Population Pharmacokinetic and Pharmacodynamic Analyses From a 4-Month Intradose Escalation and Its Subsequent 12-Month Dose Titration Studies for a Human Monoclonal Anti-FGF23 Antibody (KRN23) in Adults With X-Linked Hypophosphatemia

Xiaoping Zhang, PhD1, Thomas Peyret, PhD2, Nathalie H. Gosselin, PhD2, J. F. Marier, PhD2, Erik A. Imel, PhD3, and Thomas O. Carpenter, MD4

Abstract
X-linked hypophosphatemia (XLH) is an inherited metabolic bone disease with abnormally elevated serum FGF23 resulting in low renal maximum threshold for phosphate reabsorption, low serum phosphate (Pi) and 1,25-dihydroxyvitamin D levels with subsequent development of short stature and skeletal deformities. KRN23 is a novel human anti-FGF23 antibody for the treatment of XLH. The pharmacokinetics (PK) and pharmacodynamics (PD) models of KRN23 were assessed following subcutaneous dosing every 28 days over an initial 4-month dose escalation (0.05–0.6 mg/kg) and a subsequent 12-month titration period (0.1–1.0 mg/kg) in XLH adults. The PK of KRN23 was described by a 1-compartmental model with first-order absorption and elimination at doses ≥0.1 mg/kg. The elimination half-life was 17.8 days. Covariates did not affect KRN23 PK. Mean peak serum Pi was attained 7–10 days after dosing and progressively increased following each of the initial 4 doses with comparable peak values attained following the sixth through tenth doses with a slight decrease thereafter. A PK-PD model with a maximum effect (Emax) and a time-varying effective concentration to reach 50% of Emax (EC50,t) described data adequately. Typical Emax was 1.5 mg/dL. Typical EC50,t was 1780 ng/mL and 5999 ng/mL after first and last dose, respectively.

Keywords
X-linked hypophosphatemia (XLH), human anti-FGF23 antibody (KRN23), serum phosphorus, pharmacokinetics, pharmacodynamics

Loss-of-function mutations in PHEX cause X-linked hypophosphatemia (XLH), the most common heritable form of rickets, with an estimated prevalence of 1:20,000.1 PHEX mutations cause increased bone expression of fibroblast growth factor 23 (FGF23).2,3 FGF23 reduces renal tubular phosphate reabsorption via effects on sodium-phosphate cotransporters, causing low serum inorganic phosphorus concentration (Pi).4–6 FGF23 also alters production and degradation of 1,25-dihydroxyvitamin D [1,25(OH)2D], resulting in low or normal serum levels, which are considered an inappropriate physiologic response relative to the hypophosphatemia in XLH.1,2,7

A reduced bone mineral apposition rate results in osteomalacia, rickets, and consequent skeletal deformities including leg bowing and short stature. Additional disease features include bone pain and pseudofractures, especially in adults. Standard therapy with calcitriol and phosphate improves skeletal mineralization in XLH but does not correct the defects in renal phosphorus reabsorption or 1,25(OH)2D production.8–11 The effects of standard therapy on growth and skeletal deformity are highly variable.12 Therapeutic responses are further limited by poor compliance, due to the requirement for multiple daily doses, and the common occurrence of adverse gastrointestinal effects. Serious long-term complications of therapy (hypercalcioria, nephrocalcinosis, and hyperparathyroidism) necessitate frequent monitoring and careful dose titration.12

1Kyowa Hakko Kirin Pharma Inc, Princeton, NJ, USA
2Pharsight-A Certara™ Company, Montreal, Quebec, Canada
3Indiana University School of Medicine, Indianapolis, IN, USA
4Yale Center for X-Linked Hypophosphatemia, Yale University School of Medicine, New Haven, CT, USA

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

Submitted for publication 9 July 2015; accepted 3 August 2015.

Corresponding Author:
Xiaoping Zhang, PhD, Kyowa Hakko Kirin Pharma Inc, 212 Carnegie Center, Suite 101, Princeton, NJ 08540
Email: zhangxiaoping@aol.com
ClinicalTrials.gov identifier: NCT00830674 for KRN23-US-02, NCT01340482 for KRN23-INT-001, and NCT01571596 for KRN23-INT-002

[The copyright line for this article was changed on 22 January, 2020 after original online publication.]
KRN23 is a recombinant human IgG1 monoclonal antibody that binds FGF23 and has been demonstrated to block FGF23 activity.13 In a phex-deficient mouse, treatment with anti-FGF23 antibodies increased serum Pi and 1,25(OH)2D levels and improved rickets, osteomalacia, and skeletal growth.14 In a phase 1 double-blind, placebo-controlled study of adults with XLH, a single intravenous (IV) or subcutaneous (SC) dose of KRN23 increased phosphate reabsorption as indicated by renal tubular maximum for phosphate reabsorption ratio to glomerular filtration rate (TmP/GFR), serum Pi, and 1,25(OH)2D.15 A multicenter phase 1/2 open-label, dose-escalation study was conducted in adults with XLH to evaluate the safety and efficacy of SC KRN23 administered every 28 days for 4 doses, followed by a 12-month extension study.16 Monthly KRN23 significantly increased serum Pi, TmP/GFR, and 1,25(OH)2D in all subjects. KRN23 showed a favorable safety profile in these studies and has potential for effectively treating XLH.16 Using a noncompartmental analysis method, the mean maximum serum KRN23 concentration after SC injection (Tmax) to be 8–11 days.15,17 The terminal mean elimination half-life (t1/2) was 13–19 days.15,17 Bioavailability of KRN23 following SC administration was essentially complete based on the comparability of area under the curve (AUC∞) following IV and SC administration.15 The mean KRN23 serum PK exposures increased with increase of dose in a dose-proportional manner.15,17 The area under the effect concentration-time curve for the change from baseline in TmP/GFR, serum Pi, 1,25(OH)2D, and bone markers for each dosing interval increased linearly with increases in KRN23 area under the serum concentration-time curve over 4 dosing intervals in the 4-month dose escalation study.17

The objective was to assess population pharmacokinetics (PK) and pharmacodynamics (PD) of KRN23 given SC every 28 days over an initial 4-month dose escalation and a subsequent 12-month dose titration period in adults with XLH.16,17 The PK of KRN23 following SC single ascending doses was also included.15

Methods

Included Studies

Thirty-eight adults with XLH were randomized to receive a single dose of KRN23 or placebo by either IV or SC route of administration (KRN23-US-02).15 Of the 38, 12 subjects received KRN23 (0.1, 0.3, 0.6, and 1 mg/kg) by the SC route of administration.15 For the SC cohorts, PK samples were collected up to day 50 postdose. PK data following IV dosing was not included in the analysis because absolute bioavailability for SC administration was complete,15 and IV formulation was not used for the multiple-dose studies.

Twenty-eight adults with XLH were enrolled in a 4-month open-label, phase 1/2 dose-escalation study (KRN23-INT-001),16 of which 22 subjects continued in a 12-month extension study (KRN23-INT-002).16 In the KRN23-INT-001 study, subjects were treated with 4 SC doses of KRN23 every 28 days using stepwise dose escalation from 0.05 to 0.1 to 0.3 to 0.6 mg/kg. In the KRN23-INT-002 study, subjects started with same dose levels achieved at the last dose level in the first study. Fasting serum Pi on day 26 postdosing was used to guide dose changes.16 The primary efficacy outcome was the proportion of subjects achieving maximum fasting serum Pi within the normal range (>2.5 to ≤4.5 mg/dL). In study KRN23-INT-001, the serum Pi levels and KRN23 concentrations were measured at predose, and at days 3, 7, 12, 18, and 26 postdose for each of 4 dosing intervals. In study KRN23-INT-002, the serum Pi levels were measured at predose and at days 7, 14, and 25 for each of 12 dosing intervals, at days 38 and 81 after the last dose, and at the end of study/early withdrawal, whereas KRN23 concentrations were measured only at predose at each dose visit with postdose at visits 46, 47, 48, 49, and 50 after the last dose (follow-up visits).

All 3 studies were approved by relevant local institutional review boards at the investigative sites.15,16 All participants signed an informed consent form at the screening visit.

Analytical Methods

Serum KRN23 concentrations were measured at Kyowa Hakko Kirin California, Inc (La Jolla, California) using a validated sandwich enzyme-linked immunosorbent assay (ELISA) (data on file), which employs an anti-KRN23 mouse monoclonal antibody as the capture antibody and a biotinylated anti-KRN23 monoclonal antibody as the detection antibody (both were supplied from Kyowa Hakko Kirin Co, Ltd, Japan).15,17 With the use of a streptavidin-alkaline phosphatase conjugate to catalyze the chemiluminescent substrate, the resulting chemiluminescent light intensity was measured and the concentrations of KRN23 were calculated. The calibration range was 50 to 3000 ng/mL. The lower limit of quantification was 50 ng/mL. The assay accuracy (relative error) and precision (coefficient of variation for the mean) were within acceptance criteria (±20%) for a majority of quality control samples. Ten individual lots of pooled human serum were spiked with 50 ng/mL KRN23. The relative error of detection ranged from −11.4% to 7.2%, whereas all the unspiked individual lots yielded concentrations below the low limit of quantification (50 ng/mL), confirming the selectivity of the assay.

Serum Pi levels were analyzed by Quest Diagnostics Inc using standardized automated methodology.

Computer Software

Population PK and PK-PD analysis were performed using a nonlinear mixed-effect modeling approach (NLME)
with Phoenix\textsuperscript{TM} NLME$^\text{®}$ v1.3 (Pharsight – A Certara\textsuperscript{TM} Company). Dataset preparation, exploration, and visualization of the data as well were performed using S-PLUS$^\text{®}$ Version 8.2 (Tibco, Seattle, Washington) and R V2.15.3$^\text{18}$ with comprehensive R archive network (CRAN) packages.

**Population PK Methodology**

Various compartmental PK models were used to fit serum concentration-time profiles of KRN23. The PK model consisted of the following: description of the relationships between serum concentration and time and a variance component characterizing between- and within-subject variability in model parameters. Models had the forms

\[ C_{ij} = C(D_i, t_j, \theta_i) + e_{ij} \]  \hspace{2cm} (1)

\[ \theta_i = (\theta_{i1}, \ldots, \theta_{im}) \]  \hspace{2cm} (2)

where \( C_{ij} \) was concentration at \( j \)-th collection time for subject \( i \), \( D_i \) represents dose for subject \( i \), \( \theta_i \) was the vector of \( m \) PK parameters for subject \( i \), and \( e_{ij} \) was random error associated with the \( j \)-th concentration for subject \( i \). Between-subject variability (BSV) in parameters was modeled with a lognormal distribution as described below:

\[ \theta_{in} = \theta_{TVn} \cdot \exp(\eta_{in}) \]  \hspace{2cm} (3)

\[ (\eta_{1}, \ldots, \eta_{m}) \sim \text{MVN} \left( 0, \Omega \right) \]  \hspace{2cm} (4)

where \( \theta_{TVn} \) was the population-typical value for the \( n \)-th PK parameter, eg, apparent clearance (CL/F), and \( \eta_{in} \) was the random intersubject effect on the \( n \)-th parameter for subject \( i \). Random effects \( (\eta_{1}, \ldots, \eta_{m}) \) followed a multivariate normal distribution (MVN) with mean 0 and estimated variance \( \omega^2 \) included in the OMEGA (\( \Omega \)) matrix. Hereafter, individual random effects of CL/F and apparent volume of distribution (\( V_{c}/F \)) were included in the modeling analysis.

The population PK models included, as proposed for monoclonal antibodies,$^\text{19}$ allometric functions on CL/F and \( V_{c}/F \) of KRN23.

Model evaluation and selection were based on standard model diagnostics and goodness-of-fit criteria (eg, log-likelihood difference) and by looking at pertinent graphical representations of goodness of fit (eg, fitted and observed concentrations vs time, weighted residuals vs time). Model validation and qualification of population PK models for KRN23 were based on the standard diagnostic plots.$^\text{20}$

Internal model validation of the PK and PK-PD models was conducted by performing a visual predictive check (VPC) on PK sampling time for the PK model and on serum \( \Delta \Pi \) for PK-PD model. Based on the estimates of the final models, time-PK and PK-PD profiles were simulated using 1000 replicates of the subjects. Medians and fifth and ninety-fifth percentiles of observations within each of the 8 bins separated with equal number of data were derived and compared to the 90\% confidence interval (CI) of the corresponding simulated percentiles.

**Results**

**Patient Characteristics at Baseline**

The clinical characteristics of the patients enrolled in the single- and multiple-dose studies are listed in Table 1. In the single-dose trial (KRN23-US-02), 12 subjects received SC treatment. In the multiple-dose trials, KRN23 was administered to 28 subjects during the
dose-escalation study (KRN23-INT-001); 26 of these subjects (92.9%) received all 4 doses. Of these, 22 subjects entered the extension study (KRN23-INT-002), and 19 subjects received all 16 doses.

Baseline age, weight, sex, and height were comparable among single dose and multiple dose studies. At baseline, median intact FGF23 levels were higher than the upper limit of the normal range. Prior to dosing, mean serum Pi concentrations, and TmP/GFR were comparable among the groups and lower than the normal ranges. Baseline values for serum 1,25(OH)2D, 25-hydroxy-vitamin D [25(OH)D], total calcium, and 24-hour urine calcium were in the ranges typical of those observed in adults with XLH and did not differ among single-dose and multiple-dose study groups. As expected in patients with XLH, baseline mean values for serum parathyroid hormone (PTH) and BALP were near or above the upper limit of the normal ranges.

### Population Pharmacokinetic Database
A total of 40 subjects (12 from the single-dose study and 28 from the multiple-dose study) were included in the population PK analysis. A total of 1192 measurable KRN23 concentrations (200 from the single-SC-dose study, 719 from the multiple-SC-dose escalation study, and 273 from its extension study) were available to construct the model for population PK analysis.

### Population Pharmacodynamic Database
A total of 28 subjects who participated in the multiple-dose studies were included in the population PK-PD analysis. There were 1621 observed serum Pi concentration data. As more serum Pi data were available than were KRN23 concentration data, individual PK parameters from the final population PK model derived from PK data analysis were employed to predict KRN23 concentrations at each time point where serum Pi was available.

---

### Table 1. Characteristics of XLH Patients at Baseline

| Characteristics | KRN23-US-02 | KRN23-INT-001 | KRN23-INT-002 | Reference Range |
|----------------|-------------|---------------|---------------|----------------|
| Demographics   | N = 12b     | N = 28c       | N = 22d       | NA             |
| Age (years)    | 48 ± 11 (25-68) | 42 ± 14 (20) | 42 ± 15 (20) | NA             |
| Sex (male, female), n | 6, 6        | 9, 19         | 9, 13         | NA             |
| Race (white, African American), n | 12, 0       | 27, 1         | 21, 1         | NA             |
| Weight (kg)    | 73 ± 16 (48, 103) | 70 (46, 122)* | 75.3 (51, 124)* | NA             |
| Height (cm)    | 151 ± 6 (143, 164) | 150 ± 12 (122, 170) | 151 ± 13 (123, 170) | NA             |

Laboratory measurements

|                   | KRN23-US-02 | KRN23-INT-001 | KRN23-INT-002 | Reference Range |
|-------------------|-------------|---------------|---------------|----------------|
| Intact FGF23 (pg/mL) | 88.4 (67, 174) | 95 (36, 3520)* | 85 (36, 3520)* | 8-5421 |
| Serum Pi (mg/dL)   | 1.6 ± 0.3 (0.9, 2.1) | 1.9 ± 0.3 (0.8, 2.3) | 1.6 ± 0.3 (0.9, 2.0) | 2.5-4.522 |
| TmP/GFR (mg/dL)    | 1.9 ± 0.5 (1.1, 2.6) | 1.6 ± 0.4 (0.8, 2.3) | 1.6 ± 0.3 (0.9, 2.0) | 2.5-4.223 |
| Serum 1,25(OH)2D (pg/mL) | 42 ± 18 (13-70) | 37 ± 14 (10, 62) | 36 ± 13 (10, 61) | 15.9-55.624 |
| Serum 25(OH)D (ng/mL) | 32 ± 14 (13-53) | 25 ± 9 (12-44) | 23 ± 8.7 (12-37) | 32-10024 |
| Serum total calcium (mg/dL) | 9.0 ± 0.3 (8.5, 9.5) | 9.1 ± 0.4 (8.5, 10.2) | 9.1 ± 0.4 (8.5, 10.2) | 8.5-10.322 |
| Serum PTH (pg/mL)  | 76 ± 46 (25, 171) | 74 (38, 143) | 68.5 (40, 143) | 10-6522 |
| Serum BALP (µg/L)  | 30 ± 12 (16, 61) | 28 ± 13 (8, 52) | 31 ± 12 (13, 52) | Female: 3.7-20.9 |
|                   | 79           |               |               | Female: 2.9-14.5 (Premenopausal)22 |
| 24-hour urine calcium (mg/24 hour) | 115 ± 67 (25, 240) | 67 (11, 253)* | 79 (11, 253)* | Female: 3.8-22.6 (Postmenopausal)22 |
|                   | 79           |               |               | Female: 50-300 |
|                   |              |               |               | Female: 50-25022 |

---

1,25(OH)2D, 1,25-dihydroxyvitamin D; 25(OH)D; 25-hydroxyvitamin D; BALP, bone alkaline phosphatase; FGF23, fibroblast growth factor 23; PTH, parathyroid hormone; Pi, serum phosphorus (inorganic); TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.

*aData are presented from baseline study visits and expressed as mean ± standard deviation (range) unless otherwise noted.

*bThis column provides baseline (pre-KRN23) values on all 12 subjects who received KRN23 single dose subcutaneously in the study (KRN23-US-02). The data are included in pharmacokinetic modeling assessments.

*cThis column provides baseline (pre-KRN23) values on all 28 subjects who entered the dose-escalation trial (KRN23-INT-001).

*dThis column shows the baseline (pre-KRN23) values 22 subjects who later went on to enter the 12-month extension trial (KRN23-INT-002).

*eData distribution was skewed and median (range) is reported.

*f*n = 24 for serum 1,25(OH)2D.
PK-PD modeling used individual simulated serum KRN23 and observed serum Pi concentrations.

KRN23 Population Pharmacokinetics

Various structural models were fitted to the concentration-time data of KRN23 described by typical PK compartmental models (eg, 1- or 2-compartmental model with linear elimination). All structural PK models were parameterized in terms of apparent clearance (CL/F) and apparent volume of distribution (Vc/F) with allometric functions on CL/F and Vc/F as follows:

$$\frac{\text{CL/F}1}{\text{CL/F}i} = \left(\frac{\text{WT}i}{70}\right)^{0.75}$$  \hspace{1cm} (7)

$$\frac{\text{Vc/F}1}{\text{Vc/F}i} = \left(\frac{\text{WT}i}{70}\right)^{1.0}$$  \hspace{1cm} (8)

The absorption phase was modeled using a first-order absorption rate constant (Ks) with or without a lag time. BSV of PK parameters was assessed using a log-normal distribution with and without correlation. The details of the model discrimination are presented in Supplementary Table S1. The one-compartment model with BSV on Ks, CL/F, and Vc/F with additive and proportional errors was selected as the structural model. No lag time was necessary for KRN23 absorption.

The assumption for nonlinear clearance was formally tested. Typical CL/F values for each dose level were first integrated in the model. The model with a typical value for each dose showed that the lower dose level of KRN23 (0.05 mg/kg) was associated with a higher CL/F value (0.320 L/day) as compared to those observed from 0.1 to 1.0 mg/kg dose range (0.271 to 0.294 L/day). The above results suggest that the PK of KRN23 was linear from 0.1 to 1.0 mg/kg. Thus, the population PK modeling was performed with CL/F for the lower dose level of KRN23 (0.05 mg/kg) and by grouping the higher doses (0.1 to 1.0 mg/kg) together. This resulted in a decrease in minimum objective function (MOF; difference of 37.117, Supplementary Table S2).

Exploratory analyses were first performed to visually assess the effect of key covariates (ie, sex, dose levels, body weight at baseline, intact FGF23, BALP) on PK parameters of KRN23. Overall, there were no relevant trends that were observed for the covariate effect with the exception of the dose levels on CL/F. The inclusion of WT using the equations 1 and 2 well predicted the effect of body weight on the PK parameters of KRN23.

The performance of the final population PK model of KRN23 is presented in Figure 1. The population PK model adequately fitted the observed KRN23 concentration-time data following SC administration. The observed data and individual predicted data (Figure 1, top left) and the observed data versus population PK model predicted data (Figure 1, top right) are in the line of unity. The concentration data over time for model-predicted and observed data were superimposed (Figure 1, lower left). Conditional weighted residual (CWRES) values homogeneously distributed around 0 (Figure 1, lower right). Of the 1192 KRN23 serum concentrations (C_{KRN23}) in the population PK analysis, only 2 concentrations showed absolute values of CWRES > 4. A sensitivity analysis was performed to identify the effect of these concentrations in the model predictions. These concentrations had no significant effect on the estimates of model parameters with a relative difference ranging from ~1.83% (proportional error) to 0.27% (BSV on CL) (Supplementary Table S3).

The appropriateness of the current model to perform simulations was also evaluated using VPC on serum concentration-time profiles of KRN23 stratified by tertiles of body weight (ie, <65.5 kg, ≥65.5 and <79.4 kg, ≥79.4 kg). A total of 1000 replicates of the original observed C_{KRN23} were simulated with the final population PK model. As presented in Figure 2 for two multiple-dose studies (KRN23-INT-001 and KRN23-INT-002), the model adequately simulated the concentration-time profiles of KRN23 in adults with XLH since the vast majority of the fifth, fiftieth, and ninety-fifth percentiles of observed concentrations were within the 90%CI of the corresponding simulated percentiles.

The final population PK model parameters are shown in Table 2. The rate constant of absorption of KRN23 was very slow (0.349 day⁻¹). For a 70-kg subject, typical values of CL/F and Vc/F of KRN23 following SC dosing of doses ≥0.1 mg/kg were 0.279 L/day and 7.17 L, respectively. The typical CL/F with a dosing level of 0.05 mg/kg was 1.15-fold (95%CI: 1.109–1.194) higher than that at other higher dose levels. Relative standard errors of PK parameters were lower than 8%.

Results for interindividual variability (BSV) (41% for K_{a}, 36.8% for CL/F, and 30.5% for Vc/F) were acceptable. The residual variability (RES) of the model was low, with proportional error of 21.8% and additive error of 0.099 ng/mL.

Population PK-PD Modeling

The triangles in Figure 3 presents the serum Pi change from baseline (ΔPi) (day 0 in study KRN23-INT-001) vs time from first dose from study KRN23-INT-001. Based on LOESS lines, ΔPi increased as dose escalation occurred in the KRN23-INT-001 study. In the extension study KRN23-INT-002, ΔPi reached a plateau between 168 and 280 days after the first dose (between the sixth and tenth doses). However, a slight decrease in effect was apparent from day 280 to the end of study.
The time component was tested on $E_{\text{max}}$ and $EC_{50}$ using linear and sigmoid functions. The PK-PD data sets were best fitted to a time-varying $EC_{50,t}$ model as follows:

$$D_{Pi} = \frac{\text{simCKRN23} \cdot E_{\text{max}}}{EC_{50,t} + \text{simCKRN23}}$$

(9)

$$EC_{50,t} = tvEC_{50} + \alpha \cdot \frac{t^\gamma}{32^\gamma + t^\gamma}$$

(10)

where $\alpha$ = maximum rate of increase of $EC_{50,t}$; $EC_{50,t}$ = simulated serum KRN23 concentration to reach 50% of maximal effect at time $t$; $E_{\text{max}}$ = maximum effect; $tvEC_{50} = EC_{50,t}$ at time 0; $\gamma$ = Hill coefficient; $\text{simCKRN23} = \text{simulated KRN23 concentration}$; and $t = \text{time (weeks) after dosing}$.

Figure 3 represents the prediction of $D_{Pi}$ over time for the $E_{\text{max}}$ model with time component on $EC_{50,t}$. Figure 3 indicates that the model, including a time-varying $EC_{50,t}$, resulted in a good fit throughout treatment duration for the 16-month period. The observed (blue line) and predicted (red line) data were superimposed. The plateau observed between 168 and 280 days after the first dose and then the marked decrease from day 280 onward were well described with the population PK-PD model with time varying $EC_{50,t}$. The estimated PK-PD model parameters are shown in Table 3. Supplementary Figure S1 shows the VPC of the population PK-PD model, indicating that the PK-PD model adequately described the observed data at

Figure 1. Goodness of fit of final population PK model. CWRES, conditional weighted residuals; IDENT, identity line; IPRED, individual predicted concentrations; LOESS, locally weighted scatter plot smoothing; OBS, observed concentrations; PRED, population predicted concentrations.
The $E_{\text{max}}$ and serum KRN23 concentrations associated with 50% of the $E_{\text{max}}$ at time 0 ($t_{vE50}$) were 1.5 mg/dL and 1780 ng/mL, respectively. Relative standard errors of $t_{vE50}$, $\alpha$, and $\gamma$ were between 15.9% and 17.1%. $E_{\text{max}}$ value was fixed to achieve the covariance matrix and to obtain precision of the PK-PD parameters. The fixed value of 1.5 mg/dL was based on data exploration and previous run results. The predicted typical values of $EC_{50,1}$ in function of time from the $E_{\text{max}}$ model with time component are presented in Supplementary Figure S2. $EC_{50,1}$ increased with increase of treatment duration. At week 32 (day 224), $EC_{50,1}$ was 4102 ng/mL, and at the end of the treatment at week 72 (day 504), $EC_{50,1}$ was 5999 ng/mL. Over 560 days, the value of $EC_{50,1}$ increased from 1780 to 6098 ng/mL.

**Discussion**

Present population PK analysis resulted in PK parameters similar to other monoclonal antibody therapies. The typical CL/F with a dosing level of 0.05 mg/kg was significantly higher than that at higher dose levels. This could be because of the free circulating intact FGF23 in subjects with XLH (Table 1), which is more evident at lower doses (ie, lower KRN23 serum concentration). Nonlinear clearance of other monoclonal antibodies at lower dose levels was previously reported in the literature.25

The rate constant of absorption of KRN23 was very slow (0.349 day$^{-1}$) (Table 2). This would correspond to an absorption half-life of approximately 2.0 days (0.693/0.349 day$^{-1}$) and would further suggest that almost all the amount of drug administered would be absorbed from the SC injection site after approximately 10 days ($5 \times 2$ days), with a previously noted absolute

**Table 2. Typical Values of the Final Population PK Model of KRN23**

| PK Parameters | Typical Values | BSV % | RSE % | 90% Confidence Interval |
|---------------|----------------|-------|-------|-------------------------|
| $K_s$ (day$^{-1}$) | 0.349 | 41.0% | 7.2% | 0.300-0.398 |
| CL/F (L/day) | $0.279 \times (WT/70)^{0.75}$ for doses higher than 0.05 mg/kg | 36.8% | 5.1% | 0.247-0.311 |
| | (= 0.279 Typical) | | | |
| | $0.321 \times (WT/70)^{0.23}$ for dose of 0.05 mg/kg | 1.9% | | (1.109-1.194) |
| | (= 1.15 Typical) | | | |
| $V_c$ (L) | $7.17 \times (WT/70)^{1}$ | 30.5% | 5.1% | 6.45-7.89 |
| Error prop (%) | 21.8% | NA | 32.9% | 7.3%-35.8% |
| Error additive (ng/mL) | 0.099 | NA | 23.3% | 0.054-0.144 |

BSV, between-subject variability; CL/F, apparent clearance; $K_s$, first-order rate of absorption; PK, pharmacokinetic; prop, proportional; $V_c/F$, apparent central volume of distribution; WT, body weight (kg); NA, not applicable; RSE, relative standard error.

Shrinkage: CL/F: 4.6%, $V_c$/F: 7.4%, $K_s$: 18.8%.
bioavailability of almost 100%.\textsuperscript{15} This is consistent with $T_{\text{max}}$ previously reported for KRN23 (8 to 11 days) using model-independent analyses from both single-dose\textsuperscript{15} and multiple-dose studies.\textsuperscript{17}

For a 70-kg subject, typical values of CL/F and Vc/F of KRN23 following SC dosing of doses $\leq 0.1$ mg/kg were 0.279 L/day and 7.17 L, respectively. This would correspond to an elimination rate constant of 0.03891 day$^{-1}$ (ie, 0.279/7.17) (Table 2) and an elimination half-life of approximately 17.8 days ($0.693/0.03891$ day$^{-1}$).

This elimination half-life value is consistent with the half-life estimated from noncompartmental analysis (13 to 19 days) from both single- and multiple-dose studies\textsuperscript{15,17} as well as those previously reported for other monoclonal antibodies.\textsuperscript{25}

Assuming all patients received the 0.1 mg/kg dose, the PK model-predicted AUC at steady state appears to be independent of body weight (Supplementary Figure S3); therefore, the effect of body weight on the model adequately corrected the body weight factor to achieve uniform PK exposure across the body weight range in the study.

Despite the small sample size of subjects (ie, $N = 40$ subjects), good precision of the PK parameters was achieved with relative standard errors less than 8%.

In order to enrich the PK-PD data set and allow a good estimation of the effect of time on the PK-PD relationship, a population PK model was used to simulate KRN23 concentrations to pair with all observed serum $\Delta Pi$ concentrations. PK-PD modeling was thus performed based on the enriched PK-PD dataset. The PK-PD data were well described by a sigmoid $E_{\text{max}}$ model with time-varying EC$_{50,t}$ component. The EC$_{50,t}$ was found to increase with longer treatment duration (Supplementary Figure S2). Following the first dose, EC$_{50,t}$ required was 1780 ng/mL, whereas EC$_{50,t}$ increased to 6098 ng/mL near the end of 16 months of treatment.

In the present study, the effect of KRN23 on the increase of serum Pi appears to decrease after long-term treatment. The decrease in $\Delta Pi$ after multiple doses over time was described by an increase of EC$_{50,t}$ over time. This may be explained by the increases in total and unbound FGF23 concentrations which increased above predose levels after KRN23 administration.\textsuperscript{26}

Increased FGF23 concentrations in the circulation could result in a need for a higher KRN23 concentration (EC$_{50,t}$) to bind to the greater amount of FGF23 and inhibit its actions. It was also noted that peak serum 1,25(OH)$_2$D levels decreased gradually as number of doses increased in the extension study.\textsuperscript{17} The decreased 1,25(OH)$_2$D concentration after long-term treatment may lessen intestinal absorption of phosphate. Both increased FGF23 and decreased 1,25(OH)$_2$D concentration may thus contribute to the increased EC 50,t over time.

Increased EC$_{50,t}$ over time suggests that higher doses may be needed after long-term treatment to achieve comparable $\Delta Pi$ increase as at the beginning of the treatment. However, this assumption needs to be verified in future clinical trials with treatment duration greater than 16 months and adequate number of serum samples for Pi levels. More PK-PD data from future trials are needed to refine the model to guide dosing strategy.

In summary, population PK and PK-PD modeling indicated that there is a slow KRN23 SC absorption over 10 days, higher CL/F at the lowest dose of 0.05 mg/kg, and an elimination half-life of about 17.8 days, supporting a monthly dose. The EC$_{50,t}$ increased with longer duration of treatment, which may indicate a need for higher doses

Table 3. Typical Values of the Final Population PK-PD Model of KRN23

| PK-PD Parameters    | Typical Values | BSV (%) |
|---------------------|----------------|---------|
|                     | [RSE (%)]      | [RSE (%)] |
| EC$_{50,t}$         | 73.8% (14.8%)  |         |
| tvEC$_{50}$ (ng/mL) | 1799.6 (15.9%) |         |
| $\alpha$ (ng/mL/week)| 4605.5 (16.8%)|         |
| $\gamma$            | 2.88 (17.1%)   |         |
| E$_{\text{max}}$ (mg/dL) | 1.5 (FX)      |         |
| Residual additive error (mg/dL) | 0.310 (1.8%) |         |

BSV, between-subject variability; EC$_{50,t}$, simulated serum KRN23 concentration to reach 50% of maximal effect at time t; E$_{\text{max}}$, maximum effect; PD, pharmacodynamic; PK, pharmacokinetic; RSE, relative standard error; t, time (weeks); tvEC$_{50}$, EC$_{50,t}$ at time 0; XLH, X-linked hypophosphatemia; $\alpha$, maximum rate of increase of EC$_{50,t}$; $\gamma$, Hill coefficient. Note: E$_{\text{max}}$ value was fixed to achieve the covariance step and obtain precision of the parameters.
after a longer duration of therapy. The results from the present population PK and PK-PD model analyses are valuable for guiding further studies of KRN23 in the treatment of XHL.

Acknowledgments
This study was funded by Kyowa Hakko Kirin Pharma Inc. We are particularly grateful to the dedicated subjects who participated in the study. We thank clinical study investigators: Mary D. Ruppe, Thomas J. Weber, Francis H. Glorieux, Anthony A. Portale, Karl Insogna, and Munro Peacock. We thank clinical study coordinators and subinvestigators, Elizabeth Olear and Rebecca Sullivan, at Yale University School of Medicine, New Haven, CT; Connie Sullivan and Marian Hart at Indiana University, Indianapolis, IN; Margaret Stewart at Duke University Medical Center, Durham, NC; Nathaniel Jacob Harrison and Monika Ruscheinsky at the University of Texas Health Science Center at Houston, Houston, TX; Michaela Durigova at Shriners Hospital for Children, Montreal, Canada; and Dr Farzana Perwad, Stephanie Lemp, and Vinodhini Lakshman at the University of California, San Francisco, CA. We thank Val Barra and Yamamoto Katsuhiro for analyses of KRN23 concentrations (Kyowa Hakko Kirin California, Inc, La Jolla, CA). We thank Mark A. Klausner for medical monitoring, Maria Vergeire for clinical trial management, and Peter Todd for editing and formatting (Kyowa Hakko Kirin Pharma Inc).

Author Contributions
All authors contributed to authoring the manuscript. X.Z., N.H.G., J.F.M., and T.P. contributed to data analysis and interpretation. X.Z., E.A.I., and T.O.C. designed and performed research.

Prior Presentations
Results were presented in part at the “American Society for Clinical Pharmacology and Therapeutics 2015 Annual Meeting” in New Orleans, LA, USA, March 3–7, 2015. Poster presentation numbers: PI078 and PI097.

Disclosures
The data in this article were obtained from KRN23-US-02, KRN23-INT-001, and KRN23-INT-002 studies funded by Kyowa Hakko Kirin Pharma Inc. Drs E. A. Imel and T. O. Carpenter received research grants and/or consulting fees (other than Advisory Board or Board of Directors) during study conduct from the sponsor and from ultragenyx Pharmaceuticals, Inc for subsequent work related to KRN23. Dr X. Zhang is employed by Kyowa Hakko Kirin Pharma Inc. Drs T. Peyret, N. H. Gosselin, and J. F. Marier received consulting fees.

Funding
Kyowa Hakko Kirin Pharma Inc.

References
1. Burnett CH, Dent CE, Harper C, Warland BJ. Vitamin D-resistant rickets. Am J Med. 1964;36:222–232.
2. Imel EA, Econs MJ. Fibroblast growth factor 23: roles in health and disease. J Am Soc Nephrol. 2005;16:2565–2575.
3. Liu S, Quarles LD. How fibroblast growth factor 23 works. J Am Soc Nephrol. 2007;18:1637–1647.
4. Larsson T, Marshall R, Schipani E, et al. Transgenic mice expressing fibroblast growth factor 23 under the control of the alpha 1(1) collagen promoter exhibit growth retardation, osteomalacia, and disturbed phosphate homeostasis. Endocrinology. 2004;145:3087–3094.
5. Shimada T, Uraoka I, Yamazaki T, et al. FGF-23 transgenic mice demonstrate hypophosphatemic rickets with reduced expression of sodium phosphate cotransporter type IIa. Biochem Biophys Res Commun. 2005;314:409–414.
6. Gattinoni J, Bates C, Twombly K, et al. FGF23 decreases renal NaPi-2a and NaPi-2c expression and induces hypophosphatemia in vivo predominantly via FGF receptor 1. Am J Physiol Renal Physiol. 2009;297:F282–291.
7. Haussler M, Hughes M, Baylink D, et al. Influence of phosphate depletion on the biosynthesis and circulating level of 1α,25-dihydroxyvitamin D. Adv Exp Med Biol. 1997;81:233–250.
8. Glorieux FH, Marie PJ, Pettifor JM, Delvin EE. Bone response to phosphate salts, ergocalciferol, and calcitriol in hypophosphatemic vitamin D-resistant rickets. N Engl J Med. 1980;303:1023–1031.
9. Costa T, Marie PJ, Scrivier CR, et al. X-linked hypophosphatemia: effect of calcitriol on renal handling of phosphate, serum phosphate, and bone mineralization. J Clin Endocrinol Metab. 1981;52:460–472.
10. Harrell RM, Lyles KW, Harrelson JM, Friedman NE, Dreznner MK. Healing of bone disease in X-linked hypophosphatemic rickets/osteomalacia. J Clin Invest. 1985;75:1858–1868.
11. Petersen DJ, Boniface AM, Schranck FW, Rupich CR, Whyte MP. X-linked hypophosphatemic rickets: a study (with literature review) of linear growth response to calcitriol and phosphate therapy. J Bone Miner Res. 1992;7:583–590.
12. Carpenter TO, Imel EA, Holm IA, et al. A clinician’s guide to X-linked hypophosphatemia. J Bone Miner Res. 2011;26:1381–1388.
13. Yamazaki Y, Tamada T, Kasai N, et al. Anti-FGF23 neutralizing antibodies show the physiological role and structural features of FGF23. J Bone Miner Res. 2008;23:1509–1518.
14. Aono Y, Yamazaki Y, Yasutake J, et al. Therapeutic effects of anti-FGF23 antibodies in hypophosphatemic rickets/osteomalacia. J Bone Miner Res. 2009;24:1879–1888.
15. Carpenter TO, Imel EA, Ruppe MD, et al. Randomized trial of anti-FGF23 antibody (KRN23) in X-linked hypophosphatemia. J Clin Invest. 2014;124:1587–1597.
16. 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):2565–2573.
17. Zhang X, Imel EA, Ruppe MD, et al. Pharmacokinetics and pharmacodynamics of a human monoclonal anti-FGF23 antibody (KRN23) in the first multiple ascending dose trial treating adults with X-linked hypophosphatemia. J Clin Pharmacol. 2015. doi: 10.1002/jcph.570.
18. R Core Team. 2013. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing. http://www.R-project.org/. Accessed on July 26, 2015.
19. Dong JQ, Salinger DH, Endres CJ, et al. Quantitative prediction of human pharmacokinetics for monoclonal antibodies: retrospective analysis of monkey as a single species for first-in-human prediction. Clin Pharmacokinet. 2011;50:131–142.
20. Karlsson MO, Savic RM. Diagnosing model diagnostics. Clin Pharmacol Ther. 2007;82:17–20.
21. Yamazaki Y, Okazaki R, Shibata M, et al. Increased circulatory level of biologically active full-length FGF-23 in subjects with
hypophosphatemic rickets/osteomalacia. *J Clin Endocrinol Metab.* 2002;87(11):4957–4960.

22. Investigator Laboratory Instruction Manual. Quest Diagnostics Clinical Trials. 27027 Tourney, Suite 2E, Valencia, CA 91355.

23. Walton RJ, Bijvoet OL. Nomogram for derivation of renal threshold phosphate concentration. *Lancet.* 1979;2(7929):309–310.

24. Investigator Laboratory Instruction Manual. Cranford, NJ: Esoterix Clinical Trials Services, A Division of LabCorp.

25. Tabrizi MA, Tseng CM, Roskos LK. Elimination mechanisms of therapeutic monoclonal antibodies. *Drug Discov Today.* 2006;11:81–88.

26. Zhang X, Imel EA, Ruppe MD, et al. Pharmacokinetics (PK) and pharmacodynamics (PD) following four monthly doses of a human monoclonal anti-FGF23 (fibroblast growth factor 23) antibody (KRN23) in adults with X-linked hypophosphatemia (XLH). *Endocr Rev.* 2014;35(suppl 3):17–20.

**Supporting Information**

Additional supporting information may be found in the online version of this article at the publisher’s web-site.