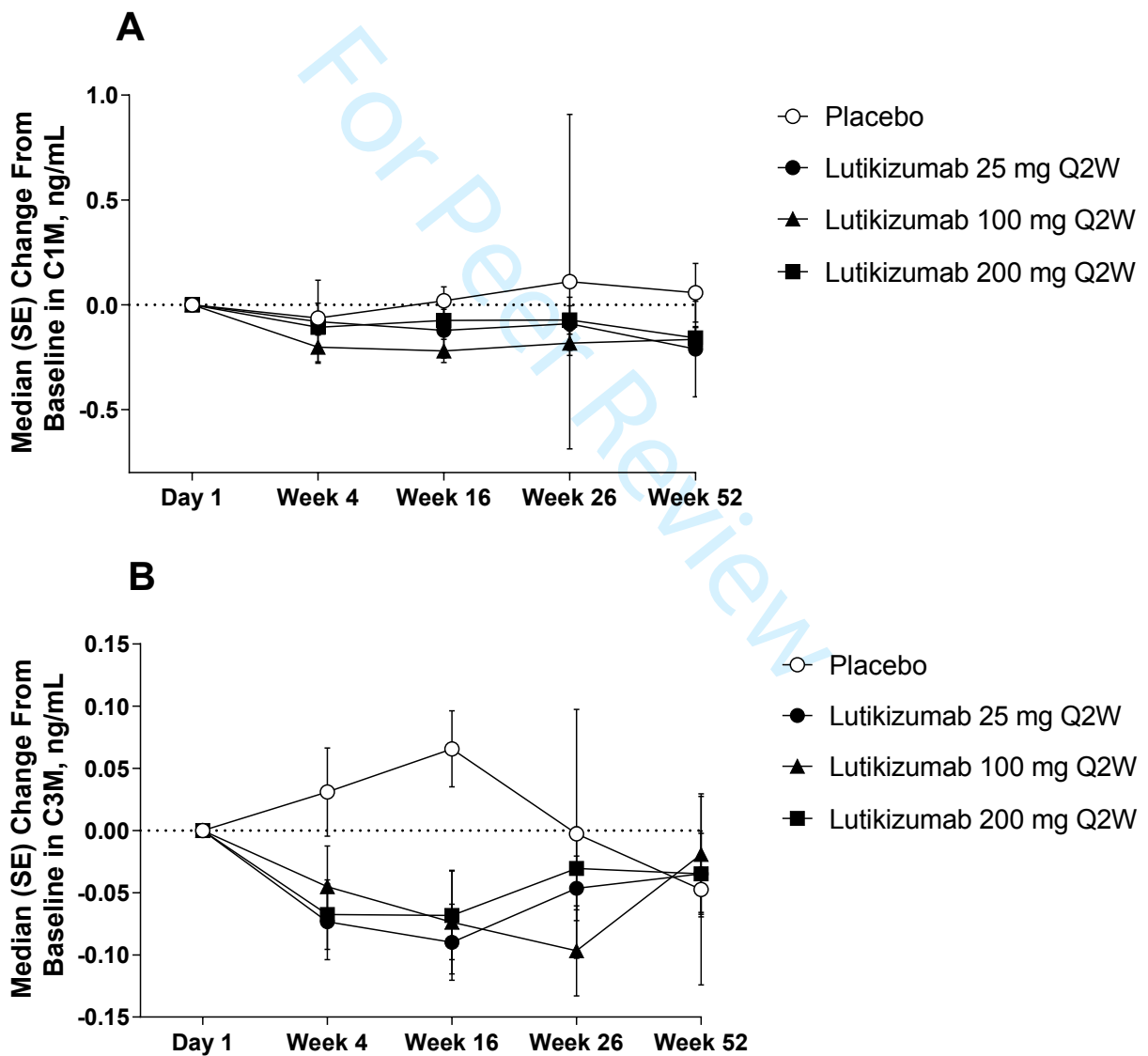
Supplemental Figure 6. Median absolute neutrophil counts for placebo and lutikizumab serum

concentration quartiles at week 52 (observed cases).
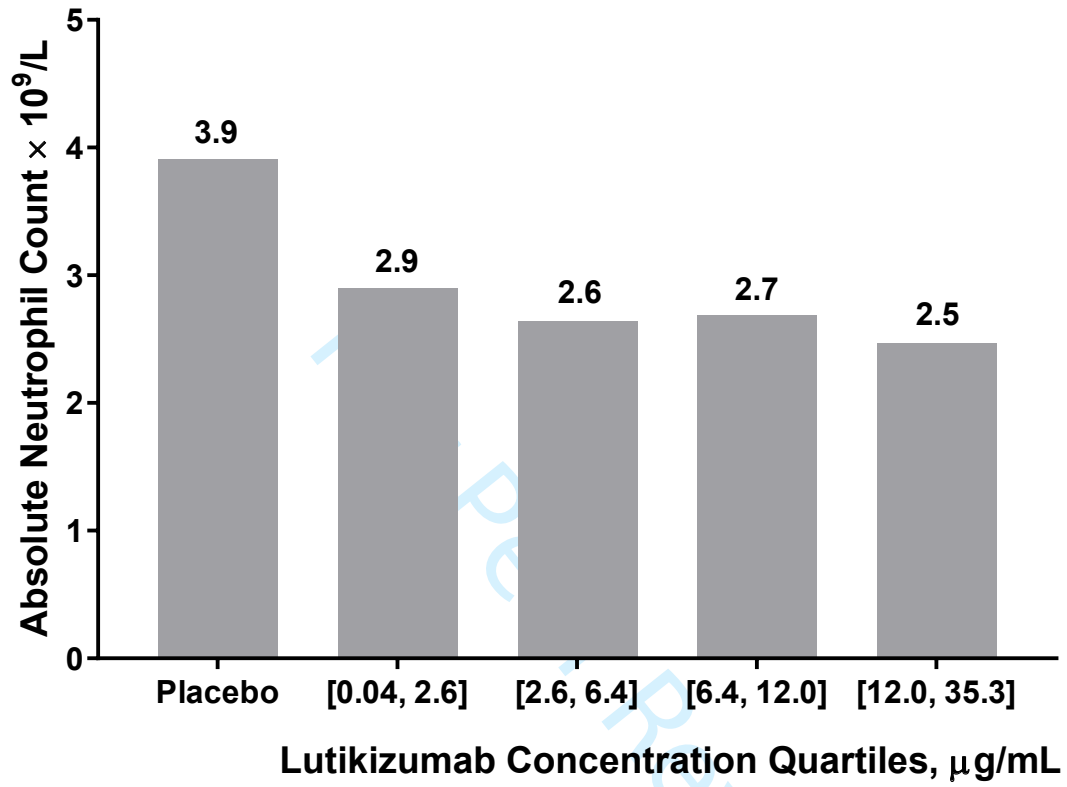
Supplemental Figure 7. Change From Day 1 in Serum IL-1α Levels at 2 Weeks After Initiating Treatment With Placebo or Lutikizumab. IL-1α=interleukin-1α; Q2W=every 2 weeks.
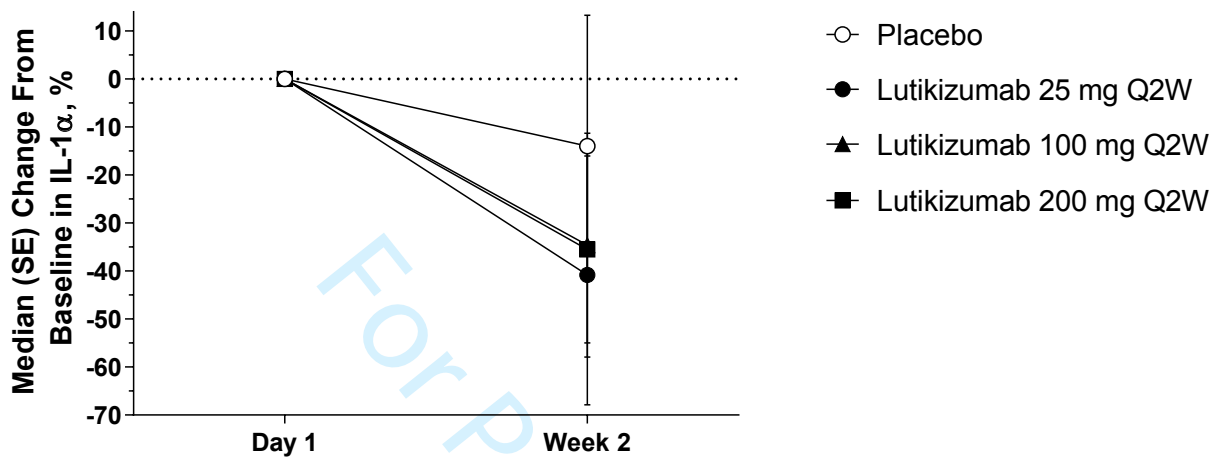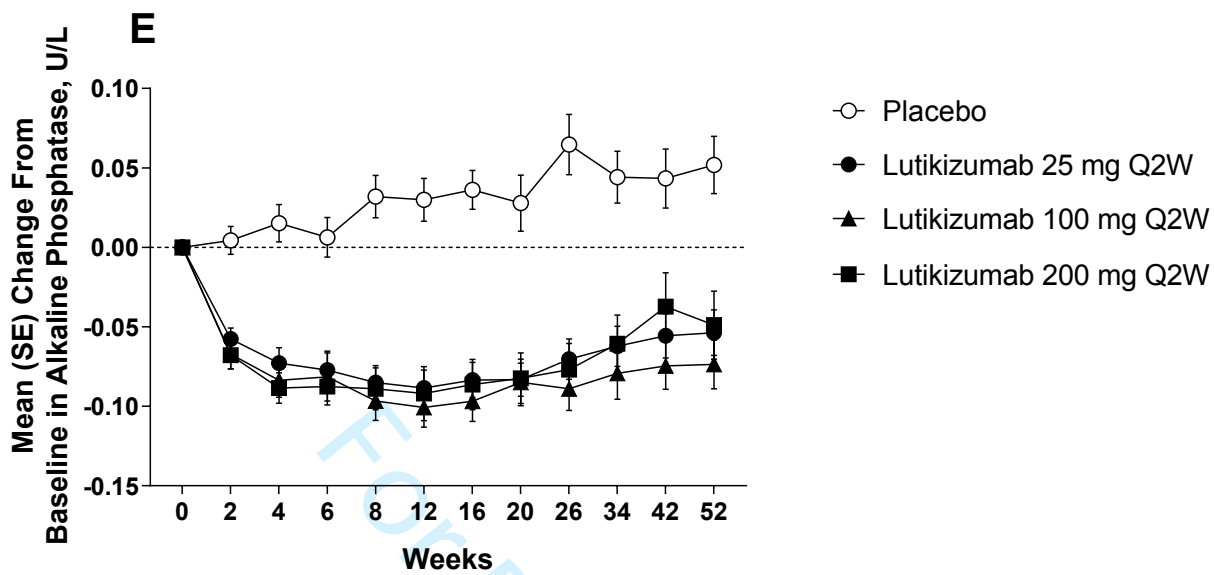
Supplemental Figure 8. Median change from baseline over time for the biomarkers C1M **(A)**, C3M **(B)**, and CRPM **(C)**, median percentage change from baseline over time in IL-6 **(D)**, and mean change from baseline over time in alkaline phosphatase **(E)**. C1M, metalloproteinase-degraded collagen type I; C3M, metalloproteinase-degraded collagen type III; CRPM, matrix metalloproteinase-generated fragment of C-reactive protein; IL-6, interleukin-6; Q2W, every 2 weeks.

Supplemental Figure 8 continued.

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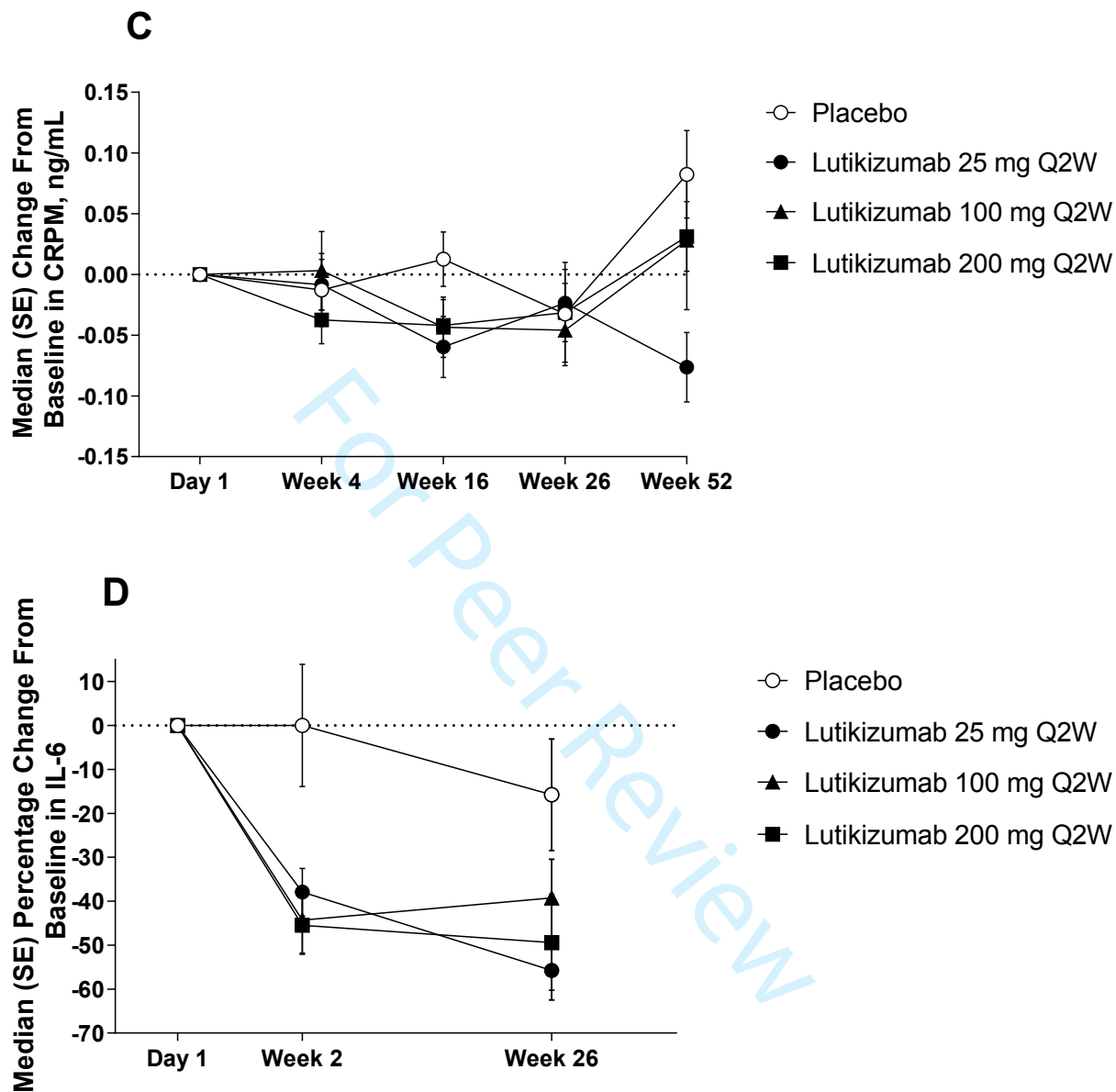
Supplemental Figure 8 continued.

Supplemental Methods

Subjects

Key exclusion criteria included significant trauma or surgery to the index knee within the last year or arthroscopy of the index knee within 6 months of the initial screening visit; severe knee malalignment, either >4.0° in varus or >8.0° in valgus angulation in the index knee; previous exposure to anti–IL-1 treatment; intra-articular corticosteroids within 3 months or via another route within 1 month before screening; and use of any investigational or immunosuppressive therapy within 1 month or 5 half-lives, whichever was longer, before the first dose of study drug.

Subject disposition was calculated for the intent-to-treat population, comprising all randomized patients.

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Ultrasound

Patients meeting criteria for B-mode synovial hypertrophy or power Doppler were selected for further screening. For selection and quality assurance, sonographers were asked to submit test scans for B-mode and power Doppler images. B-mode frequency started at 12 MhZ and could be reduced for penetration. Gain was set at approximately 50%. Using the index fingertip, Doppler settings were optimized for respective equipment to show vascularity in at least one third of the finger pulp. The pulse repetition frequency was set in a range between 400 to 600 Hz, with Doppler box to cover recess and superficial tissue, and gain just above noise. Doppler frequency was adjusted for the highest sensitivity. The knee was placed in 30° flexion, and the B-mode synovial hypertrophy was measured at the suprapatellar recess and parapatellar recesses.(3, 4) The following cutoffs were used to select patients for further screening: distension of midline suprapatellar recess ≥3.6 mm, medial parapatellar recess ≥3.0 mm, lateral parapatellar recess ≥3.3 mm. Since the suprapatellar recess is insensitive to Doppler inspection for synovitis, the medial and lateral parapatellar recesses were chosen. Scans were acquired using a gel standoff assuring minimal pressure on the recess. Semiquantitative scoring was used: grade 0, no signal; grade 1, ≤3 single vessels; grade 2, >3 single or confluent blood vessels in less than half the synovial area; grade 3, vessel signals in more than half the synovial area. In addition to meeting the B-mode criteria for inclusion, a power Doppler score of ≥2 at the parapatellar recesses was one of the independent cutoffs for further screening.

Posteroanterior weight-bearing radiographs of the target knee were acquired at screening and at week 52, using the fixed-flexion technique with 10° caudal beam angulation using a positioning frame and calibration phantom. Knee joint space width (JSW) measurements were made using a computer-assisted algorithm (KneeAnalyzer, Optasia Medical, Manchester, United Kingdom) that identified the contours of the femoral condyles and tibia plateau. A single radiologist (O.V.) reviewed computer-generated contours and adjusted them, if necessary. Software then computed the minimum JSW in the weight-bearing region of the medial and lateral compartments. The method was highly reproducible, with intraclass correlation coefficient (ICC) of 0.98 and root mean square coefficient of variation of 0.18 mm.

Magnetic Resonance Imaging

Pulse sequences included sagittal 2-dimensional (2D) proton-density weighted (PD-w) fast spin echo (FSE) with spectral fat saturation (FS), axial 2D in/out-of-phase T1-weighted gradient echo (GRE), sagittal 3-dimensional T1-weighted GRE with FS or selective water excitation, coronal 2D PD-w FSE with FS, and axial 2D PD-w FSE with FS. The same scanners were used at baseline and follow-up.

All WORMS assessments were performed with multiple time points viewed simultaneously by a single central radiologist who was blinded to time point order. WORMS inter-reader ICCs have previously been shown to range from 0.61 to 0.99, depending on the feature.(2)

The extent of synovitis was assessed by measuring its thickness in mm in 4 regions of interest: the medial and lateral recess and the medial and lateral border of the suprapatellar bursa.(5) The intra-reader and inter-reader correlation coefficients were r=0.91 and r=0.82, respectively (*P*<0.0001).

The synovial fluid volume was assessed using a fully automated system as described (6) with a correlation coefficient with manual quantification of r=0.98 (*P*<0.0001) and direct aspiration r=0.88 (*P*=0.0008).

Semi-automated cartilage volume/thickness was measured as previously described.(7-9). The change in knee cartilage volume/thickness was obtained by subtracting the initial (baseline) volume/thickness from follow-up volume/thickness and calculated compared with initial (baseline) volume/thickness in percentage values. Between-reader agreement of measurement had ICCs ranging from 0.958 to 0.997 (*P*<0.0001) for global knee cartilage. Test-retest reliability of within-reader measurements had Pearson correlation coefficients ranging from 0.978 to 0.999 (*P*<0.0001).(8) Automated cartilage volume/thickness was assessed as described and validated (10, 11) with a test-retest measurement error of 0.3%±1.6% for the global knee (10).

Dynamic Contrast Enhanced Magnetic Resonance Imaging

The following parameters were defined for quantitative analysis of the dynamic changes in synovial fluid using dynamic contrast enhanced MRI (DCE-MRI):

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- Initial rate of enhancement (IRE): slope of the enhancement versus time curve from initial onset of enhancement to the time of plateau or until the end of acquisition if a plateau pattern is not observed. IRE is computed for each voxel in the image stack and the mean IRE is calculated for all voxels within the measurement volume of interest

- Maximum enhancement (ME): maximum increase in enhancement from precontrast images to the time point of maximum enhancement. ME is computed for each voxel in the image stack and the mean ME is calculated for all voxels within the measurement volume of interest

- Enhancing volume (E_VOL): volume of tissue (in mL) demonstrating plateau or washout characteristics. E_VOL is equal to the product of the number of enhancing voxels within the volume of interest, plateau, or washout) and the volume of an individual voxel, determined by the area of a voxel in-plane and the slice separation

    o E_VOL with persistent uptake: E_VOL × number of pixels with persistent uptake/classified pixels

    o E_VOL with plateau uptake: E_VOL × number of pixels with plateau uptake/classified pixels

    o E_VOL with washout uptake: E_VOL × number of pixels with washout uptake/classified pixels

- IAUGC60: initial area under the gadolinium curve at time 60 seconds

- DEMRIQ ME Score (DEMRIQ_ME): mean ME × E_VOL

- DEMRIQ IRE score (DEMRIQ_IRE): mean IRE × E_VOL

- Ktrans: rate contrast for the diffusion of contrast between locally defined blood and tissue regions of interest

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- VE: proportion (0–1) of extravascular extracellular space present in the tissue region of interest

The analysis of the DCE MRI scans used 2 independent readers and a third reader for adjudication of discrepancies. For each single measurement, if there was only 1 raw read, it was used as the measurement value; if there were 2 reads, they were averaged; if there were 2 raw reads and 1 adjudicator read, the adjudicator read was averaged with the closest raw read.

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Supplemental Table 1. Qualification for Study Entry Based on Evidence of Knee Synovitis

Evidence Source, n	Placebo	Lutikizumab SC Q2W, mg			Untreated	Total
		25	100	200		
Ultrasound or MRI	84	89	84	87	3	347
Ultrasound	53	56	55	63	2	229
Without MRI	48	56	54	60	2	220
With positive MRI	3	0	1	2	0	6
With negative MRI	2	0	0	1	0	3
MRI	31	33	29	24	1	118
None	0	0	1*	0	0	1*
Missing	1	0	1*	0	0	2*

MRI, magnetic resonance imaging; Q2W, every 2 weeks; SC, subcutaneous.

*Inadvertently enrolled despite the lack of evidence of synovitis; 1 of these 3 subjects had synovitis at baseline based on the Whole-Organ Magnetic Resonance Imaging Score assessment.

Supplemental Table 2. Changes From Baseline in Efficacy Endpoints in Subjects With Positive WORMS Synovitis/Effusion at Baseline

Signs and Symptoms Endpoints (LOCF Data)

Endpoint (Full Scale)	Week 16				Week 52			
		Lutikizumab SC Q2W, mg				Lutikizumab SC Q2W, mg		
	PBO (n=72)	25 (n=78)	100 (n=65)	200 (n=79)	PBO (n=72)	25 (n=78)	100 (n=65)	200 (n=79)
Co-primary endpoint								
WOMAC pain (0–50; higher scores indicate worse condition)	−8.1 (−10.44, −5.79)	−10.3 (−12.58, −8.08)	−11.7 (−14.17, −9.26)	−11.9 (−14.11, −9.66)	−9.1 (−11.60, −6.57)	−12.1 (−14.48, −9.62)	−11.9 (−14.58, −9.27)	−12.5 (−14.88, −10.07)
Difference vs PBO		−2.2 (−5.43, 1.00)	−3.6 (−6.98, −0.22)	−3.8 (−6.97, −0.56)		−3.0 (−6.44, 0.51)	−2.8 (−6.50, 0.81)	−3.4 (−6.86, 0.07)
P value vs PBO		0.176	**0.037***	**0.021***		0.094	0.127	0.055
Secondary endpoint								
WOMAC function (0–170; higher scores indicate worse condition)	−29.1 (−36.46, −21.82)	−31.4 (−38.53, −24.34)	−36.3 (−43.99, −28.53)	−32.6 (−39.61, −25.52)	−31.0 (−39.62, −22.36)	−39.2 (−47.59, −30.87)	−39.0 (−48.13, −29.91)	−40.4 (48.65, −32.05)
Difference vs PBO		−2.3 (−12.44, 7.84)	−7.1 (−17.76, 3.52)	−3.4 (−13.53, 6.68)		−8.2 (−20.18, 3.70)	−8.0 (−20.57, 4.50)	−9.4 (−21.27, −2.55)
P value vs PBO		0.656	0.189	0.506		0.176	0.208	0.123
Exploratory endpoint								
OMERACT/OARSI response, %[†]	59.7 (48.4, 71.1)	68.8 (58.5, 79.2)	71.9 (60.9, 82.9)	65.4 (54.8, 75.9)	68.1 (57.3, 78.8)	74.0 (64.2, 83.8)	73.4 (62.6, 84.3)	73.4 (63.7, 83.2)
Difference vs PBO		9.1 (−6.2, 24.5)	12.2 (−3.6, 28.0)	5.7 (−9.8, 21.1)		6.0 (−8.6, 20.5)	5.4 (−9.9, 20.6)	5.4 (−9.2, 19.9)
P value vs PBO		0.253	0.123	0.457		0.448	0.577	0.484

Structural Endpoints (Observed Data)

Endpoint	Week 26				Week 52			
		Lutikizumab SC Q2W, mg				Lutikizumab SC Q2W, mg		
	PBO	25	100	200	PBO	25	100	200
Co-primary endpoint								
Synovial membrane thickness, mm	−0.06 (−0.12, 0.01)	0.03 (−0.03, 0.09)	−0.07 (−0.13, −0.003)	−0.00 (−0.06, 0.06)	−0.08 (−0.15, −0.01)	−0.03 (−0.09, 0.03)	−0.05 (−0.12, 0.02)	−0.02 (−0.09, 0.04)
n	49	56	49	59	44	52	46	50
Difference vs PBO		0.08 (−0.01, 0.17)	−0.01 (−0.10, 0.08)	0.05 (−0.03, 0.14)		0.05 (−0.04, 0.14)	0.03 (−0.07, 0.12)	0.05 (−0.04, 0.15)
P value vs PBO		0.064	0.819	0.219		0.291	0.554	0.247

Synovial fluid volume, mL	−0.01 (−2.99, 2.96)	0.07 (−2.58, 2.73)	−0.75 (−3.60, 2.10)	−2.15 (−4.82, 0.52)	−2.38 (−5.94, 1.18)	0.88 (−2.38, 4.13)	−0.45 (−3.92, 3.02)	−2.33 (−5.64, 0.98)
n	49	62	53	61	46	55	48	53
Difference vs PBO		0.09 (−3.87, 4.04)	−0.74 (−4.84, 3.37)	−2.13 (−6.11, 1.84)		3.26 (−1.52, 8.04)	1.93 (−3.04, 6.90)	0.05 (−4.78, 4.88)
P value vs PBO		0.966	0.724	0.291		0.181	0.444	0.984
WORMS semiquantitative synovitis/effusion volume	0.09 (−0.05, 0.23)	−0.02 (−0.16, 0.11)	−0.08 (−0.23, 0.06)	−0.12 (−0.25, 0.02)	−0.06 (−0.20, 0.09)	−0.07 (−0.21, 0.07)	−0.08 (−0.23, 0.07)	−0.06 (−0.20, 0.07)
n	58	67	57	68	51	58	51	61
Difference vs PBO		−0.11 (−0.31, 0.08)	−0.17 (−0.37, 0.03)	−0.20 (−0.40, −0.01)		−0.02 (−0.22, 0.19)	−0.02 (−0.23, 0.18)	−0.01 (−0.20, 0.19)
P value vs PBO		0.255	0.098	0.039		0.880	0.813	0.955

Data are least squares means (95% CI).

KL, Kellgren-Lawrence; LOCF, last observation carried forward; OMERACT/OARSI, Outcome Measures in Rheumatology Clinical Trials/Osteoarthritis Research Society International; PBO, placebo; Q2W, every 2 weeks; SC, subcutaneous; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

*$P\leq0.05$ vs PBO. Continuous variables compared with analysis of covariance with treatment, age, and KL grade as main factors and baseline as covariate. Categorical variables compared with Cochran-Mantel-Haenszel test with age group and KL grade as stratification factors.

†OMERACT/OARSI response defined as either (1) ≥50% relative and ≥20% absolute improvement from baseline in WOMAC pain or function or (2) ≥20% relative and ≥10% from baseline in at least 2 of 3 measures (WOMAC pain, WOMAC function, and patient global assessment).

Supplemental Table ~~2~~3. Change From Baseline in DCE-MRI Parameters in Synovial Membrane at Week 26

Parameter, LS Mean (%) Change	Placebo (n=7)	Lutikizumab SC Q2W, mg		
		25 (n=10)	100 (n=10)	200 (n=12)
IRE*	−0.002 (−25.0)	−0.002 (−5.5)	0.002 (46.3[‡])	−0.003 (−7.9)
ME*	−0.040 (−3.2)	0.030 (2.4)	0.119 (9.5)	−0.096 (−3.3)
E_VOL, mL*	0.337 (67.7)	0.711 (273.9)	7.001 (28.6)	−6.950 (15.3)
Persistent uptake*	0.049 (7.1)	0.129 (113.0)	−0.136 (27.1)	−0.023 (59.2)
Plateau uptake*	−0.363 (87.3)	0.672 (379.2)	7.254 (37.6)	−5.091 (18.1)
Washout uptake*	0.872 (106.3)	−0.214 (244.5)	−0.240 (16.4)	−1.519[§] (37.1)
IAUGC60, mmol•s[†]	−0.519 (−20.4)	0.191 (0.8)	1.241 (61.6)	−1.134 (7.2)
DEMRIQ ME score, mL*	−0.140 (67.2)	4.025 (318.6)	20.505 (35.1)	−18.734 (34.0)
DEMRIQ IRE score, mL*	−0.134 (35.9)	−0.091 (342.9)	0.188 (56.1)	−0.242 (49.8)
Ktrans, min^{-1}[†]	−0.058 (−51.5)	−0.003 (25.5)	0.052 (329.9)	−0.045 (319.1)
VE[†]	−0.391 (23.6)	0.169 (20.9)	−0.515 (5.5)	−0.545 (38.3)

DCE-MRI, dynamic contrast-enhanced magnetic resonance imaging; DEMRIQ, DCE-MRI quantification; E_VOL, enhancing volume; IAUGC60, initial area under the gadolinium concentration-time curve over 60 s; IRE, initial rate of enhancement; KL, Kellgren-Lawrence; Ktrans, volume transfer coefficient; LS, least squares; ME, maximal enhancement; Q2W, every 2 weeks; SC, subcutaneous; VE, fractional extracellular extravascular space.

*n=5, 8, 10, and 10 and [†]n= 5, 6, 10, and 10 for placebo and lutikizumab 25 mg, 100 mg, and 200 mg, respectively.

[†]P=0.016 and [§]P=0.043 vs placebo from analysis of covariance with treatment, age, and KL grade as main factors and baseline as covariate.

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Supplemental Table 4̶3̶. Concomitant Medication Use During the Treatment Period*

n (%)	Placebo (n=85)	Lutikizumab SC Q2W, mg		
		25 (n=89)	100 (n=85)	200 (n=88)
Any concomitant medication, n (%)	84 (98.8)	87 (97.8)	82 (96.5)	87 (98.9)
Ibuprofen	37 (43.5)	26 (29.2)	29 (34.1)	39 (44.3)
Acetaminophen	75 (88.2)	66 (74.2)	68 (80.0)	78 (88.6)

Q2W, every 2 weeks; SC, subcutaneous.

*First study drug dose through 14 days after the final study drug dose.

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Supplemental Table 54. Proportion of Subjects With Decreases From Baseline in IL-1β 2

Weeks After Initiating Treatment With Placebo or Lutikizumab

Treatment	Subjects, n/N (%)
Placebo	4/40 (10.0)
Lutikizumab 25 mg SC Q2W	17/37 (45.9)
Lutikizumab 100 mg SC Q2W	10/34 (29.4)
Lutikizumab 200 mg SC Q2W	11/40 (27.5)

IL-1β=interleukin-1β; Q2W, every 2 weeks; SC, subcutaneous.

Supplemental Table 5. ORs for Factors Associated With the Development of Neutropenia During Lutikizumab Treatment Based on a Multiple Logistic Regression Model

Variable	OR	95% CI	*P* Value
Treatment group			<0.0001
Lutikizumab 25 mg SC Q2W	5.99	1.52–23.69	
Lutikizumab 100 mg SC Q2W	14.84	3.80–57.98	
Lutikizumab 200 mg SC Q2W	24.36	6.24–95.05	
Baseline ANC	0.19	0.12–0.30	<0.0001
Age <62 y	2.11	1.09–4.09	0.027
Female sex	2.86	1.34–6.10	0.007

ANC, absolute neutrophil count; OR, odds ratio; Q2W, every 2 weeks; SC, subcutaneous.

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Supplemental Figure 1. Study design. JSN, joint space narrowing; MRI, magnetic resonance imaging; Q2W, every 2 weeks; SC, subcutaneous; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

Supplemental Figure 2. LS mean percentage change from baseline over time for WOMAC pain **(A)** and WOMAC function **(B)**. LS, least squares; Q2W, every 2 weeks; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index. *$P \leq 0.05$ vs placebo; [†]$P \leq 0.01$ vs placebo.
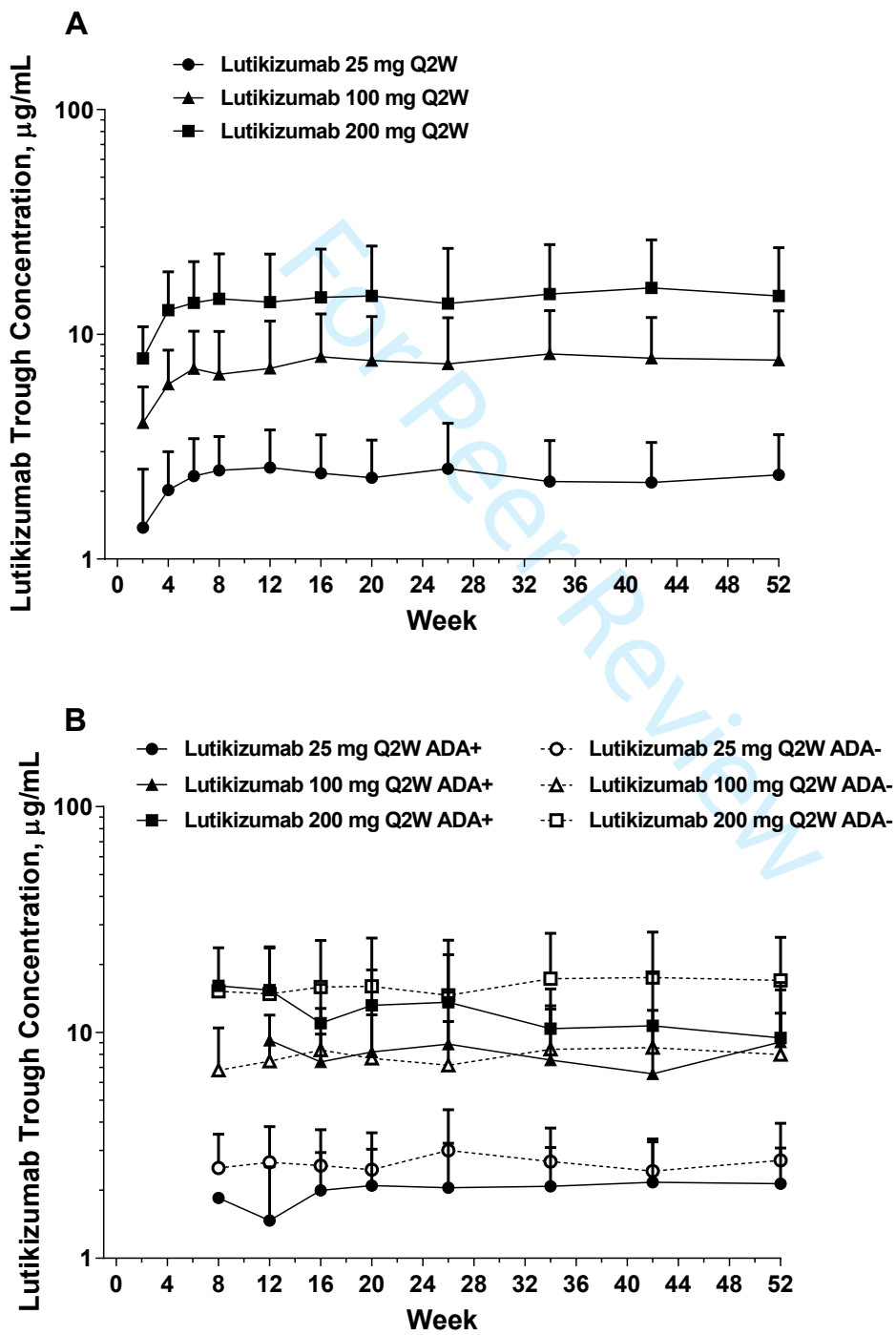

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Supplemental Figure 3. LS mean change from baseline over time for WOMAC pain using MI. LS, least squares; MI, multiple imputation; Q2W, every 2 weeks; WOMAC, Western Ontario and McMaster Universities Osteoarthritis index. *P≤0.05 vs placebo; †P≤0.01 vs placebo.
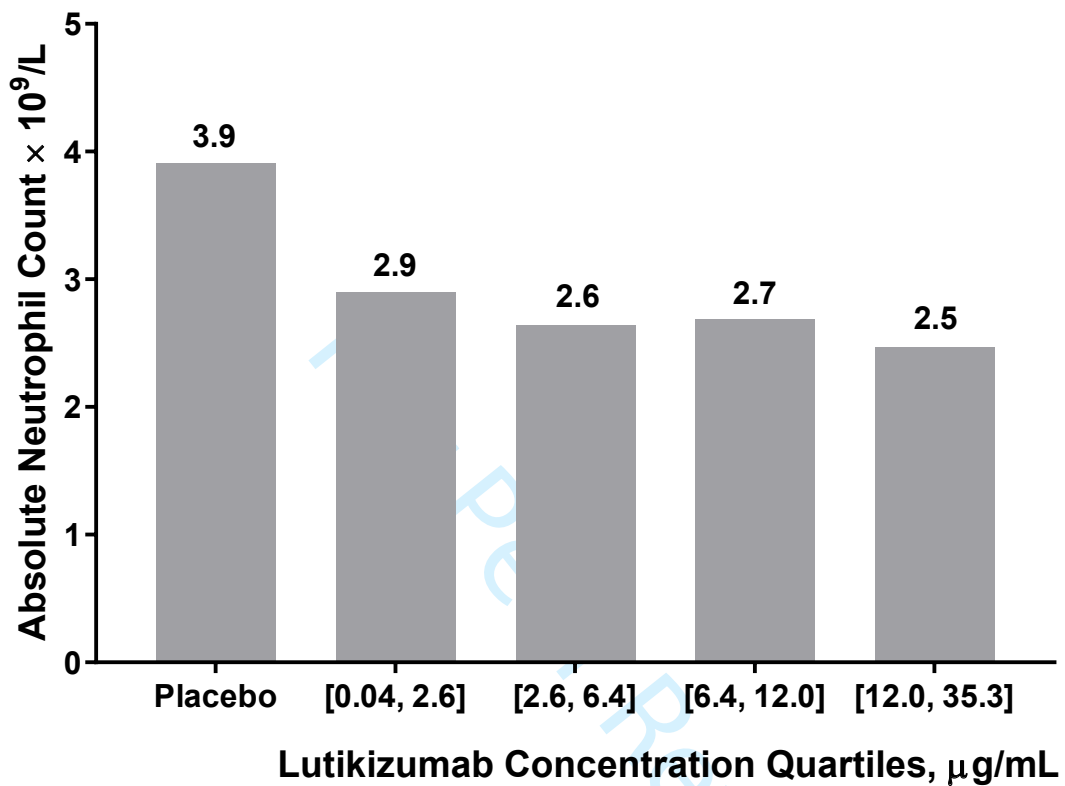
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Supplemental Figure $\underline{5}$3. Mean (SD) trough serum concentrations of lutikizumab over time for all subjects **(A)** and separated by presence of ADAs **(B)**. ADA, anti-drug antibody; Q2W, every 2 weeks.
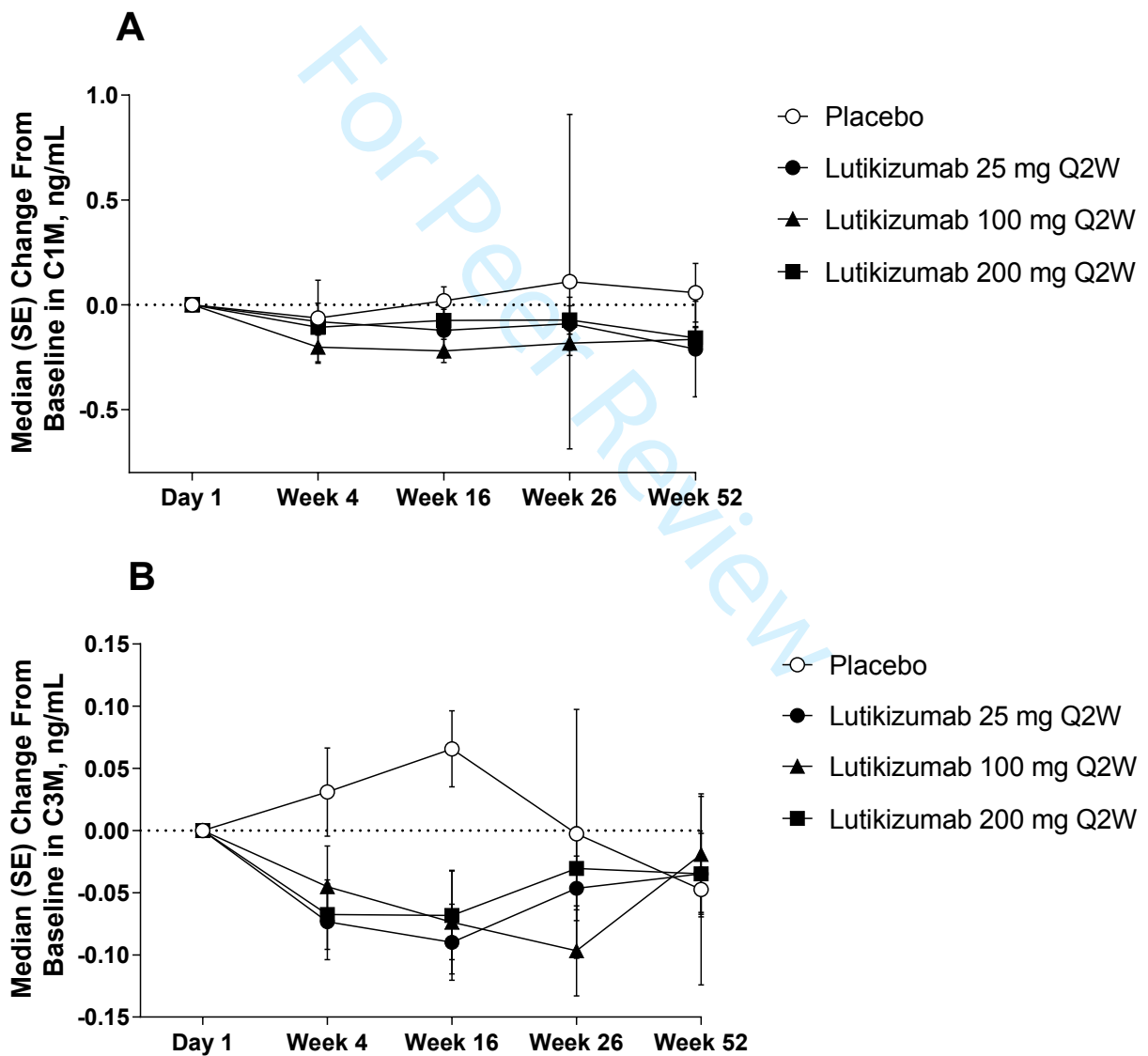
Supplemental Figure <u>64</u>. Median absolute neutrophil counts for placebo and lutikizumab serum concentration quartiles at week 52 (observed cases).
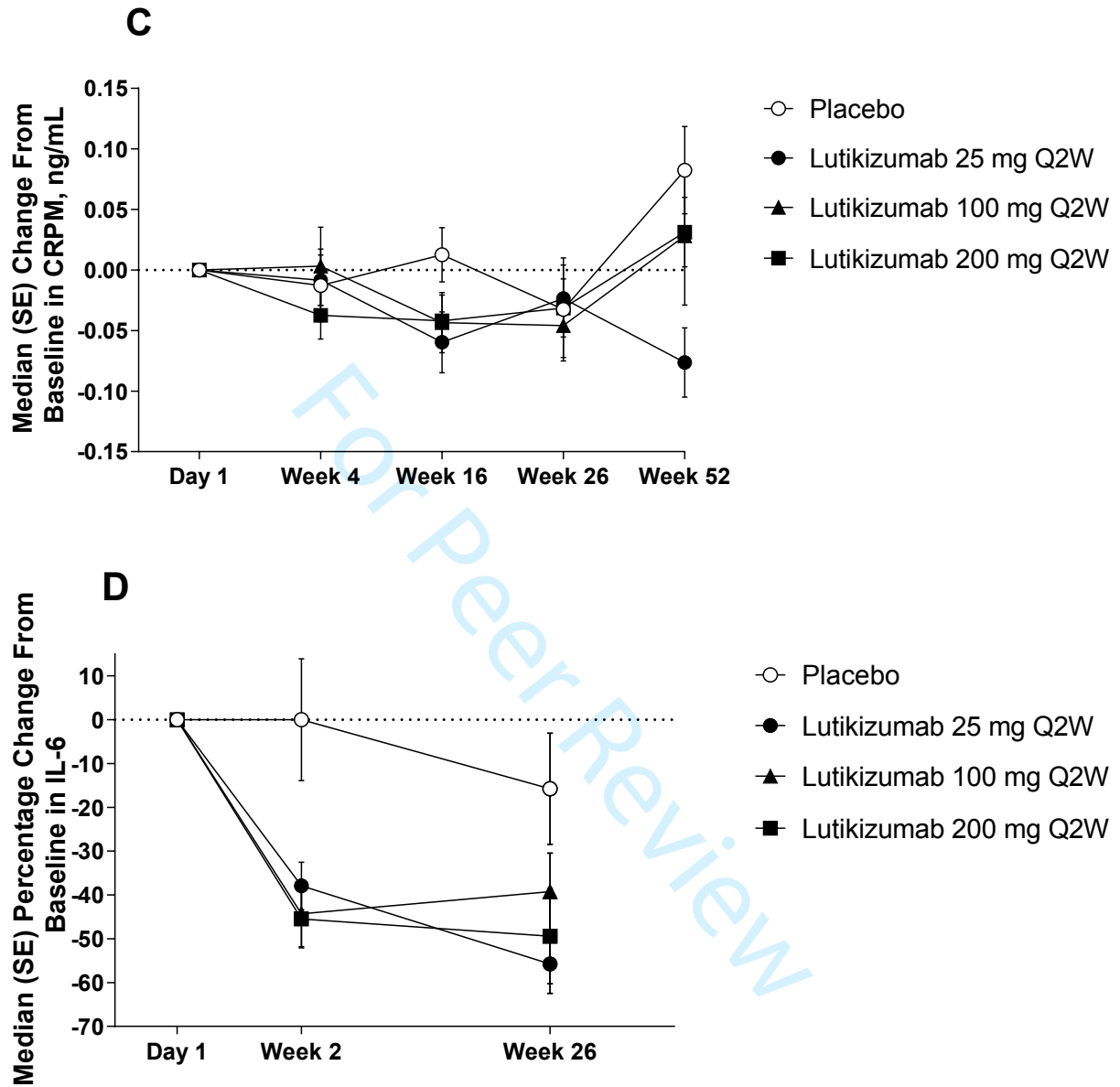
Supplemental Figure 75. Change From Day 1 in Serum IL-1 Levels at 2 Weeks After Initiating Treatment With Placebo or Lutikizumab. IL-1α=interleukin-1α; Q2W=every 2 weeks.
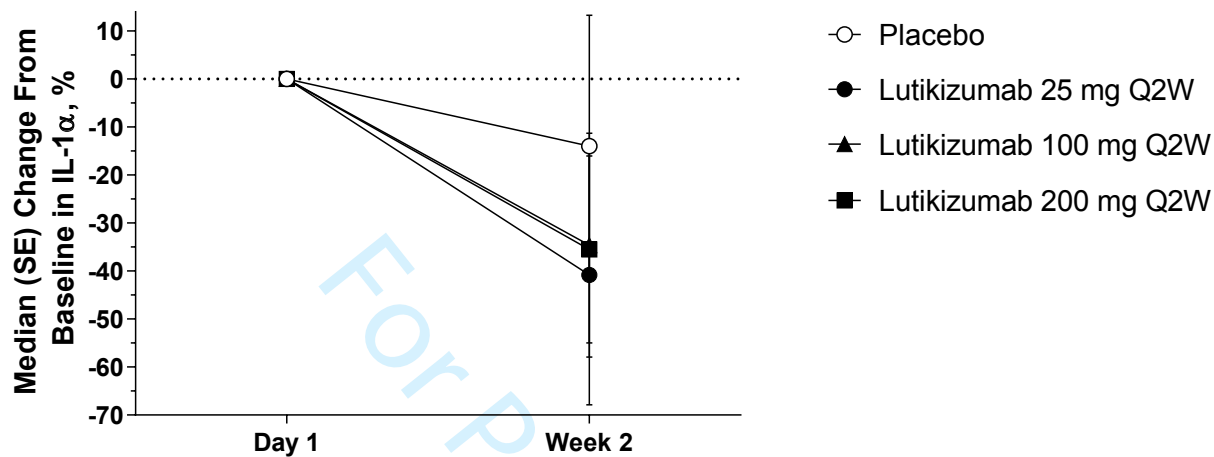
Supplemental Figure 86. Median change from baseline over time for the biomarkers C1M **(A)**, C3M **(B)**, and CRPM **(C)**, median percentage change from baseline over time in IL-6 **(D)**, and mean change from baseline over time in alkaline phosphatase **(E)**. C1M, metalloproteinase-degraded collagen type I; C3M, metalloproteinase-degraded collagen type III; CRPM, matrix metalloproteinase-generated fragment of C-reactive protein; IL-6, interleukin-6; Q2W, every 2 weeks.

Supplemental Figure 6̶8̶ continued.

C

D

Supplemental Figure 6̶8 continued.

E

Mean (SE) Change From Baseline in Alkaline Phosphatase, U/L

Weeks

- ○ Placebo
- ● Lutikizumab 25 mg Q2W
- ▲ Lutikizumab 100 mg Q2W
- ■ Lutikizumab 200 mg Q2W