

A Randomized, Double-Blind, Placebo-Controlled, Phase 3 Trial Evaluating the Efficacy of Burosumab, an Anti-FGF23 Antibody, in Adults With X-Linked Hypophosphatemia: Week 24 Primary Analysis

Karl L Insogna,¹ Karine Briot,² Erik A Imel,³ Peter Kamenický,⁴ Mary D Ruppe,^{5*} Anthony A Portale,⁶ Thomas Weber,⁷ Pisit Pitukcheewanont,⁸ Hae Il Cheong,⁹ Suzanne Jan de Beur,¹⁰ Yasuo Imanishi,¹¹ Nobuaki Ito,¹² Robin H Lachmann,¹³ Hiroyuki Tanaka,¹⁴ Farzana Perwad,⁶ Lin Zhang,¹⁵ Chao-Yin Chen,¹⁵ Christina Theodore-Oklota,¹⁵ Matt Mealiffe,¹⁵ Javier San Martin,¹⁵ and Thomas O Carpenter¹ on behalf of the AXLES 1 Investigators

¹Yale School of Medicine, New Haven, CT, USA

²Centre d'Evaluation des Maladies Osseuses, Hôpital Cochin, Paris, France

³Indiana University School of Medicine, Indianapolis, IN, USA

⁴Université Paris-Sud, Le Kremlin Bicêtre, France

⁵Houston Methodist Hospital, Houston, TX, USA

⁶University of California, San Francisco, CA, USA

⁷Duke University Medical Center, Durham, NC, USA

⁸Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA, USA

⁹Seoul National University Children's Hospital, Seoul, South Korea

¹⁰Johns Hopkins University School of Medicine, Baltimore, MD, USA

¹¹Osaka City University Graduate School of Medicine, Osaka, Japan

¹²University of Tokyo Hospital, Tokyo, Japan

¹³University College London Hospitals, London, UK

¹⁴Okayama Saiseikai General Hospital, Okayama, Japan

¹⁵Ultragenyx Pharmaceutical Inc., Novato, CA, USA

ABSTRACT

In X-linked hypophosphatemia (XLH), inherited loss-of-function mutations in the *PHEX* gene cause excess circulating levels of fibroblast growth factor 23 (FGF23), leading to lifelong renal phosphate wasting and hypophosphatemia. Adults with XLH present with chronic musculoskeletal pain and stiffness, short stature, lower limb deformities, fractures, and pseudofractures due to osteomalacia, accelerated osteoarthritis, dental abscesses, and enthesopathy. Burosumab, a fully human monoclonal antibody, binds and inhibits FGF23 to correct hypophosphatemia. This report summarizes results from a double-blind, placebo-controlled, phase 3 trial of burosumab in symptomatic adults with XLH. Participants with hypophosphatemia and pain were assigned 1:1 to burosumab 1 mg/kg ($n = 68$) or placebo ($n = 66$) subcutaneously every 4 weeks (Q4W) and were comparable at baseline. Across midpoints of dosing intervals, 94.1% of burosumab-treated participants attained mean serum phosphate concentration above the lower limit of normal compared with 7.6% of those receiving placebo ($p < 0.001$). Burosumab significantly reduced the Western Ontario and the McMaster Universities Osteoarthritis Index (WOMAC) stiffness subscale compared with placebo (least squares [LS] mean \pm standard error [SE] difference, -8.1 ± 3.24 ; $p = 0.012$). Reductions in WOMAC physical function subscale (-4.9 ± 2.48 ; $p = 0.048$) and Brief Pain Inventory worst pain (-0.5 ± 0.28 ; $p = 0.092$) did not achieve statistical significance after Hochberg multiplicity adjustment. At week 24, 43.1% (burosumab) and 7.7% (placebo) of baseline active fractures were fully healed; the odds of healed fracture in the burosumab group was 16.8-fold greater than that in the placebo group ($p < 0.001$). Biochemical markers of bone formation and resorption increased significantly from baseline with burosumab treatment compared with placebo. The safety

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Address correspondence to: Karl L Insogna, MD, Department of Medicine, Endocrine Section, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8020, USA. E-mail: karl.insogna@yale.edu

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profile of burosumab was similar to placebo. There were no treatment-related serious adverse events or meaningful changes from baseline in serum or urine calcium, intact parathyroid hormone, or nephrocalcinosis. These data support the conclusion that burosumab is a novel therapeutic addressing an important medical need in adults with XLH. © 2018 The Authors. *Journal of Bone and Mineral Research* Published by Wiley Periodicals, Inc.

KEY WORDS: BUROSUMAB; FGF23; X-LINKED HYPOPHOSPHATEMIA (XLH); OSTEOMALACIA; VITAMIN D

Introduction

X-linked hypophosphatemia (XLH) is characterized by lifelong hypophosphatemia caused by loss-of-function mutations in the *PHEX* gene (phosphate-regulating gene with homologies to endopeptidases on the X chromosome)⁽¹⁾ that result in excess circulating levels of fibroblast growth factor 23 (FGF23).⁽²⁾ Predominantly synthesized by osteocytes, FGF23 functions in the kidney as a critical regulator of phosphate and vitamin D homeostasis.^(3,4) Excess circulating levels of FGF23 in patients with XLH lead to impaired renal phosphate reabsorption and consequent hypophosphatemia. FGF23 inhibits the enzyme CYP27B1 and stimulates CYP24A1, thereby reducing circulating levels of 1,25-dihydroxyvitamin D (1,25(OH)₂D), the active metabolite of vitamin D.⁽⁵⁾

XLH is a disabling disease with a significant musculoskeletal morbidity. Chronic hypophosphatemia results in defective bone mineralization that in adults is manifested by persistent osteomalacia, leading to impaired bone turnover and poor bone quality.⁽⁶⁾ Adults with XLH are prone to pseudofractures and fractures due to a combination of osteomalacia and skeletal deformities resulting from rickets in childhood that expose long bones to abnormal biomechanical stress.^(7,8) In addition, many patients have early and accelerated osteoarthritis in weight-bearing joints, lower limb deformities, acquired joint malalignment, enthesopathy, and muscle function deficits.^(8–10) These musculoskeletal abnormalities are prominent and debilitating features in adults with XLH that cause pain and stiffness, impairing mobility and physical function, and reducing health-related quality of life.⁽¹¹⁾

No current therapy for XLH specifically addresses the underlying pathophysiology: elevated circulating concentrations of bioactive FGF23. Symptomatic adults with XLH may be treated with supplemental oral phosphate and active vitamin D metabolites or analogs, but treatment is burdensome, adherence is difficult, and not infrequently is associated with adverse side effects. Although there are no randomized controlled trials of phosphate and vitamin D metabolites in adults with XLH, some observational studies have reported benefit from conventional therapy with reduction in pain and improvement in osteomalacia in adults.^(7,12,13) Long-term complications from conventional therapy, particularly nephrocalcinosis, hyperparathyroidism, and chronic kidney disease, appear to increase with dose and duration of treatment,⁽¹⁴⁾ and serum FGF23 levels are higher in treated versus untreated adults.⁽¹⁵⁾ Thus, the benefit/risk profile for long-term conventional therapy in adults with XLH is uncertain.

Burosumab (previously KRN23) is a recombinant, fully human IgG1 monoclonal antibody that inhibits FGF23 activity, thereby directly addressing the pathologic mechanism of hypophosphatemia in XLH.^(16,17) In phase 1 and phase 2 studies in adults with XLH, burosumab doses of up to 1.0 mg/kg every 4 weeks (Q4W) increased renal phosphate reabsorption, thereby increasing serum phosphate and circulating levels of 1,25(OH)₂D.^(16–18) This report summarizes the key results from a phase 3 trial evaluating the efficacy and safety of burosumab in adults with XLH.

Participants and Methods

Study design

This randomized, double-blind, placebo-controlled, phase 3 trial was conducted at 25 centers in the United States, France, United Kingdom, Ireland, Italy, Japan, and South Korea. Eligible participants were randomized 1:1 to receive burosumab or placebo administered subcutaneously (sc) every 4 weeks for 24 weeks. Per protocol, randomization was to be stratified based on mean Brief Pain Inventory (BPI) worst pain score for the 7 days preceding the baseline visit. However, because of an error, BPI average pain data instead of BPI worst pain data were used to stratify randomization in the interactive web response system. The BPI worst pain score was highly correlated with the average pain score and had minimal impact on the study results. Randomization was also stratified by region (North America/European Union, Japan, or South Korea). After completing all double-blind assessments at week 24, subjects entered a treatment continuation period in which all subjects received burosumab. This report includes the results for the double-blind period at the time of the database lock of this ongoing study. A sample size of 60 per group (total sample size of 120) was determined to provide >95% power to detect a 50% difference between groups in the proportion of subjects achieving the primary efficacy endpoint.

Participants

Eligible participants were adults between 18 and 65 years of age with a diagnosis of XLH supported by a confirmed *PHEX* mutation (self or family member consistent with X-linked inheritance) and/or prespecified clinical findings and laboratory features: serum phosphate concentration below the lower limit of normal (LLN; <2.5 mg/dL [0.81 mmol/L]), ratio of renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) of <2.5 mg/dL, and a BPI worst pain score of ≥4. If a participant was receiving pain medication, the regimen had to be stable for ≥21 days before screening, and the participant must have been willing to maintain the same dose (maximum of 60 mg/d oral morphine equivalents) and schedule during the double-blind period.

The full list of inclusion criteria and exclusion criteria is provided in Supplemental Methods. Key exclusion criteria included corrected serum calcium ≥10.8 mg/dL (2.7 mmol/L), serum intact parathyroid hormone (iPTH) ≥2.5-fold the upper limit of normal (ULN), and/or use of medication to suppress parathyroid hormone within 60 days before screening; and a recent history (≤6 months) of traumatic fracture or orthopedic surgery. Subjects receiving therapies affecting phosphorus metabolism (eg, oral phosphate, active vitamin D metabolites, or analogs) could enroll only after a wash-out period of at least 2 weeks. If serum 25-hydroxyvitamin D (25(OH)D) levels fell below 20 ng/mL during the study, oral supplementation (eg, cholecalciferol, ergocalciferol) could be provided. If a study participant's condition deteriorated

during the study, then the investigator could withdraw the study participant and start treatment with oral phosphate and/or active vitamin D.

This study was designed, conducted, recorded, and reported in accordance with the principles established by the World Medical Association Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects. The Institutional Review Board or Ethics Committee for each site approved the study protocol. Investigators obtained written informed consent from each study participant. The clinical trial was registered as NCT02526160.

Treatments

Participants received either burosumab 1.0 mg/kg or matching placebo, administered Q4W. Each dose was rounded to the nearest 10 mg, up to a maximum of 90 mg. Participants received study drug via sc injection to the abdomen, upper arms, or thighs; the site was rotated with each injection. The concentration of study drug was 30 mg/mL and the maximum volume per injection was 1.5 mL (45 mg).

Treatment assignment was unblinded and the absolute dose of burosumab was reduced by half if the serum phosphate concentration was >5.0 mg/dL (1.61 mmol/L) at any time or if two sequential serum phosphate concentrations were between 4.5 and 5.0 mg/dL. After a dose was reduced, the investigator and the medical monitor determined any further adjustment.

Outcomes

For a detailed description of outcome measurements, refer to the Supplemental Methods. Clinical laboratory tests for efficacy were fasting serum phosphate and serum $1,25(\text{OH})_2\text{D}$. TmP/GFR was calculated from fasting blood and urine measurements. Biochemical markers of bone remodeling were procollagen type 1 N-propeptide (P1NP), carboxy-terminal cross-linked telopeptide of type I collagen (CTX), and bone-specific alkaline phosphatase (BALP). Patient-reported outcomes were assessed using the short-form BPI questionnaire⁽¹⁹⁾ and the full Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) questionnaire, designed for the assessment of lower-extremity pain and function in osteoarthritis of the knee or hip.⁽²⁰⁾ Mobility was assessed with the 6-minute walk test.⁽²¹⁾ Active (unhealed) bone fractures, including pseudo-fractures, were identified at baseline by a complete radiographic skeletal survey. If an active fracture was detected, follow-up X-rays of that fracture were obtained post-baseline to assess healing. Safety measurements included the incidence and severity of adverse events and serious adverse events, development of anti-burosumab antibodies, results of echocardiograms and electrocardiograms (ECG), renal ultrasound nephrocalcinosis scores, fasting serum calcium, plasma iPTH, and 24-hour urine calcium excretion.

Statistical analysis

Statistical analyses were conducted in SAS v9.4 or higher (SAS Institute, Cary, NC, USA). Continuous data were summarized as mean and standard deviation (SD) (or standard error [SE], as noted), and categorical data were summarized as n (%). The primary efficacy endpoint was the proportion of subjects achieving a mean serum phosphate concentration above the LLN of 2.5 mg/dL (0.81 mmol/L); a single value for this endpoint was calculated as the average of values at the midpoints of the

Q4W dosing intervals (ie, at weeks 2, 6, 10, 14, 18, and 22). The primary efficacy endpoint was analyzed using the Cochran-Mantel-Haenszel test, adjusting for randomization stratification factors (BPI average pain and region), tested at the two-sided alpha level of 0.05.

Key secondary efficacy endpoints were the changes from baseline to week 24 in patient-reported outcomes: BPI worst pain score, WOMAC stiffness subscale score, and WOMAC physical function subscale score. If the test of the primary efficacy endpoint was statistically significant, the key secondary efficacy endpoints were then analyzed as a group at an alpha level of 0.05 using a generalized estimating equation (GEE) repeated-measures analysis, with the Hochberg adjustment applied for multiple testing. The model included treatment, actual randomization stratification factor based on BPI average pain (except the model for BPI worst pain), region, visit, and interaction of treatment-by-visit as fixed factors, adjusted for baseline measurements. The covariance structure used for the model is compound symmetry, which specifies constant variance for the assessments and constant covariance between the assessments over time. Sensitivity analyses for the primary and key secondary efficacy endpoints were performed using the planned randomization stratification factors in the models instead of the actual randomization stratification factors.

Analyses of other continuous efficacy endpoints over time used similar GEE methods. For the fracture analysis, a generalized linear mixed model was used for binomial distribution with the logit link function that included treatment, visit, treatment-by-visit, and fracture type as fixed factors, accounting for nesting of fractures within subjects.

Safety endpoints included the participant incidence of any treatment-emergent adverse events and treatment-emergent adverse events of interest in the following categories: injection site reactions, hypersensitivity, hyperphosphatemia (when reported by the investigator as an adverse event), ectopic mineralization, and restless legs syndrome. The following safety endpoints were analyzed at week 24: echocardiographic parameters (left ventricular mass index and left ventricular ejection fraction; ectopic mineralization), ECG results, and changes from baseline in renal ultrasound nephrocalcinosis score. Other safety endpoints were the development of new anti-burosumab antibodies at week 4 or 24, and changes in serum or 24-hour urine calcium excretion and iPTH concentration.

Results

Participants and treatment

Of the 163 participants who were screened, 134 were randomly assigned to receive burosumab ($n = 68$) or placebo ($n = 66$); 133 participants completed the 24-week double-blind period, and 1 burosumab-treated participant withdrew consent after approximately 6 months of study treatment (Supplemental Fig. S1). Actual randomization stratification based on BPI average pain and planned randomization stratification based on BPI worst pain were highly correlated (Pearson correlation = 0.82, $p < 0.001$).

Baseline characteristics were similar between the burosumab and placebo groups (Table 1). Overall, 64.9% of participants were female, which is consistent with XLH being an X-linked dominant disorder, and 80.6% were white. Sex-specific percentile for height was $6.8\% \pm 12.5\%$, indicating impaired stature in XLH. Overall, *PHEX* mutations were pathogenic for 70.9% of

Table 1. Baseline Demographics and Characteristics

	Placebo (n = 66)	Burosumab (n = 68)	Total (n = 134)
Age (years)			
Mean ± SD	38.7 ± 12.8	41.3 ± 11.6	40.0 ± 12.2
Range	18.5–65.5	20.0–63.4	18.5–65.5
Female, n (%)	43 (65.2)	44 (64.7)	87 (64.9)
Race, n (%)			
White	53 (80.3)	55 (80.9)	108 (80.6)
Asian	9 (13.6)	12 (17.6)	21 (15.7)
Black	3 (4.5)	0	3 (2.2)
Other	1 (1.5)	1 (1.5)	2 (1.5)
Geographic region, n (%)			
North America/Europe	58 (87.9)	58 (85.3)	116 (86.6)
Japan	5 (7.6)	6 (8.8)	11 (8.2)
South Korea	3 (4.5)	4 (5.9)	7 (5.2)
Height, ^a mean ± SD			
Centimeters	153 ± 11.8	152 ± 9.5	152 ± 10.7
Z-score ^b	−2.3 ± 1.3	−2.3 ± 1.2	−2.3 ± 1.3
Percentile	7.2 ± 12.1	6.4 ± 12.9	6.8 ± 12.5
Body mass index ^a (kg/m ²), mean ± SD	30.6 ± 7.8	30.0 ± 7.5	30.3 ± 7.6
PHEX mutation, n (%)			
Pathogenic	50 (75.8)	45 (66.2)	95 (70.9)
Likely pathogenic	7 (10.6)	8 (11.8)	15 (11.2)
Variant of uncertain significance	8 (12.1)	9 (13.2)	17 (12.7)
No mutation	1 (1.5)	6 (8.8)	7 (5.2)
Serum phosphate (mg/dL), ^c mean ± SD	1.9 ± 0.32	2.0 ± 0.30	2.0 ± 0.31
TmP/GFR (mg/dL), ^c mean ± SD	1.6 ± 0.37	1.7 ± 0.40	1.6 ± 0.39
Serum 1,25(OH) ₂ D (pg/mL), ^c mean ± SD	33.5 ± 15.6	32.4 ± 13.0	33.0 ± 14.3
Serum calcium (mg/dL), ^c mean ± SD	9.1 ± 0.41	9.2 ± 0.49	9.2 ± 0.45
Serum iPTH (pg/mL), ^c mean ± SD	95.2 ± 38.8	98.9 ± 60.8	97.0 ± 50.9
Conventional therapy ever, n (%)			
Phosphate + vitamin D metabolites or analogs	62 (93.9)	59 (86.8)	121 (90.3)
Phosphate alone	1 (1.5)	3 (4.4)	4 (3.0)
Vitamin D metabolites or analogs alone	3 (4.5)	3 (4.4)	6 (4.5)
Conventional therapy before age 18 years, n (%)			
Phosphate + vitamin D metabolites or analogs	48 (72.7)	45 (66.2)	93 (69.4)
Phosphate alone	2 (3.0)	5 (7.4)	7 (5.2)
Vitamin D metabolites or analogs alone	4 (6.1)	5 (7.4)	9 (6.7)
Conventional therapy duration (years), mean ± SD			
Phosphate ^d	16.2 ± 10.2	16.8 ± 10.7	16.5 ± 10.4
Vitamin D metabolites or analogs ^e	17.5 ± 11.9	19.0 ± 10.0	18.2 ± 11.0
BPI worst pain >6.0, n (%)	43 (65.2)	53 (77.9)	96 (71.6)
Any pain medication at baseline, n (%)	44 (66.7)	47 (69.1)	91 (67.9)
Any opioid at baseline, n (%)	13 (19.7)	17 (25.0)	30 (22.4)
Enthesopathy on X-ray, n (%)	65 (98.5)	68 (100.0)	133 (99.3)
Nephrocalcinosis score >0, ^f n (%)	39 (59.1)	34 (50.0)	73 (54.5)
Medical history, n (%)			
Orthopedic surgery	47 (71.2)	45 (66.2)	92 (68.7)
Osteoarthritis	38 (57.6)	47 (69.1)	85 (63.4)

Data are mean ± SD or n (%).

TmP/GFR = ratio of renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate; iPTH = intact parathyroid hormone; BPI = Brief Pain Inventory.

^aHeight and body mass index were not recorded at baseline for 1 participant in each group.

^bZ-score adjusted for sex.

^cNormal ranges: phosphate, 2.5–4.5 mg/dL; 1,25(OH)₂D, 18–72 pg/mL; calcium, 8.6–10.2 mg/dL; iPTH, 14–72 pg/mL; TmP/GFR, 2.5–4.2 mg/dL.

^dAmong participants with any prior use of phosphate (n = 63 placebo; n = 62 burosumab).

^eAmong participants with any prior use of vitamin D metabolites or analogs (n = 65 placebo; n = 62 burosumab).

^fOn a 5-point scale; see Supplemental Methods for additional details.

participants, a variant of uncertain significance for 12.7% of participants, and likely pathogenic for 11.2% of participants. Seven (5.2%) participants had no identified *PHEX* mutation.

Overall, 68.7% of participants had undergone orthopedic surgery and 63.4% had a history of osteoarthritis. Enthesopathy was present on X-ray in nearly all participants (99.3%). Before age 18 years, most participants had received conventional therapy with oral phosphate salts in combination with an active vitamin D metabolite or analog (69.4%), phosphate alone (5.2%), or active vitamin D metabolite or analog alone (6.7%). The lifetime duration of conventional therapy (childhood plus adulthood) was 16.5 ± 10.4 years for phosphate and 18.2 ± 11.0 years for vitamin D metabolites or analogs. More than half of the participants (54.5%) had evidence of nephrocalcinosis (score >0) on baseline renal ultrasound. Most participants had severe pain (71.6%; BPI worst pain >6.0) and most were using analgesics (67.9%), including opioids in 22.4% of participants.

At baseline, serum phosphate concentration was 2.0 ± 0.31 mg/dL (0.64 ± 0.10 mmol/L) (normal range, 2.5–4.5 mg/dL [0.81–1.45 mmol/L]), TmP/GFR was 1.6 ± 0.39 mg/dL (0.52 ± 0.13 mmol/L) (normal range, 2.5–4.2 mg/dL [0.81–1.36 mmol/L]), and serum 1,25(OH)₂D concentration was 33.0 ± 14.3 pg/mL (85.8 ± 37.1 pmol/L) (normal range, 18–72 pg/mL [46.8–187.2 mmol/L]).

Most participants received study treatment at the initial dose of 1.0 mg/kg (up to 90 mg maximum total dose) without dose adjustment. In the burosumab group, 5 (7.4%) participants required protocol-specified dose reduction due to high serum phosphate; 1 participant required two dose reductions. After dose reduction(s), serum phosphate normalized rapidly.

Efficacy

A significantly greater percentage of participants in the burosumab group than in the placebo group (94.1% versus 7.6%; $p < 0.001$) achieved a mean serum phosphate concentration above the LLN averaged across the midpoints between monthly doses, which was the primary efficacy endpoint. The odds ratios were consistent ($p = 0.904$ by Breslow-Day test) across the strata by the randomization stratification factors for BPI average pain and region. A greater percentage of participants in the burosumab group than in the placebo group (67.6% versus 6.1%) maintained a mean serum phosphate concentration above the LLN just before the next dose. In the burosumab group, the mean serum phosphate concentration was within the normal range at both the midpoint (Fig. 1A) and the end (Fig. 1B) of the monthly dosing intervals.

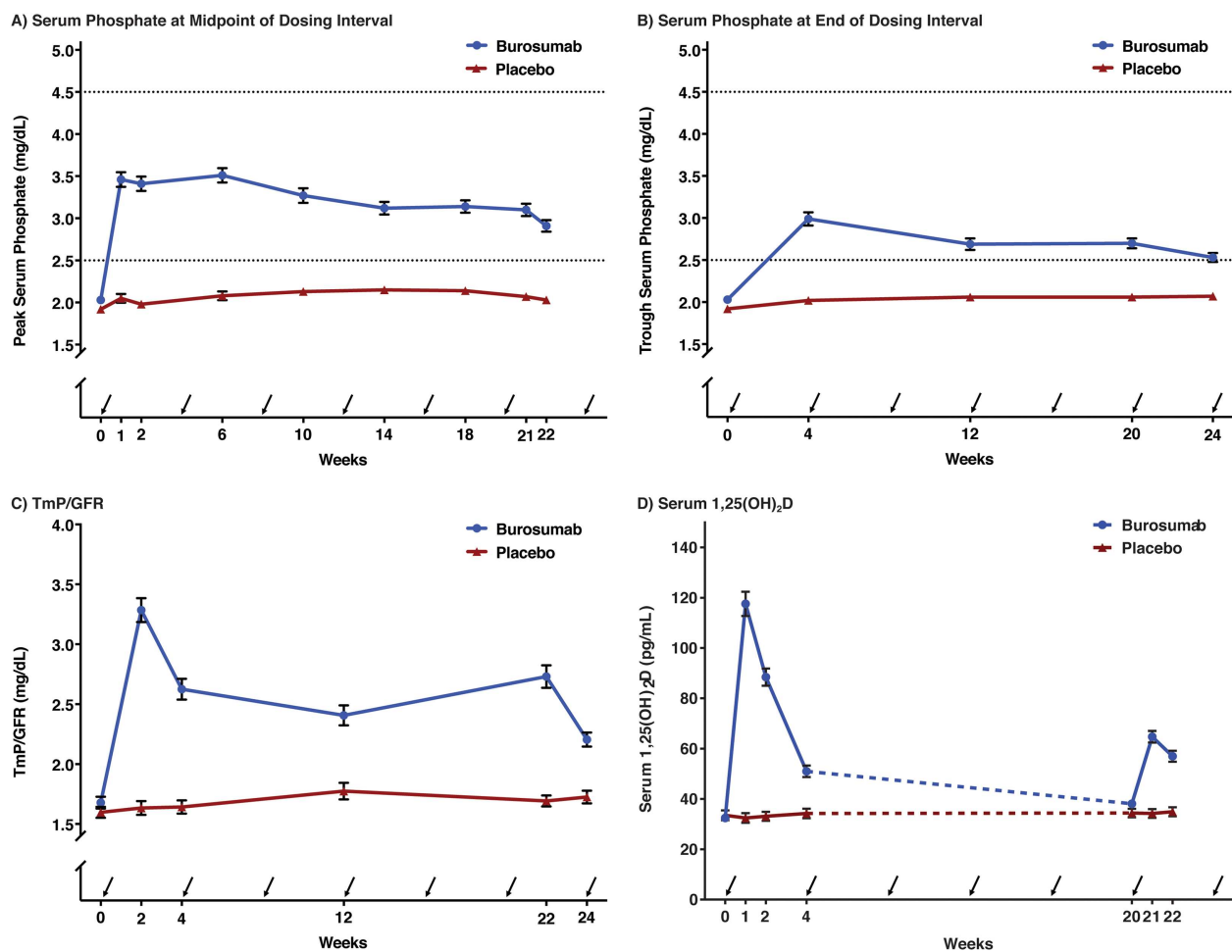


Fig 1. Effect of burosumab on pharmacodynamic markers. Data are expressed as mean \pm standard error. The dashed lines in panels A and B are the lower and upper limits of normal. Arrows indicate administration of study drug (burosumab or placebo). 1,25(OH)₂D = 1,25 dihydroxyvitamin D; TmP/GFR = ratio of the maximum rate of tubular phosphate reabsorption to the glomerular filtration rate.

In the burosumab group, Tmp/GFR (Fig. 1C) increased from 1.7 ± 0.40 mg/dL at baseline to 2.7 ± 0.75 mg/dL at week 22 (peak effect) and 2.2 ± 0.48 mg/dL at week 24 (trough effect), but showed minimal change in the placebo group, being 1.6 ± 0.37 mg/dL at baseline, 1.7 ± 0.37 mg/dL at week 22, and 1.7 ± 0.42 mg/dL at week 24. The least squares (LS) mean \pm SE difference of 0.43 ± 0.067 mg/dL between treatment groups for the change from baseline to week 24 was statistically significant ($p < 0.001$).

Burosumab also increased serum $1,25(\text{OH})_2\text{D}$ significantly compared with placebo (Fig. 1D). In the burosumab group, the serum $1,25(\text{OH})_2\text{D}$ concentration increased from 32.4 ± 12.96 pg/mL at baseline to 57.0 ± 18.02 pg/mL at week 22; the LS mean \pm SE change from baseline was 25.5 ± 3.52 pg/mL ($100.9\% \pm 17.84\%$). In the placebo group, serum $1,25(\text{OH})_2\text{D}$ concentration was 33.5 ± 15.61 pg/mL at baseline and 34.9 ± 14.52 pg/mL at week 22; the LS mean \pm SE change from baseline was 2.7 ± 2.82 pg/mL ($19.3\% \pm 14.33\%$). The LS mean \pm SE difference between groups for change from baseline was 22.7 ± 2.40 pg/mL ($81.6\% \pm 11.67\%$; $p < 0.001$). Serum $25(\text{OH})\text{D}$ did not change notably in either treatment group. In the burosumab group, the serum $25(\text{OH})\text{D}$ concentration was 26.2 ± 9.45 ng/mL at baseline and 25.5 ± 11.24 ng/mL at week 24. In the placebo group, the serum $25(\text{OH})\text{D}$ concentration was 26.2 ± 10.45 ng/mL at baseline and 25.1 ± 12.07 ng/mL at week 24.

Over the 24 weeks of study, burosumab was associated with improvement in the key secondary endpoints of BPI worst pain (Fig. 2A), WOMAC physical function subscale (Fig. 2B), and WOMAC stiffness subscale (Fig. 2C) when compared with the placebo-treated group. Burosumab significantly reduced the WOMAC stiffness subscale score at week 24 relative to placebo (LS mean \pm SE difference, -8.1 ± 3.24 ; $p = 0.012$) when tested at the significance level of 0.0167 required with Hochberg adjustment. Differences favoring burosumab over placebo for WOMAC physical function subscale score (LS mean \pm SE difference, -4.9 ± 2.48 ; $p = 0.048$) and reduction in BPI worst pain score (LS mean \pm SE difference, -0.5 ± 0.28 ; $p = 0.092$) at week 24 did not achieve the significance levels of 0.025 and 0.05, respectively, required with Hochberg adjustment. No meaningful changes from baseline were observed for the 6-minute walk test in either group (Supplemental Table S1).

Burosumab was associated with significantly greater fracture healing than placebo. At baseline, 32 (47.1%) subjects in the burosumab group had 65 active fractures (including fractures and pseudofractures) and 38 (57.6%) subjects in the placebo group had 91 active fractures. At week 24, a greater percentage of baseline active fractures were fully healed in the burosumab group than in the placebo group (43.1% versus 7.7%, respectively) (Fig. 3A). The odds of full healing at week 24 was 16.8-fold greater in the burosumab group than in the placebo

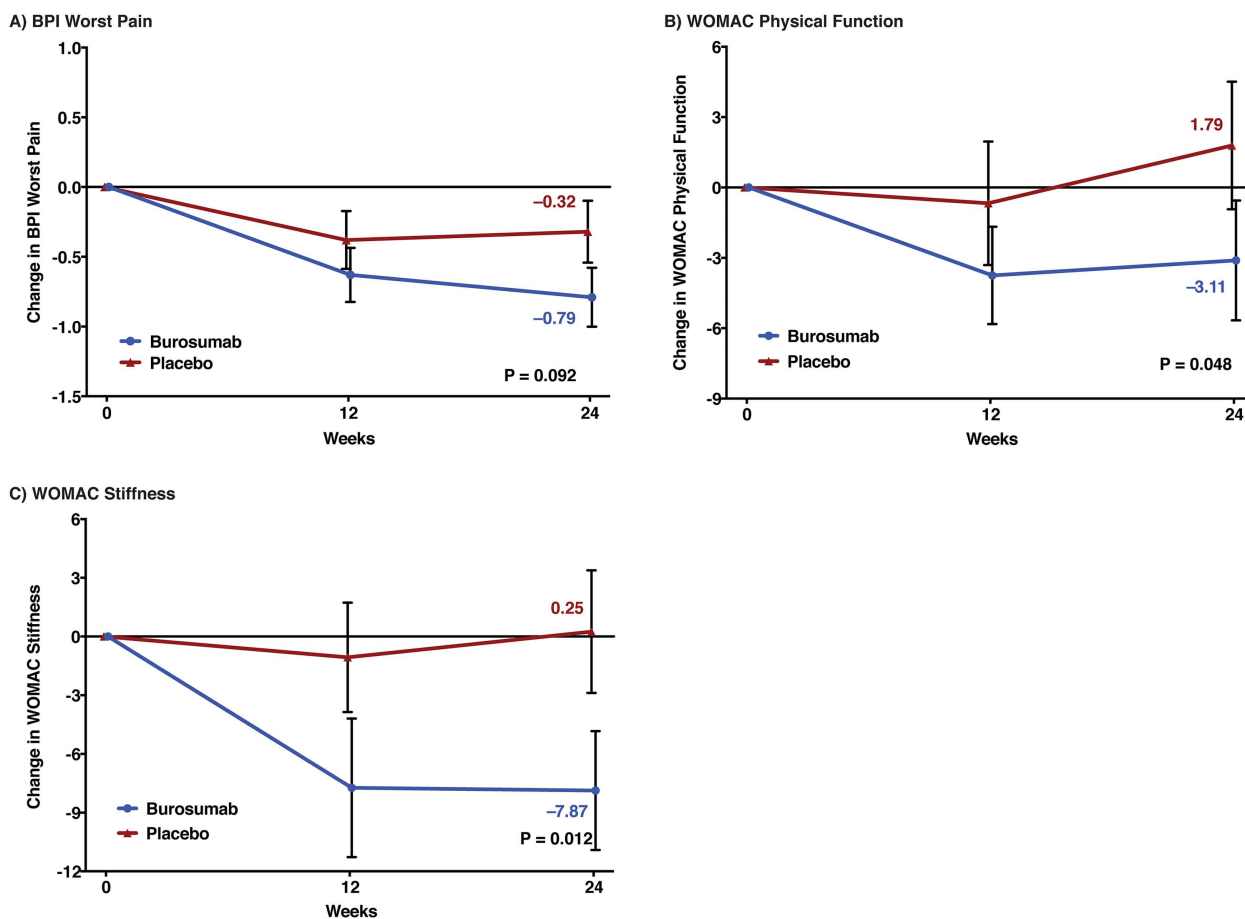


Fig 2. Effect of burosumab on participant-reported outcomes. Data are expressed as least squares mean change from baseline \pm standard error. BPI = Brief Pain Inventory; WOMAC = Western Ontario McMaster Universities Osteoarthritis Index.

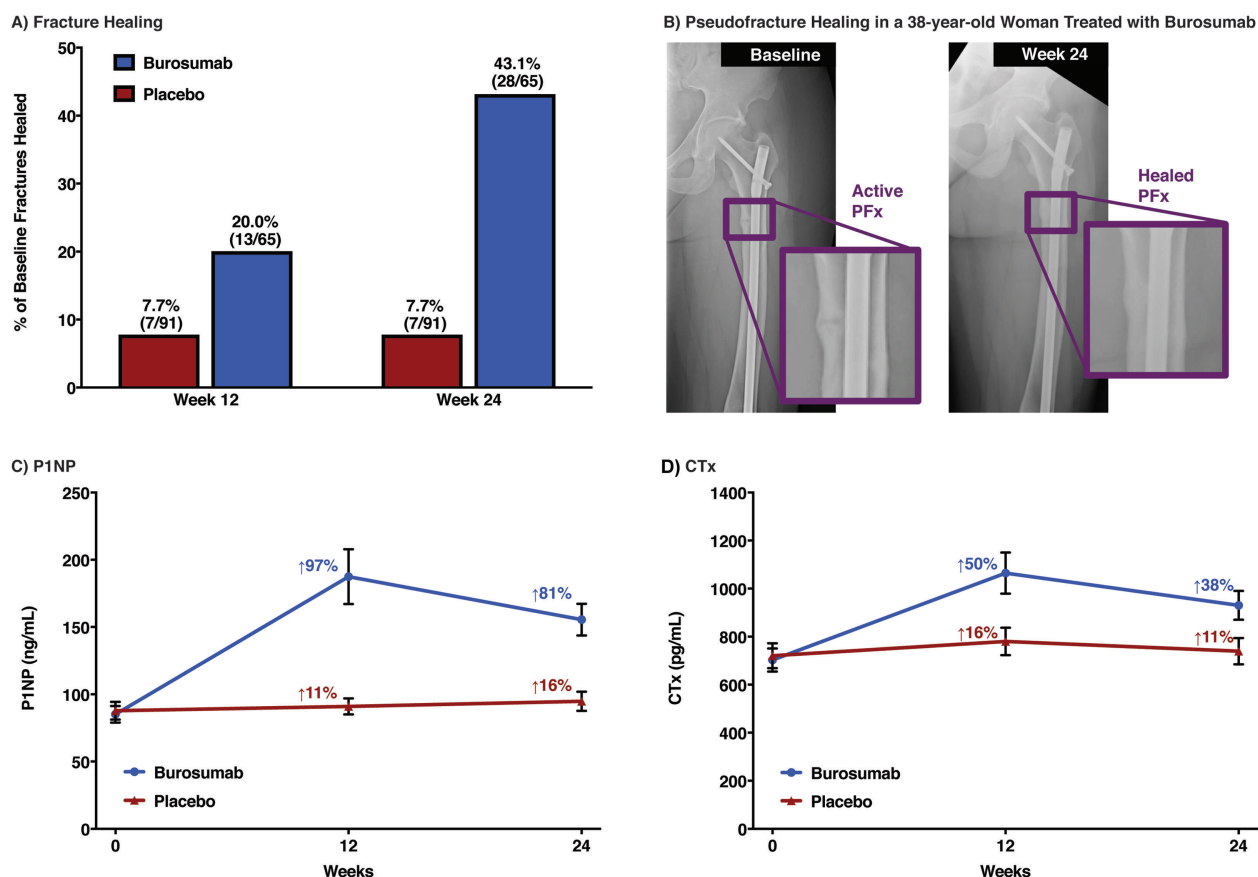


Fig 3. Effect of burosumab on fracture/pseudofracture healing and bone turnover markers. In *A*, the numbers in parentheses are total number of fractures or pseudofractures healed/total number of fractures or pseudofractures identified at baseline. (*B*) Complete healing of a pseudofracture after 24 weeks of burosumab treatment. Data in *C* and *D* are expressed as mean \pm standard error, with the percentage changes from baseline; CTx = carboxy terminal cross-linked telopeptide of type I collagen; P1NP = procollagen type 1 N-propeptide.

group ($p < 0.001$). Sample radiographs are provided for a subject with a fully healed pseudofracture at week 24 of burosumab treatment (Fig. 3B).

Compared with baseline values, serum P1NP (Fig. 3C), a marker of bone formation, increased by 81%, and serum CTx (Fig. 3D), a marker of bone resorption, increased by 38%, at week 24 of burosumab treatment, whereas little change was observed in the placebo group. The LS mean \pm SE difference between the burosumab and placebo groups for the change from baseline to week 24 was 62 ± 7.5 ng/mL for P1NP ($p < 0.001$) and 190 ± 41.2 pg/mL for CTx ($p < 0.001$). At week 24, serum BALP increased from baseline by 43% in the burosumab group and by 33% in the placebo group; the LS mean \pm SE difference between the burosumab and placebo groups for the change from baseline to week 24 was 4.4 ± 2.48 μ g/L.

Results for the key study efficacy outcomes are summarized in Supplemental Table S1. The study results were robust using either worst pain or average pain to stratify subjects.

Safety

Most participants in each group (94.1% burosumab, 92.4% placebo) had at least one adverse event through week 24 of

treatment (Table 2). Two participants in each group had serious adverse events, none of which were considered by the investigator to be related to study treatment. No deaths, discontinuations due to adverse events, or dose-limiting toxicities occurred. In both treatment groups, most adverse events were mild or moderate in severity.

For the adverse events of interest, incidences of injection site reactions and hypersensitivity events were similar between treatment groups. Investigators reported adverse events of hyperphosphatemia (all mild in severity) for 5.9% of participants in the burosumab group; no participant in the placebo group experienced hyperphosphatemia. Restless legs syndrome events were reported for 11.8% and 7.6% of participants in the burosumab and placebo groups, respectively.

Plasma iPTH decreased from 98.9 ± 60.8 pg/mL at baseline (normal range, 14–72 pg/mL) to 81.5 ± 38.4 pg/mL at week 24 in the burosumab group and increased from 95.2 ± 38.8 pg/mL at baseline to 99.0 ± 42.6 pg/mL at week 24 in the placebo group. No clinically relevant renal or cardiac ectopic mineralization was evident based on renal ultrasound or echocardiography, and no participant had an increase of >1 point in the renal ultrasound nephrocalcinosis score or echocardiography calcium score. None of the 1-point increases in nephrocalcinosis score were associated with an adverse event, an increase in urinary calcium,

Table 2. Summary of Safety During Double-Blind Treatment

	Placebo (n = 66)	Burosumab (n = 68)
Any adverse event	61 (92.4)	64 (94.1)
Most common adverse events ^a		
Back pain	6 (9.1)	10 (14.7)
Nasopharyngitis	6 (9.1)	9 (13.2)
Tooth abscess	5 (7.6)	9 (13.2)
Headache	5 (7.6)	8 (11.8)
Nausea	6 (9.1)	7 (10.3)
Dizziness	4 (6.1)	7 (10.3)
Arthralgia	16 (24.2)	6 (8.8)
Pain in extremity	10 (15.2)	5 (7.4)
Oropharyngeal pain	7 (10.6)	1 (1.5)
Adverse events of interest ^b		
Injection site reaction	8 (12.1)	8 (11.8)
Hypersensitivity	4 (6.1)	4 (5.9)
Hyperphosphatemia	0	4 (5.9)
Restless legs syndrome ^c	5 (7.6)	8 (11.8)
Ectopic mineralization	0	0
Serious adverse event	2 (3.0)	2 (2.9)
Adverse event leading to discontinuation	0	0
Adverse event leading to death	0	0
Change in renal ultrasound nephrocalcinosis score		
Increased by 1 point	12 (18.2)	11 (16.2)
Increased by >1 point	0	0
Decreased by 1 point	4 (6.1)	4 (5.9)
Decreased by >1 point	0	0
Change in echocardiographic calcium score		
Increased by 1 point	7 (10.6)	1 (1.5)
Increased by >1 point	0	0
Decreased by 1 point	1 (1.5)	3 (4.4)
Decreased by >1 point	0	0

Values are n (%).

^aListed in descending order of incidence for the burosumab group are preferred terms for events that were reported for at least 10% of participants in either treatment group.

^bListed are adverse events of interest that were identified from searches for groups of related preferred terms.

^cAdverse events of interest for restless legs syndrome included not only adverse events with the specific term of “restless legs syndrome” (4 [6.1%] placebo; 8 [11.8%] burosumab) but also adverse event terms of akathisia, formication, limb discomfort, muscle cramp, neuromuscular pain, psychomotor hyperactivity, restlessness, or sensory disturbance.

any clinically meaningful changes in iPTH, or a reduction in renal function.

No clinically significant changes occurred in left ventricular mass index as assessed by echocardiography. No participant developed anti-burosumab antibodies post-baseline. No clinically significant changes from baseline through week 24 were observed in serum calcium concentration (Fig. 4A), 24-hour urine calcium excretion (Fig. 4B), or plasma iPTH (Fig. 4C).

Discussion

This double-blind, placebo-controlled, phase 3 trial demonstrates that burosumab, by binding excess circulating FGF23, increased renal phosphate reabsorption and normalized serum phosphate levels throughout the dosing interval in symptomatic adults with XLH. Burosumab also increased serum 1,25 (OH)₂D concentrations. These improvements in phosphate homeostasis and vitamin D metabolism are consistent with the pharmacologic action of burosumab to attenuate FGF23 activity in vivo.

Osteomalacia, which is the hallmark of XLH in adult patients,⁽¹²⁾ is characterized by severe mineralization defects that impair bone quality and bone remodeling. As a consequence, low-trauma fractures and pseudofractures are commonly observed in adults with XLH. By restoring phosphate homeostasis, burosumab treatment is expected to improve mineralization and restore normal skeletal physiology. At study entry, more than half of the subjects had active fractures or pseudofractures on radiographic skeletal survey, even though many subjects had received phosphate salts and active vitamin D metabolites in the past. Burosumab treatment for 24 weeks was associated with full healing of nearly half of the fractures identified at baseline. In fact, the odds of healing a fracture for the participants treated with burosumab was 16.8-fold greater than for those receiving placebo. To our knowledge, this is the first controlled trial to investigate fracture healing in adults with XLH. Improvement in the underlying osteomalacia may be reflected in increases in bone formation and resorption markers. This is currently being investigated further in an ongoing, complementary study examining the effect of burosumab on

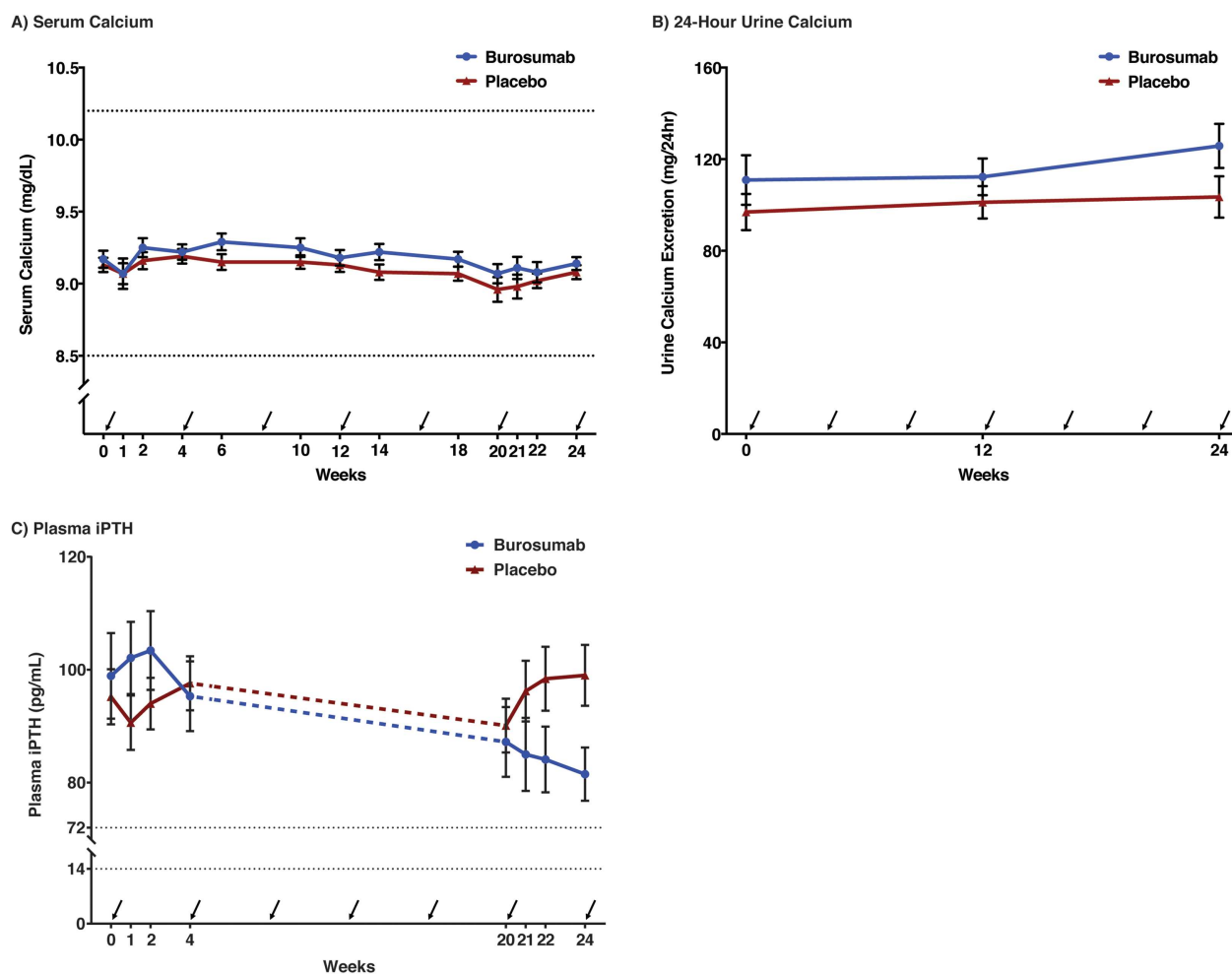


Fig 4. Effect of burosumab on calcium homeostasis. Data are expressed as mean \pm standard error. Dashed lines for serum calcium and iPTH are the lower and upper limits of normal. Arrows indicate administration of study treatment (burosumab or placebo). iPTH = intact parathyroid hormone.

skeletal histology and histomorphometry in XLH. Collectively, these results suggest that as phosphate homeostasis improves with burosumab treatment, the underlying osteomalacia also improves, allowing for normal bone remodeling and by that mechanism fracture healing.

The baseline characteristics of study participants highlight the substantial disease burden in adults with XLH. Lower-limb deformities, early osteoarthritis, and enthesopathy lead to chronic pain, stiffness, and impaired mobility. Consequently, normal physical activity is very difficult in symptomatic adults with XLH and may contribute to the high prevalence of overweight/obesity in this disease, as evidenced by the mean body mass index of $>30 \text{ kg/m}^2$ in this study. From baseline to week 24, all three of the key secondary patient-reported outcomes in this study improved in the burosumab group. After adjusting for multiplicity, burosumab treatment led to a statistically significant reduction in stiffness relative to placebo using the WOMAC score. Stiffness in adults with XLH may have multiple causes, including not only musculoskeletal contributors but also psychological factors and fatigue; nonetheless, improvements in muscle function with burosumab treatment in this study may have contributed to the reported reduction in stiffness. In particular, correction of hypophosphatemia has

been shown to improve muscle ATP synthesis.⁽²²⁾ Pain lessened and physical function improved but neither achieved statistical significance relative to placebo. Multiple causes of musculoskeletal pain are encountered in XLH. Improving phosphate metabolism, osteomalacia, and fracture healing with burosumab treatment may address a major cause of pain in adults with XLH, but phosphate homeostasis is not expected to treat every cause of pain or physical impairment of individuals with longstanding disease. Neither osteoarthritis nor enthesopathy-related pain would be expected to improve with burosumab based on its mechanism of action. This may explain the absence of a more substantial effect on pain during the 24-week duration of burosumab exposure in this study. On the other hand, several normal bone remodeling cycles would likely be required to fully restore skeletal metabolism and correct osteomalacia, which would require treatment for longer than the 24 weeks of this study.

Burosumab is the first treatment that specifically addresses the underlying pathophysiology of XLH: chronic hypophosphatemia caused by elevated circulating levels of FGF23. Repeated administration of supplemental phosphate throughout the day, as is done with conventional therapy, leads to wide daily swings in serum phosphate and a large

phosphorus load delivered to the kidney. These two factors are thought to contribute to the frequent occurrence of hyperparathyroidism and nephrocalcinosis, potentially complicating conventional therapy.^(23–25) In contrast, 24 weeks of burosumab treatment was not associated with any increase in plasma iPTH or the development or progression of renal or cardiac ectopic mineralization as assessed by renal ultrasound and echocardiography. Burosumab had an acceptable safety profile. The overall incidence, nature, and severity of adverse events were comparable in the two treatment groups. The incidence of injection site reactions was similar in the two groups, and no participant developed new anti-burosumab antibodies during treatment.

In conclusion, this study demonstrates that burosumab can address the central pathophysiologic mechanism in XLH through blockade of excess circulating levels of FGF23, thereby increasing renal phosphate reabsorption and normalizing serum phosphate. The improvement in phosphate metabolism was associated with significant improvement in fracture healing and increased bone formation and resorption markers, consistent with restored bone remodeling. Improvements in phosphate metabolism were accompanied by significant reductions in stiffness, with a trend toward increased physical function and reduced pain. In aggregate, the results from the double-blind period of this phase 3 trial support the clinically meaningful efficacy and safety profile of burosumab, a novel therapy for the treatment of symptomatic adults with XLH.

Disclosures

KLI received consulting fees and travel support for this study from Ultragenyx. KB received consulting fees for this study from Kyowa Hakko Kirin; consulting fees not related to this study from Lilly, Amgen, MSD, and Pfizer; and travel support unrelated to this study from Lilly and Amgen. EAI received travel support for this study from Ultragenyx and Kyowa Hakko Kirin, and consulting fees not related to this study from Ultragenyx. MDR received payment from Ultragenyx for advisory board participation. AAP received travel support and payment from Ultragenyx for advisory board participation and payment for educational presentations not related to this study. TW received consulting fees and travel support not related to this study from Ultragenyx. PP received consulting fees and travel support related to this study from Ultragenyx, and consulting fees not related to this study from Ultragenyx. SJdB received consulting fees and travel support related to this study from Ultragenyx, and payment for development of educational presentations not related to this study from Ultragenyx. YI received travel support for this study and consulting fees not related to this study from Kyowa Hakko Kirin. RHL received travel support related to this study from Ultragenyx and payment for advisory board participation not related to this study from SanofiGenzyme and Audentes Therapeutics. FP received consulting fees not related to this study from Ultragenyx. LZ, CYC, CTO, MM, and JSM are employees and stockholders of Ultragenyx. TOC received consulting fees and payment for advisory board membership and lectures from Ultragenyx, and payment for development of educational presentations from Premier and Ultragenyx. The institutions that employ KLI, EAI, PK, MDR, AAP, TW, PP, SJdB, NI, HT, and TOC received research grants from Ultragenyx for this study or other studies.

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References

1. A gene (PEX) with homologies to endopeptidases is mutated in patients with X-linked hypophosphatemic rickets. The HYP Consortium. *Nat Genet.* 1995;11(2):130–6.
2. Jonsson KB, Zahradnik R, Larsson T, et al. Fibroblast growth factor 23 in oncogenic osteomalacia and X-linked hypophosphatemia. *N Engl J Med.* 2003;348(17):1656–63.
3. Liu S, Quarles LD. How fibroblast growth factor 23 works. *J Am Soc Nephrol.* 2007;18(6):1637–47.
4. Bonewald LF, Wacker MJ. FGF23 production by osteocytes. *Pediatr Nephrol.* 2013;28(4):563–8.
5. Shimada T, Hasegawa H, Yamazaki Y, et al. FGF-23 is a potent regulator of vitamin D metabolism and phosphate homeostasis. *J Bone Miner Res.* 2004;19(3):429–35.
6. Shore RM, Chesney RW. Rickets: part I. *Pediatr Radiol.* 2013;43(2):140–51.
7. Linglart A, Biosse-Duplan M, Briot K, et al. Therapeutic management of hypophosphatemic rickets from infancy to adulthood. *Endocr Connect.* 2014;3(1):R13–30.
8. Hardy DC, Murphy WA, Siegel BA, Reid IR, Whyte MP. X-linked hypophosphatemia in adults: prevalence of skeletal radiographic and scintigraphic features. *Radiology.* 1989;171(2):403–14.
9. Reid IR, Hardy DC, Murphy WA, Teitelbaum SL, Bergfeld MA, Whyte MP. X-linked hypophosphatemia: a clinical, biochemical, and histopathologic assessment of morbidity in adults. *Medicine (Baltimore).* 1989;68(6):336–52.
10. Veilleux LN, Cheung M, Ben Amor M, Rauch F. Abnormalities in muscle density and muscle function in hypophosphatemic rickets. *J Clin Endocrinol Metab.* 2012;97(8):E1492–8.
11. Che H, Roux C, Etcheto A, et al. Impaired quality of life in adults with X-linked hypophosphatemia and skeletal symptoms. *Eur J Endocrinol.* 2016;174(3):325–33.
12. Carpenter TO, Imel EA, Holm IA, Jan de Beur SM, Insogna KL. A clinician's guide to X-linked hypophosphatemia. *J Bone Miner Res.* 2011;26(7):1381–8.
13. Sullivan W, Carpenter T, Glorieux F, Travers R, Insogna K. A prospective trial of phosphate and 1,25-dihydroxyvitamin D3 therapy in symptomatic adults with X-linked hypophosphatemic rickets. *J Clin Endocrinol Metab.* 1992;75(3):879–85.
14. Verge CF, Lam A, Simpson JM, Cowell CT, Howard NJ, Silink M. Effects of therapy in X-linked hypophosphatemic rickets. *N Engl J Med.* 1991;325(26):1843–8.
15. Imel EA, DiMeglio LA, Hui SL, Carpenter TO, Econs MJ. Treatment of X-linked hypophosphatemia with calcitriol and phosphate increases circulating fibroblast growth factor 23 concentrations. *J Clin Endocrinol Metab.* 2010;95(4):1846–50.
16. Carpenter TO, Imel EA, Ruppe MD, et al. Randomized trial of the anti-FGF23 antibody KR23 in X-linked hypophosphatemia. *J Clin Invest.* 2014;124(4):1587–97.
17. Imel EA, Zhang X, Ruppe MD, et al. Prolonged correction of serum phosphorus in adults with X-linked hypophosphatemia using

- monthly doses of KRN23. *J Clin Endocrinol Metab.* 2015;100(7): 2565–73.
18. Ruppe M, Peacock M, Weber T, et al. Clinical and radiographic characteristics of adult X-linked hypophosphatemia (XLH) in a cohort of patients treated with KRN23, an antibody to FGF23. Presented at: ASBMR 2016 Annual Meeting, September 16–19, 2016; Atlanta, GA, USA. Presentation #MO0319.
 19. Daut RL, Cleeland CS, Flanery RC. Development of the Wisconsin Brief Pain Questionnaire to assess pain in cancer and other diseases. *Pain.* 1983;17(2):197–210.
 20. Bellamy N. WOMAC osteoarthritis index user guide. Version X. Brisbane, Australia; 2012.
 21. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med.* 2002;166(1):111–7.
 22. Pesta DH, Tsirigotis DN, Befroy DE, et al. Hypophosphatemia promotes lower rates of muscle ATP synthesis. *FASEB J.* 2016;30(10):3378–87.
 23. Rivkees SA, el-Hajj-Fuleihan G, Brown EM, Crawford JD. Tertiary hyperparathyroidism during high phosphate therapy of familial hypophosphatemic rickets. *J Clin Endocrinol Metab.* 1992;75(6): 1514–8.
 24. Taylor A, Sherman NH, Norman ME. Nephrocalcinosis in X-linked hypophosphatemia: effect of treatment versus disease. *Pediatr Nephrol.* 1995;9(2):173–5.
 25. Carpenter TO, Insogna KL, Zhang JH, et al. Circulating levels of soluble klotho and FGF23 in X-linked hypophosphatemia: circadian variance, effects of treatment, and relationship to parathyroid status. *J Clin Endocrinol Metab.* 2010;95(11):E352–7.