Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Carpenter TO, Whyte MP, Imel EA, et al. Burosumab therapy in children with X-linked hypophosphatemia. N Engl J Med 2018;378:1987-98. DOI: 10.1056/NEJMoa1714641

Supplementary Appendix

This appendix has been provided by authors to give readers additional information about the work.

Table of Contents

List of Investigators

1. Principal Investigator: Agnès Linglart, MD, PhD; APHP Hôpital Bicêtre Paris Sud, France
    a. Sub-Investigators: Anya Rothenbuhler, MD and Claire Morel-Bouvattier, MD
2. Principal Investigator: Annemieke M. Boot, MD, PhD; University of Groningen, Groningen, Netherlands
    a. Sub-Investigator: Valentina Gracchi, MD
3. Principal Investigator: Anthony A. Portale, MD; University of California, San Francisco, California, USA
    a. Sub-Investigator: Farzana Perwad, MD
4. Principal Investigator: Erik A. Imel, MD, MS; Indiana University School of Medicine, Indianapolis, Indiana, USA
    a. Sub-Investigators: Linda DiMeglio, MD and Munro Peacock, MD
5. Principal Investigator: Michael P. Whyte, MD; Shriners Hospital for Children, St. Louis, Missouri, USA
    a. Sub-Investigator: Gary S. Gottesman, MD
6. Principal Investigator: Raja Padidela, MD; Royal Manchester Children's Hospital, Manchester, UK
    a. Sub-Investigator: Zulf Mughal, MD
7. Principal Investigator: Thomas O. Carpenter, MD; Yale University School of Medicine, New Haven, Connecticut, USA
    a. Sub-Investigators: Karl Insogna, MD and Clemens Bergwitz, MD
8. Principal Investigator: William van’t Hoff, MD; Great Ormond Street Hospital, London, UK
    a. Sub-Investigators: Detlef Bockenhaur, MD and Wesley Hayes, MD
9. Principal Investigator: Wolfgang Högler, MD; Birmingham Children's Hospital, Birmingham, UK
    a. Sub-Investigators: Nick Shaw, MD, Vrinda Saraff, MD, and Sophia Sakka, MD

Supplementary Text

Methods

Biochemistry parameters: All biochemistry assessments were performed by Covance Central Laboratory Services. Phosphorus, calcium, and alkaline phosphatase assays were performed on Roche Modular and Cobas analyzers. Intact parathyroid hormone (iPTH) assay was performed using the iPTH reagent packs for the ADVIA Centaur XP instruments. $1,25(OH)_2D$ was assessed using Diasorin's 1,25-dihydroxyvitamin D ^{125}I RIA kit.

Tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) was calculated as follows: If tubular reabsorption of phosphate (TRP) ≤ 0.86, then TmP/GFR = TRP x serum phosphorus (mg/dL); if TRP > 0.86, then TmP/GFR = [0.3 x TRP/{1-(0.8 x TRP)}] x serum phosphorus (mg/dL).[1]

Rickets and Lower Extremity Bowing: Bilateral anteroposterior knee radiographs and bilateral posteroanterior hand/wrist radiographs were obtained at Screening Visit 1 and at the week 40 and 64 study visits. Standing long leg radiographs were obtained at Screening Visit 1 and at the Week 64 study visit.

Rickets severity was assessed as the change from baseline at week 40 in Total Thacher Rickets Severity Score, which is based on the combined assessment of wrist and knee radiographs. The Thacher Rickets Severity Score was developed for assessment of nutritional rickets, with higher scores (range 0-10) indicating greater rickets severity.[2] Scoring was based on the degree of metaphyseal fraying, lucency, cupping, and the proportion of growth plate affected.

Complementary to the Thacher Rickets Severity Score, the Radiographic Global Impression of Change was also used to assess rickets healing. Radiographic Global Impression of Change is a side-by-side comparison of radiographs obtained before and during treatment, utilizing a 7-point ordinal scale to assess relative change of the same radiographic abnormalities evaluated in the Thacher Rickets Severity Score, as well as an assessment of lower extremity of bowing. Radiographic Global Impression of Change scores are rated as: +3=complete healing, +2=substantial healing, +1=minimal healing, 0=unchanged, -1=minimal worsening, -2=moderate worsening, -3=severe worsening (adapted from previous use in children with hypophosphatasia).[3]

Radiographs were evaluated by pediatric radiologists, contracted through Biomedical Systems, who were presented radiographs in a blinded random order (pairs for Radiographic Global Impression of Change). Thacher Rickets Severity Score was determined by one reader, and Radiographic Global Impression of Change was determined by three readers.

Growth: Growth was evaluated using age- and gender-adjusted Z scores at the study visits at which height was measured.

Six-Minute Walk Test: The six-minute walk test was administered by a trained clinician in accordance with general principles set forth in the American Thoracic Society guidelines (ATS 2002).[4,5] Patients were instructed to walk the length of a pre-measured course for 6 consecutive minutes. The total distance walked at the end of 6 minutes was recorded in meters. The percent of predicted normal values was calculated using published normative data based on age, gender, and height.

Functional Disability and Pain: The Pediatric Outcomes Data Collection Instrument, developed by the Pediatric Orthopedic Society of North America (POSNA), was used to assess functional disability.[6] The Pediatric Outcomes Data Collection Instrument was developed to measure the functional health of pediatric and adolescent patients with a variety of musculoskeletal disorders. The instrument is designed to assess overall health, pain, and ability to participate in normal daily activities, and more vigorous activities associated with young people. The questionnaire yields a global function score, a happiness score, and four functional assessment scores (upper extremity functioning, transfers and basic mobility, sports and physical functioning, and comfort/pain). Raw, mean, standardized, and normative scores were calculated for each scale. The global function score is an average of the four functional scores. Standardized scores range from 0 to 100, with 0 representing the poorest outcome or worst health and 100 the best possible outcome or best health. Normative scores were calculated so that higher scores indicate better functioning. All scores were referenced to the general, healthy population with a normative mean score of 50 and a standard deviation of 10. Normative scores are referred to as “scores” in the text.

A parent or legal guardian completed the questionnaire (if possible, the same individual completed the assessment throughout the study for consistency). For patients 10 years of age and under at Baseline, the Pediatric Outcomes Questionnaire was used; for patients older than 10 years of age at Baseline, the Adolescent (parent reported) Questionnaire was used. Scores were calculated using spreadsheets provided by developers of the instrument at the following: http://www.aaos.org/outcomesinstruments

Safety assessment:

A comprehensive serum metabolic panel (Chem-20), complete blood count, and urinalysis were obtained to assess burosumab safety.

To determine the immunogenicity profile of KRN23 in children with X-linked hypophosphatemia, blood samples were obtained for analysis of anti-burosumab antibodies. Concentration of anti-KRN23 antibodies in human serum was determined using a validated sandwich ELISA and a 2-tiered strategy: screening assay and specificity confirmation assay.

Dose Adjustment

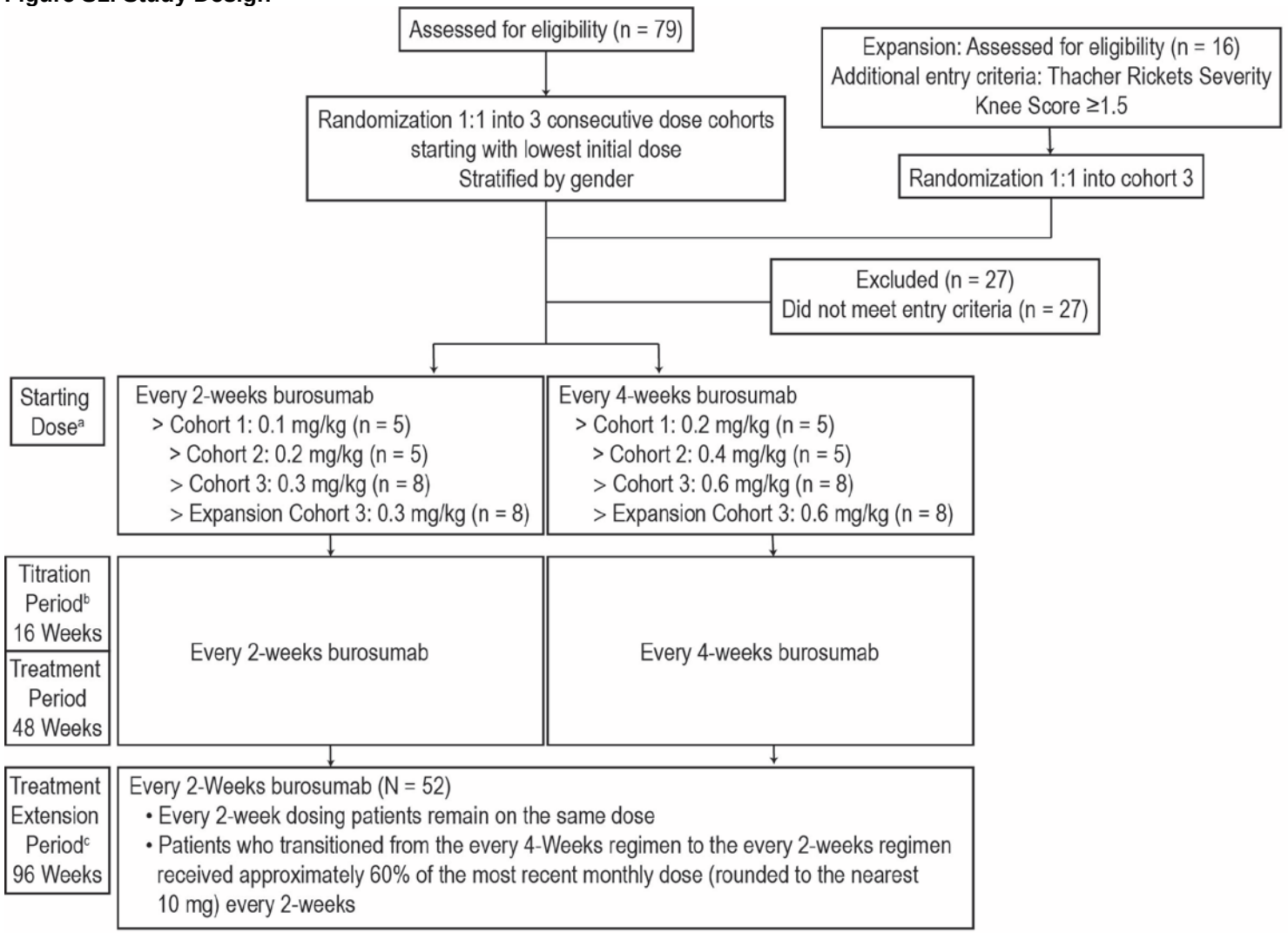
During the Titration Period, burosumab dose was adjusted every 4 weeks based on the subject's fasting serum phosphorus level obtained two weeks after their previous dose (target serum phosphorus 3.5-4.5 mg/dL; 1.13-1.45 mmol/L), up to a maximum dose of 2 mg/kg per injection. Dose adjustment continued into the Treatment Period until the target low-normal phosphorus level was reached, or no further increase in serum phosphorus was observed after dose escalation, provided there were no safety concerns.

Results

There were no impairments in 3 of the Pediatric Orthopedic Society of North America - Pediatric Outcomes Data Collection Instrument domains (Upper Extremity, Transfer and Basic Mobility, Happiness) at baseline. However, 64 weeks of treatment with burosumab improved scores in each domain (least squares mean change from baseline: Upper Extremity 2.6; Transfer and Basic Mobility 1.99; and Happiness 2.76).

Supplemental Figures

Figure S1. Study Design



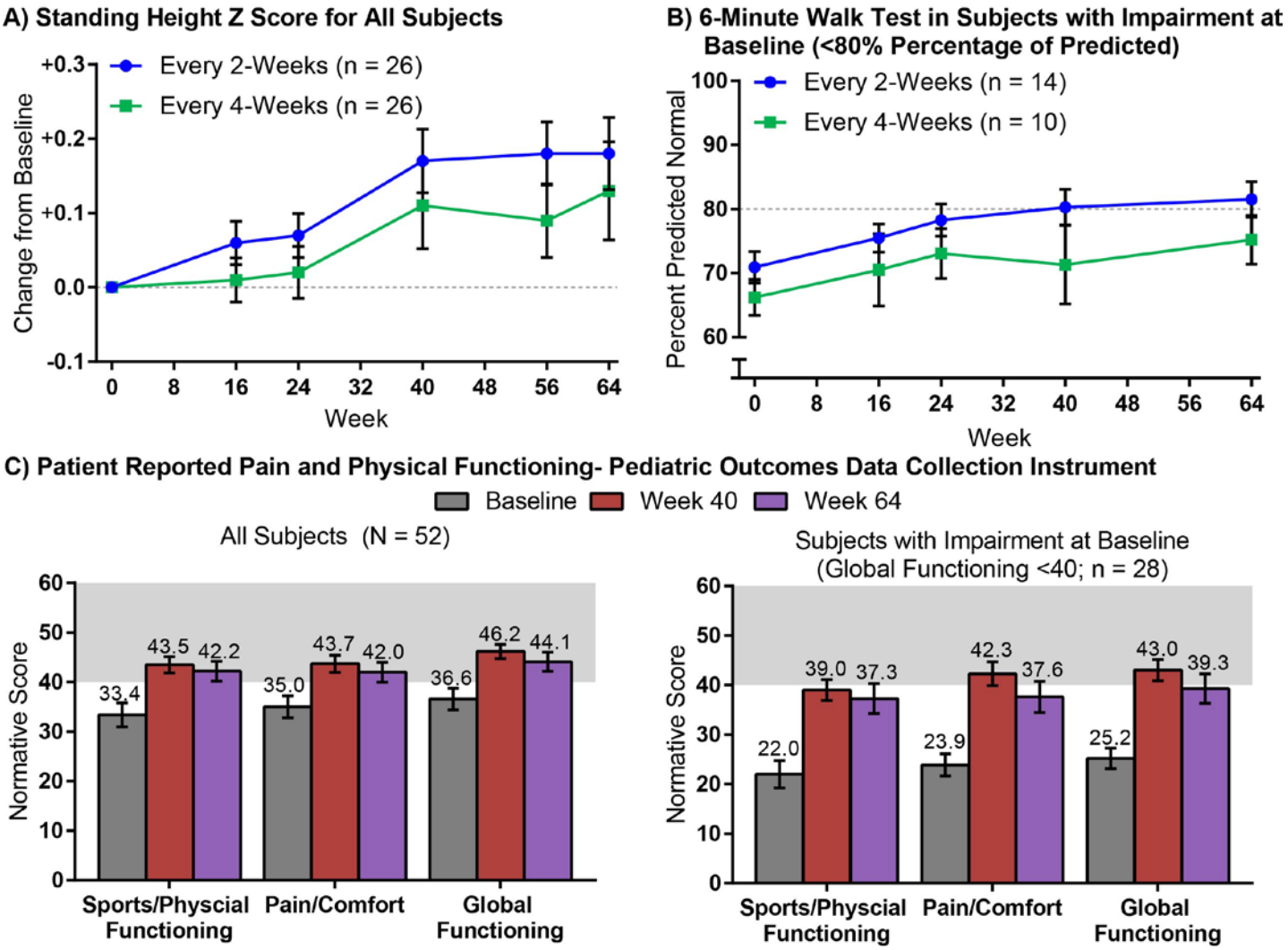
Study UX023-CL201 began in June of 2014 and is ongoing.

[a]Patients were enrolled sequentially into cohorts defined by the initial dose of burosumab. As a precautionary measure, patients in Dose Cohort 2 were not administered burosumab until the fourth subject in Dose Cohort 1 completed the Week 4 visit.

[b]During the Titration Period, the burosumab dose was adjusted every 4 weeks based on the subject's fasting serum phosphorus level obtained two weeks after their previous dose (target serum phosphorus 1.13-1.45 mmol/L), up to a maximum dose of 2mg/kg. Dose adjustment continued into the Treatment Period until the target phosphorus level was reached or no further increase was observed after dose escalation, provided there were no safety concerns.

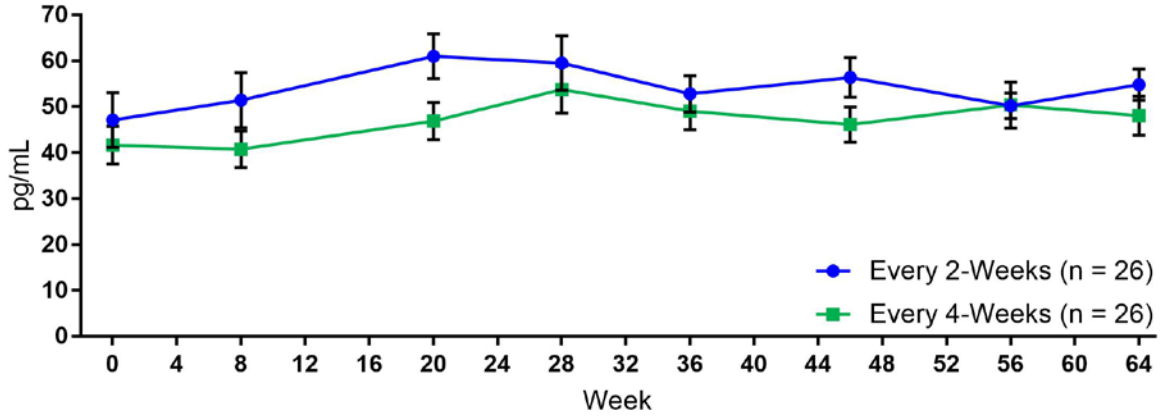
[c]Patients had the option to enroll into the Treatment Extension period; this portion of the study is still ongoing and not included in this analysis. During the Extension Period, patients in the every 2-weeks arm remained on the same dose. Patients who transitioned from the every 4-weeks regimen to the every 2-weeks regimen received approximately 60% of the most recent monthly dose (rounded to the nearest 10 mg).

Figure S2. Effects of Burosumab on Growth, Exercise Capacity, and Patient-reported Pain and Physical Function



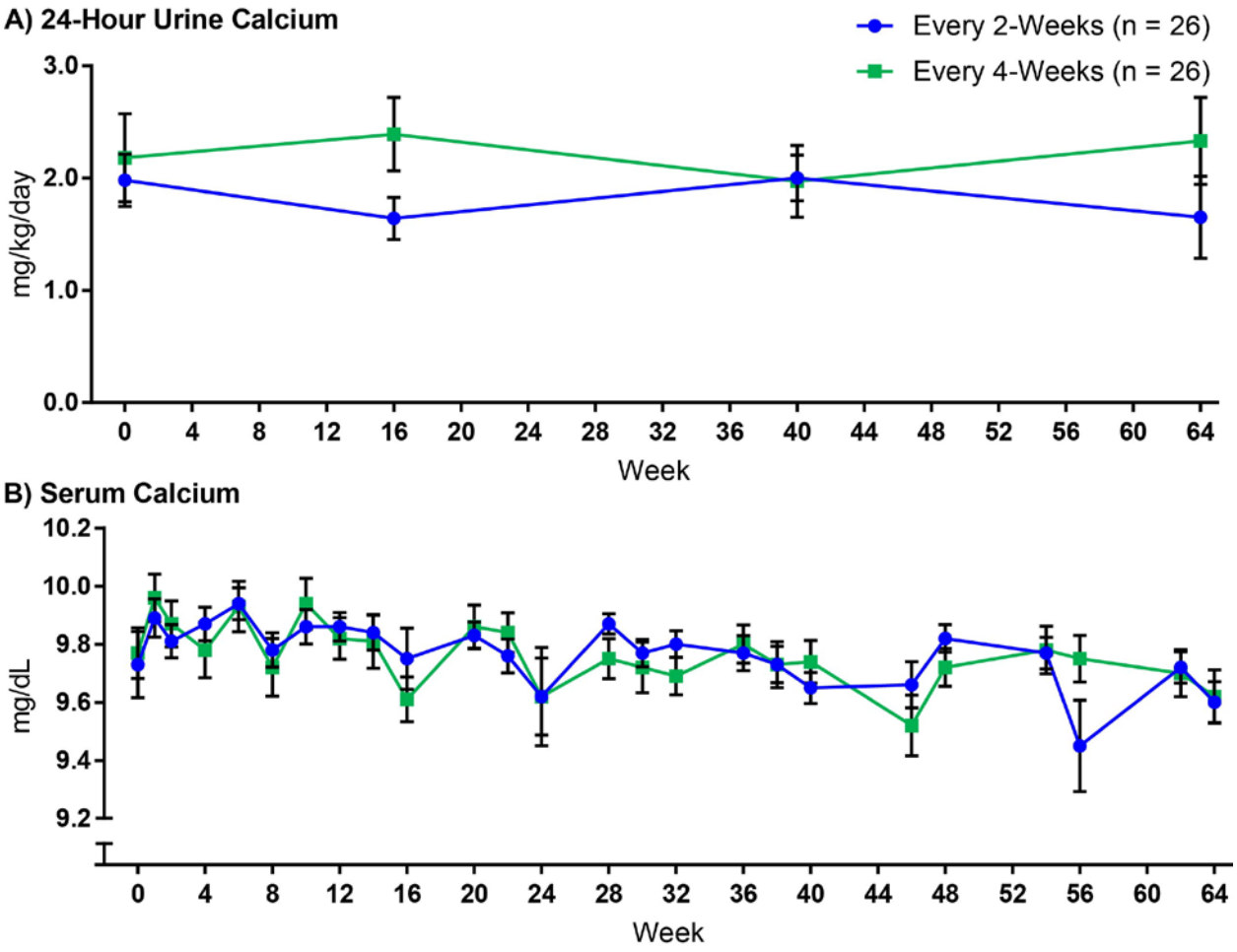
Standing height z-score is expressed as least squares mean ± SE. Percent predicted 6-minute walk test and Pediatric Outcomes Data Collection Instrument scores are expressed as Mean ± SE.

Figure S3. Serum Parathyroid Hormone



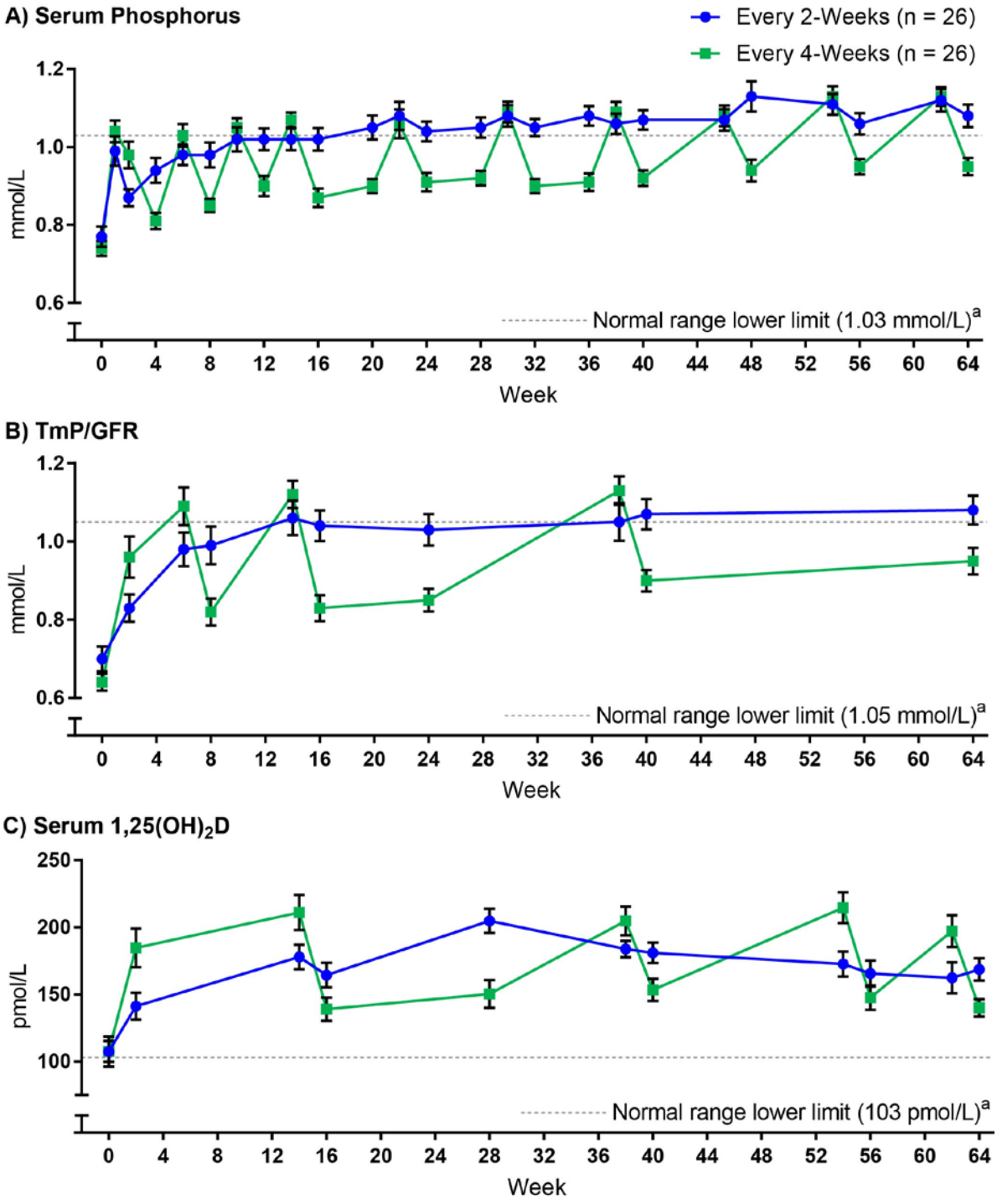
Data are expressed as mean ± SE. Conversion from SI units to conventional units, 1 pmol/L:9.4 pg/mL. Normal range for PTH is 6 – 59 pg/mL (0.64 – 6.26 pmol/L).

Figure S4. Serum and Urine Calcium



Data are expressed as mean ± SE.

Figure S5. Effects of Burosumab on Serum Phosphorus, TmP/GFR, and 1,25(OH)$_2$D (SI Units)



Data are expressed as mean ± SE. All specimens were obtained after patients had fasted. For serum phosphorus (A) and TmP/GFR (B), there was a significant increase from baseline at week 40 (n=26 for

both regimens) for both regimens ($p<0.001$). For serum $1,25(OH)_2D$ (C), there was a significant increase from baseline in both regimens at week 40 ($p<0.001$; n=26 for both regimens).

[a]Reference ranges were provided by Covance Laboratories and from the literature: Serum Phosphorus,[7] TmP/GFR,[8] $1,25(OH)_2D$[9]

LS, least squares; TmP/GFR, the ratio of the maximum rate of tubular phosphate reabsorption to the glomerular filtration rate

Supplementary Tables

Table S1. Schedule of Key Assessments

Assessment	**Week**
Serum Phosphorus	Screening, 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 28, 30, 32, 36, 38, 40, 46, 48, 54, 56, 62, and 64
2-hour urine (phosphorus, calcium, creatinine, TmP/GFR, and TRP)	0, 2, 6, 8, 14, 16, 24, 38, 40, and 64
24-hour urine (phosphorus, calcium, and creatinine)	0, 16, 40, and 64
$1,25(OH)_2D$	0, 2, 14, 16, 28, 38, 40, 54, 56, 62, 64
Alkaline Phosphatase	0, 16, 40, and 64
Bilateral anteroposterior knee radiographs and bilateral posteroanterior hand/wrist radiographs	Screening, 40 and 64
Standing long leg radiographs	Screening and 64
Growth (standing height)	0, 16, 24, 40, 56, and 64
6MWT	Screening, 0, 16, 24, 40, and 64
Pediatric Outcomes Data Collection Instrument	0, 24, 40, and 64
Adverse Events	Screening, 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, and 64
Anti-burosumab antibodies	0, 16, 24, 36, 56, and 64
Serum iPTH	Screening, 8, 20, 28, 36, 46, 56, and 64
Serum calcium	Screening, 0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 20, 22, 24, 28, 30, 32, 36, 38, 40, 46, 48, 54, 56, 62, and 64
Serum FGF23	Screening, 8, 16, 28, 38, and 64
Renal ultrasound	Screening and 16, 40, 64
ECHO & 12-lead ECG	0, 16, 40, 64

Table S2. Effects of Burosumab on Rickets Severity in All Patients

Assessment	Burosumab Every 2-Weeks (n = 26)	Burosumab Every 4-Weeks (n = 26)	Burosumab All Patients (N = 52)
Total Thacher Rickets Severity Score			
Baseline, Mean (SD)	1.92 (1.17)	1.67 (1.00)	1.80 (1.09)
Median (Q1, Q3)	2.00 (1.00, 2.50)	1.75 (0.50, 2.50)	2.00 (0.75, 2.50)
Week 40, Mean (SD)	0.75 (0.55)	1.06 (0.54)	0.90 (0.56)
Median (Q1, Q3)	1.00 (0.00, 1.00)	1.00 (0.50, 1.50)	1.00 (0.50, 1.25)
Change from baseline, LSM (SE)	-1.06 (0.11)[a]	-0.73 (0.10)[a]	-0.89 (0.07)
Change from baseline, median (Q1, Q3)	-1.00 (-2.00, -0.50)	-0.50 (-1.50, 0.00)	-1.00 (-1.50, 0.00)
Week 64, Mean (SD)	0.81 (0.60)	0.94 (0.52)	0.88 (0.56)
Median (Q1, Q3)	1.00 (0.00, 1.00)	1.00 (0.50, 1.50)	1.00 (0.50, 1.25)
Change from baseline, LSM (SE)	-1.00 (0.11)	-0.84 (0.10)	-0.92 (0.07)
Change from baseline, median (Q1, Q3)	-1.00 (-1.50, -0.50)	-0.50 (-2.00, 0.00)	-0.75 (-1.75, 0.00)
Radiographic Global Impression of Change			
Week 40, LSM (SE)	+1.66 (0.09)	+1.47 (0.14)	+1.56 (0.08)
Median (Q1, Q3)	+2.00 (1.33, 2.00)	+1.67 (1.00, 2.00)	+2.00 (1.00, 2.00)
Week 64, LSM (SE)	+1.56 (0.11)	+1.58 (0.11)	+1.57 (0.08)
Median (Q1, Q3)	+2.00 (1.00, 2.33)	+1.83 (1.00, 2.00)	+2.00 (1.00, 2.00)

[a]P < 0.001. LSM, least squares mean; Thacher Rickets Severity Score, Rickets Severity Score; Radiographic Global Impression of Change, radiographic Global Impression of Change; SD, standard deviation; SE, standard error.

Table S3. Effects of Burosumab on Rickets Severity in Patients with Greater Rickets Severity at Baseline (Total Thacher Rickets Severity Score ≥1.5)

	Burosumab Every 2-Weeks (n = 26)	Burosumab Every 4-Weeks (n = 26)	Burosumab All Patients (N = 52)
Total Thacher Rickets Severity Score in patients with a score ≥1.5 at baseline			
n	17	17	34
Baseline, Mean (SD)	2.62 (0.78)	2.29 (0.56)	2.46 (0.69)
Median (Q1, Q3)	2.50 (2.00, 3.00)	2.50 (2.00, 2.50)	2.50 (2.00, 3.00)
Week 40, Mean (SD)	0.76 (0.47)	1.18 (0.61)	0.97 (0.58)
Median (Q1, Q3)	1.00 (0.50, 1.00)	1.00 (1.00, 1.50)	1.00 (0.50, 1.50)
Change from baseline, LSM (SE)	-1.68 (0.11)	-1.29 (0.15)	-1.49 (0.09)
Change from baseline, median (Q1, Q3)	-1.50 (-2.50, -1.00)	-1.00 (-1.50, -0.50)	-1.50 (-2.00, -1.00)
Week 64, Mean (SD)	1.00 (0.56)	1.03 (0.57)	1.01 (0.56)
Median (Q1, Q3)	1.00 (0.50, 1.50)	1.00 (0.50, 1.50)	1.00 (0.50, 1.50)
Change from baseline, LSM (SE)	-1.44 (0.13)	-1.44 (0.14)	-1.44 (0.09)
Change from baseline, median (Q1, Q3)	-1.50 (-2.00, -1.00)	-1.50 (-2.00, -0.50)	-1.50 (-2.00, -1.00)
Radiographic Global Impression of Change in patients with a Thacher Rickets Severity Score ≥1.5 at baseline			
Week 40, LSM (SE)	+2.02 (0.04)	+1.80 (0.11)	+1.91 (0.06)
Median (Q1, Q3)	+2.00 (2.00, 2.00)	+2.00 (1.67, 2.00)	+2.00 (1.67, 2.00)
Week 64, LSM (SE)	+2.06 (0.07)	+1.90 (0.10)	+1.98 (0.06)
Median (Q1, Q3)	+2.00 (2.00, 2.33)	+2.00 (1.33, 2.00)	+2.00 (2.00, 2.33)

LSM, least squares mean.

References

1. Kenny AP, Glen AC. Tests of phosphate reabsorption. Lancet 1973;2:158.

2. Thacher TD, Fischer PR, Pettifor JM, Lawson JO, Manaster BJ, Reading JC. Radiographic scoring method for the assessment of the severity of nutritional rickets. J Trop Pediatr 2000;46:132-9.

3. Whyte MP, Fujita KP, Moseley S, Thompson DD, McAlister WH. Validation of a Novel Scoring System for Changes in Skeletal Manifestations of Hypophosphatasia in Newborns, Infants, and Children: The Radiographic Global Impression of Change Scale. Journal of Bone and Mineral Research 2018. 10.1002/jbmr.3377. [Epub ahead of print]

4. ATS. American Thoracic Society Statement: Guidelines for the six-minute walk test. AmJRespir, CritCare Med 2002;166:111-7.

5. Geiger R, Strasak A, Treml B, et al. Six-minute walk test in children and adolescents. J Pediatr 2007;150:395-9. S0022-3476(07)00014-5 [pii];10.1016/j.jpeds.2006.12.052 [doi]

6. Daltroy LH, Liang MH, Fossel AH, Goldberg MJ. The POSNA pediatric musculoskeletal functional health questionnaire: report on reliability, validity, and sensitivity to change. Pediatric Outcomes Instrument Development Group. Pediatric Orthopaedic Society of North America. J Pediatr Orthop 1998;18:561-71.

7. Lockitch G, Halstead A, Albersheim S, MacCallum C, Quigley G. Age-and sex-specific pediatric reference intervals for biochemistry analytes as measured with the Ektachem-700 analyzer. Clinical chemistry 1988;34:1622-5.

8. Kruse K, Kracht U, Göpfert G. Renal threshold phosphate concentration (TmPO4/GFR). Archives of disease in childhood 1982;57:217-23.

9. Chesney RW, Rosen M, Hamstra M. Serum 1,25-Dihydroxyvitamin D Levels in Normal Children and in Vitamin D Disorders. Am J Dis Child 1980;134:135-9.