Denosumab Dose Selection for Patients with Bone Metastases from Solid Tumors.

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TRANSLATIONAL RELEVANCE

Inhibition of RANKL by denosumab decreases urinary N-telopeptide normalized to urinary creatinine (uNTx/Cr) levels, which have been associated with reduced incidence of skeletal-related events (SRE) in patients with bone metastases from solid tumors. In this population, we characterized the relationship between serum denosumab concentration and uNTx/Cr suppression and explored the role of denosumab dosing regimens. The typical maximum uNTx/Cr suppression (efficacy) and the denosumab concentration providing half-maximal uNTx/Cr suppression (potency) was 93.7% and 31.8 ng/mL, respectively. Simulation analyses indicated that a subcutaneous denosumab dose of 120 mg administered every 4 weeks provides >90% suppression of uNTx/Cr in the maximum proportion of patients relative to other monthly or quarterly doses evaluated. This dosing regimen was selected to investigate the denosumab effect in preventing SRE in Phase 3 studies and is now approved in the US, EU and other countries for patients with bone metastases from solid tumors.
ABSTRACT

Purpose: To quantitatively characterize the longitudinal dose-exposure-response (urinary N-telopeptide normalized to urinary creatinine [uNTx/Cr] suppression) relationship for denosumab in patients with bone metastases from solid tumors.

Experimental Design: Data from 373 patients who received denosumab as single or multiple subcutaneous (SC) doses ranging from 30 to 180 mg (or 0.01 to 3 mg/kg) administered every 4 or 12 weeks (Q4W or Q12W) for up to 3 years were used in this analysis. An inhibitory sigmoid I_{Max} model was used to characterize the time course of uNTx/Cr as a function of serum denosumab concentrations and the M3 method was used to analyze the 52% of uNTx/Cr values below the limit of quantification in the context of a mixed-effects model. Age, weight, sex, race and cancer type were evaluated as potential covariates for model parameters. Model-based simulations were undertaken to explore and predict the role of denosumab dose and dosing intervals on uNTx/Cr suppression.

Results: The typical value (between-subject variability; %) for uNTx/Cr at baseline was 49.2 nM/mM (76.8%), denosumab maximal uNTx/Cr suppression (efficacy) was 93.7% (127%) and the denosumab concentration providing half-maximal uNTx/Cr suppression (potency) was 31.8 ng/mL (287%). No effect of covariates on denosumab efficacy and potency was identified. Simulations indicated that a denosumab SC dose of 120 mg administered Q4W provides >90% suppression of uNTx/Cr in the maximum proportion of patients relative to other Q4W and Q12W doses evaluated.

Conclusions: Over the wide range of dosing regimens examined, a denosumab SC dose of 120 mg administered Q4W is the optimal dosing regimen to suppress uNTx/Cr in patients with bone metastases from solid tumors.
INTRODUCTION

The most common metastatic site for breast cancer, prostate cancer and other solid tumors such as lung, thyroid, and kidney cancers is bone [1]. Bone metastases can result in skeletal-related events (SREs) including pathological fractures, spinal cord compression and the need to undergo radiation to or surgery of the bone [2-4]. The pathophysiology of bone metastases includes locally increased osteoclast-mediated bone breakdown, which results in elevated levels of bone turnover markers (BTM) such as urinary N-telopeptide normalized to urinary creatinine (uNTx/Cr). BTM are not only indicative of excessive bone resorption but have also been associated with disease progression and death [5-8].

RANK ligand (RANKL) plays a critical role in bone remodeling [9]. By binding to its receptor, RANK, on osteoclast precursors and mature osteoclasts, it promotes the terminal differentiation, activation, and survival of osteoclasts, which in turn stimulate bone resorption and increase BTM levels, including uNTx/Cr [10-12]. By secreting cytokines and growth factors that induce osteoblasts to release RANKL into the microenvironment, tumor cells in bone contribute to an imbalance that favors increased bone resorption and elevated BTM. This is reflected clinically as an increased risk of fractures or other SRE [5], and a potentially increased susceptibility of the bone microenvironment to implantation and proliferation of circulating tumor cells [13, 14].

Denosumab (AMG 162, XGEVA™, PROLIA™) is a fully human IgG2 monoclonal antibody with high affinity (K_D = 3·10^{-12} M) [15] and specificity for RANKL [16] that neutralizes the activity of human membrane-bound or soluble RANKL by blocking its binding to RANK [17, 18]. In clinical studies, denosumab has consistently reduced uNTx/Cr levels in patients with bone metastases from solid tumors, reflecting its anti-resorptive effect (19-24).
patients with breast cancer-related bone metastases or multiple myeloma, levels of uNTx/Cr decreased within one day after a single subcutaneous (SC) dose of denosumab and the duration of maximal uNTx/Cr suppression generally increased with dose and persisted for up to 12 weeks following a 3.0 mg/kg dose [21]. Similarly, in patients with bone metastases from breast cancer [22] or bone metastases from solid tumors (including breast and prostate cancers) [23], denosumab demonstrated a rapid, sustained and consistent suppression of uNTx/Cr levels over the dose range of 30 to 180 mg SC administered every 4 weeks (Q4W), or 60 to 180 mg SC administered every twelve weeks (Q12W). The reversibility of the effects on BTM was observed after stopping the denosumab treatment [21, 22].

Three phase 3 studies also assessed suppression of uNTx/Cr levels along with prevention of SRE in patients with bone metastases from breast cancer, prostate cancer, or other solid tumors [19-20, 24-26]. In these studies, denosumab 120 mg SC administered Q4W was more effective in preventing SREs and suppressing uNTx/Cr compared with monthly intravenous zoledronic acid, the prior standard of care in this indication. Currently, denosumab 120 mg administered SC Q4W is approved in the U.S., E.U. and other countries for the prevention of SRE in patients with bone metastases from solid tumors.

Denosumab pharmacokinetics (PK) and the time course of uNTx/Cr suppression have been extensively investigated in multiple clinical studies in cancer patients with bone metastases. Following denosumab SC administration, the absolute bioavailability was 61% [27] and the absorption was slow, reaching peak serum concentrations (C_{max}) within 4 weeks post dose. After reaching C_{max}, serum denosumab concentrations decline over 4 to 5 months with a mean half-life of approximately 25 to 30 days, with sustained concentrations higher than 2000 ng/mL. Consequently, the RANKL-mediated clearance pathway is almost fully saturated [27, 28].
Following repeat monthly administration, approximately 2.7-fold accumulation at steady-state and no evidence of time-dependent PK was observed under the dosage regimens examined.

This paper aims to quantitatively characterize the time course of uNTx/Cr following denosumab administration in patients with bone metastases from solid tumors using a mixed-effect model that accounts for uNTx concentrations below the lower limit of quantification (BLQ). The objectives are four-fold: 1) to characterize the efficacy and potency of denosumab in suppressing uNTx/Cr following SC administration; 2) to quantify the degree of between-subject variability of denosumab pharmacodynamic (PD) parameters; 3) to evaluate patient-related variables as potential sources of PD variability, and 4) to assess the activity of the 120 mg Q4W dosing regimen relative to other dose levels and regimens in suppressing uNTx/Cr.
MATERIALS AND METHODS

Clinical Data. Data collected from the six clinical studies conducted in patients with bone metastases from solid tumors for the development of denosumab were used in this analysis. The dataset included 2013 uNTx/Cr samples from 373 patients treated with single doses of denosumab ranging from 0.1 to 3.0 mg/kg or multiple SC doses of denosumab ranging from 30 to 180 mg and administered Q4W or Q12W for up to 3 years. The relevant characteristics of each clinical study are summarized in Table 1. Additional details of these clinical trials are reported elsewhere [19-22,25,29]. All studies, sponsored by Amgen Inc., were conducted in accordance with principles for human experimentation as defined in the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines and were approved by the respective Investigational Review Boards. Informed consent was obtained from each subject after being told the potential risks and benefits, as well as the investigational nature of the study.

Bioanalysis of uNTx and Creatinine. A modified commercial ELISA, Osteomark® NTx Urine (Wampole Laboratories) was used to measure uNTx concentrations. Microtiter plates coated with NTx were used to capture NTx from urine. After incubation and washing, a monoclonal antibody specific for NTx conjugated to horseradish peroxidase was used to detect uNTx bound to the plate. The lower limit of quantification (LOQ) was 30.0 nM Bone Collagen Equivalents (BCE) for study 2001023 and 62.5 nM BCE for all other studies. The upper LOQ was 2857 nM BCE. Creatinine was measured photometrically utilizing a modification of the Jaffe reaction on Roche Modular Analyzers, with a linear range of the assay between 0.00884 and 2.21 mM (0.1 – 25.0 mg/dL).
Software. Nonlinear mixed effects modeling for the population pharmacodynamic analysis of denosumab was performed using NONMEM Version 7.1.0 (ICON Development Solutions, Ellicott City, MD) with gfortran 4.4 compiler. The stochastic approximation expectation maximization (SAEM) method was used for parameter estimation. Graphical data visualization, evaluation of NONMEM outputs, construction of goodness-of-fit plots, and graphical model comparisons were conducted using S-Plus Version 8 (TIBCO Software Inc., Palo Alto, CA).

Pharmacokinetic and Pharmacodynamic (PKPD) Model. The development of the PKPD model was performed using a sequential process described previously [32]. Therefore individual Bayesian estimates of PK parameters obtained from the denosumab population PK [27] model and the available individual serum concentration data were used to predict the individual denosumab serum concentration-time profile, which in turn was used as an input function into the PD model. Complete details regarding development of the denosumab PK model have been reported elsewhere [27].

A sigmoid maximum inhibition (I_max) model was selected as the structural PD model that best characterized the time course of uNTx/Cr as a function of denosumab serum concentrations. The structure of the inhibitory sigmoid I_max model is as follows:

\[
\frac{uNTx}{Cr} = \frac{uNTx_0}{Cr_0} \left[ 1 - \frac{I_{max} \cdot C^2}{IC_{50}^2 + C^2} \right]
\]

where \( uNTx_0/Cr_0 \) represents the baseline uNTx/Cr; \( C \) represents the predicted denosumab concentration; \( I_{max} \) represents the maximal denosumab inhibition of uNTx/Cr (efficacy), \( IC_{50} \) represents the denosumab serum concentration that produces half-maximal inhibition of
uNTx/Cr (potency), and \( \lambda \) is the Hill coefficient accounting for the sigmoidicity of the concentration-response relationship. The predicted fractional RANKL inhibition as estimated from the population PK model was also tested as a predictor of uNTx/Cr suppression, instead of denosumab serum concentration.

**Statistical Model.** Between-subject variability (BSV) in model parameters was assumed to follow an independent log-normal distribution. However, as \( I_{\text{Max}} \) represents the fraction of maximal suppression, an additive error model in the logit domain was used to constrain the individual \( I_{\text{Max}} \) values to be between 0 to 1. As uNTx/Cr are distributed log-normally rather than normally, all measured uNTx/Cr values and corresponding individual model predictions were converted into natural logarithms and the magnitude of residual variability (RV) in the log domain was modeled using an additive error model.

Since the proportion of uNTx values BLQ was high (52%) and was related to the predicted denosumab concentration (Figure 1A), uNTx values BLQ could not be ignored in this analysis and strategies for handling measurements reported as BLQ were needed. In previous denosumab studies, uNTx concentrations BLQ were substituted by the lower limit of quantification (LOQ). However, common approaches for handling of concentrations BLQ, such as data exclusion or substitution by LOQ, zero, or LOQ divided by two, have been shown to introduce bias in parameter estimates [30, 31]. Thus, methods based on simultaneous modeling of continuous and categorical data, where the BLQ observations are treated as censored data, are preferred from a statistical point of view. Therefore, simultaneous modeling of the continuous uNTx observations and the uNTx values BLQ treated as a categorical variable was conducted. In this analysis, the uNTx values BLQ were treated as censored data and the likelihood for BLQ
observations was maximized with respect to the model parameters (M3 method) [33-36] in order to minimize the impact of the uNTx values BLQ on the efficacy and potency estimates.

The improvement in the fit obtained for each model evaluated was assessed by the likelihood ratio test (LRT, $p = 0.005$), the reduction in the BSV and RV, the precision in parameter estimates, the examination of diagnostic plots, and the shrinkage [37].

Model Evaluation. Two predictive checks (PC) [38] were performed to evaluate the model predictive performance. The first PC evaluated the relationship between uNTx/Cr and denosumab concentration. In this case, uNTx/Cr values of 10000 patients were simulated based on the model parameters and a range of denosumab concentrations, encompassing the expected concentrations of the patients included in the analysis dataset. At each denosumab concentration, the 5th, 50th and 95th percentiles of the simulated uNTx/Cr values were computed and plotted. The observed data were then overlaid and visually compared with the model-based prediction. A similar analysis was conducted to assess the relationship between the proportion of uNTx/Cr BLQ and denosumab concentration by simulating 500 replicates of the analysis dataset.

The second PC evaluated the time course of changes in quantifiable uNTx/Cr and the proportion of uNTx concentrations BLQ following SC administration of 120 mg denosumab Q4W. A total of 500 replicates of the analysis dataset containing the patients with uNTx/Cr time course following 120 mg denosumab Q4W were simulated. For each replicate, the median (and 90% prediction interval) of the simulated uNTx/Cr values for uNTx concentrations above the LOQ and the proportion of uNTx concentrations BLQ (and the 95% confidence interval) were computed and compared with observed data. Baseline concentrations were excluded for evaluation of the proportion of uNTx concentrations BLQ.
**Model Based Simulations.** Based on the final estimates of model parameters, simulations were conducted to explore the role of denosumab dose level and dosing regimen on uNTx/Cr suppression. In this context, simulations were conducted to evaluate: 1) the proportion of patients with at least 90% uNTx/Cr suppression as a function of denosumab serum concentration, and 2) the proportion of patients with at least 90% suppression of uNTx/Cr levels at the trough level achieved at week 25 of treatment across doses ranging from 0 to 180 mg, and Q4W and Q12W dosing regimens. Using the final parameters of the population PK model, individual trough denosumab concentrations at steady-state (week 25) were simulated for each dose level and dosing regimen (N=5000/dose/regimen) and were used to calculate the subsequent suppression of uNTx/Cr based on the model developed, which was summarized by dose and dosing regimen. Furthermore, to investigate the influence of denosumab weight-based dosing vs. fixed dosing, model-based simulations comparing the time course of uNTx/Cr following 6 denosumab doses of 2 mg/kg or 120 mg Q4W were conducted (n=1000/group). Individual body weights and uNTx0/Cr0 were sampled from the analysis dataset.

**RESULTS**

The inhibitory sigmoid I\textsubscript{Max} model was suitable to describe the time course of uNTx/Cr in patients with bone metastases from solid tumors following denosumab SC administration at different dosing schedules. The model parameter estimates and their relative standard error (RSE) are presented in Table 2. Both fixed and random effects were estimated with acceptable precision. The typical value of \( uNTx_0/Cr_0 \) was 49.2 nM/mM and its associated BSV was 77\%. Given the uNTx/Cr data available and the several approaches suggested to handle baseline values [39], an attempt was made to estimate \( uNTx_0/Cr_0 \), its associated variability, and to acknowledge the uNTx/Cr residual variability. However, the goodness of fit was not improved
and the running time was substantially increased with respect to the model that fixed $uNTx_0/Cr_0$ to the observed individual value. Consequently, only $I_{Max}$ and $IC_{50}$ were estimated directly from the data. Furthermore, the model with serum denosumab concentration as the driver of the denosumab direct effect on the $uNTx/Cr$ suppression provided significantly better fit to the data than the model based on fractional RANKL inhibition. Although both models provided similar efficacy estimates ($I_{Max} > 90\%$), the model based on fractional RANKL inhibition estimated a higher potency ($IC_{50} = 3.49\% \sim 7.52$ ng/mL) compared to the model based on denosumab concentration ($IC_{50} = 31.8$ ng/mL). Table 2 also shows that the variability in the denosumab efficacy and potency is high and evidenced a certain degree of shrinkage. Age, sex, race, and body weight had no notable effect on denosumab $I_{Max}$ and $IC_{50}$.

The PC shown in Figure 1 indicates excellent predictive ability of the model to describe $uNTx/Cr$ suppression following single and multiple doses of denosumab. Figure 1A indicates that, at steady state, serum denosumab concentrations during the entire dosing interval following 120 mg Q4W dosing in patients with bone metastases from solid tumors are associated with greater than 95% of patients achieving $uNTx/Cr$ values below 50 nM BCE/mM, a cut-off value that has been associated with a 2-fold increased risk for SRE and disease progression [10]. Furthermore, the model-predicted incidence of $uNTx/Cr$ values BLQ was 54.3% (90% CI: 50.9% – 57.5%), consistent with the observed value (52%) in the analysis dataset. The model-predicted incidence of $uNTx/Cr$ values BLQ as a function of the predicted denosumab concentration (Figure 1B) support the adequacy of the model to describe the $uNTx/Cr$ values BLQ, an indirect marker of maximal suppression. In fact, the percent of $uNTx/Cr$ values BLQ following 120 mg Q4W dosing is 14% higher compared to 30 mg Q4W (63.7% versus 55.9%). The PC results for $uNTx/Cr$ in those patients who received 120 mg denosumab Q4W are
summarized in Figure 1C and 1D, and demonstrate that the model-based predictions are appropriate to describe the overall distribution of the time courses for uNTx/Cr following denosumab 120 mg Q4W administration. Overall, the model has adequately characterized the time course of uNTx/Cr suppression following different denosumab schedules and was deemed appropriate to explore the effect of denosumab dose and dosing regimen on the uNTx/Cr suppression through model-based simulations.

Simulations illustrating the relationship between denosumab trough concentration at steady state and the proportion of patients who achieve at least 90% of uNTx/Cr suppression (Figure 2A) demonstrate that the proportion of patients with maximal suppression rises steadily as denosumab concentrations increase with approximately 60% of patients achieving at least 90% suppression at the upper range of trough concentrations following denosumab dosing at 120 mg Q4W. This level of uNTx suppression is higher than that achieved following a denosumab 30 mg Q4W dose regimen (area between vertical dotted lines in Figure 2A). To further establish the relationship between denosumab dosing regimen and proportion of patients with at least 90% suppression of uNTx/Cr at the trough level achieved at week 25 of treatment, model-based simulations were conducted across the range of doses and dosing regimens evaluated in the analysis dataset. Figure 2B demonstrates that, for a given dose or a given cumulative dose, the proportion of patients achieving at least 90% of uNTx/Cr suppression is greater for Q4W versus Q12W dosing across the entire dose range and continues to rise until it starts to plateau at 120 mg Q4W. While Q4W dosing regimens with doses higher than 120 mg provide limited additional benefit in terms of the proportion of patients with at least 90% of uNTx/Cr suppression, the 120 mg Q4W dosing regimen provides approximately 14.0% and 13.7% increases in the proportion of patients achieving the target uNTx inhibition as compared to 30
mg Q4W and 180 mg Q12W dosing, respectively. Additionally, model-based simulations of uNTx/Cr time courses are presented in Figure 2C for denosumab weight-based dosing (2 mg/kg Q4W) and fixed dosing (120 mg Q4W). At week 25, uNTx/Cr values are similar for both regimens with median (25th -75th percentile, Q1-Q3) concentrations of 4.91 (2.26 – 10.16) and 5.21 (2.31 – 12.01) nM BCE/mM, for 2 mg/kg and 120 mg, respectively. The suppression of uNTx/Cr as related to the proportion of observations BLQ was also consistent across both dosing regimens. When comparing dosing regimens at the upper and lower quartiles of body weight, both weight-based and fixed dosing resulted in similar uNTx/Cr values. For the lower quartile of body weight (<=59.7 kg), median (Q1-Q3) uNTx/Cr values at weeks 25 were 6.10 (2.58 – 13.17) nM BCE/mM for 2 mg/kg and 6.39 (2.54 – 13.0) nM BCE/mM for 120 mg. For the upper quartile of body weight (>=83 kg), median (Q1-Q3) values were 4.75 (2.17 – 10.1) nM BCE/mM for 2 mg/kg and 5.67 (2.54 – 12.48) nM BCE/mM for 120 mg. Overall, the difference in denosumab dosing (weight-based versus fixed dose) did not translate to any relevant difference in uNTx/Cr values or their variability over time.

DISCUSSION

Elevated BTM have been associated with disease progression and poor prognosis in breast cancer, prostate cancer, and other solid tumors with bone metastases [40]. After a single subcutaneous (SC) dose, denosumab caused rapid and sustained suppression of bone turnover in postmenopausal women with low bone mass and in patients with breast cancer or multiple myeloma [16,21]. A direct effect of denosumab on bone resorption was evidenced by a decrease in bone resorption markers such as uNTx/Cr [22]. Therefore, the primary objective of this analysis was to characterize the time course of uNTx/Cr as a function of denosumab serum
concentration following SC administration in patients with bone metastases from solid tumors and to quantify the degree of unexplained BSV on denosumab PD efficacy and potency.

In patients with bone metastases from solid tumors, the uNTx/Cr suppression induced directly by denosumab serum concentration was characterized by two parameters, $I_{\text{Max}}$ and $I_{C_{50}}$, which were estimated to be 93.7% and 31.8 ng/mL, respectively. As expected from the method used to handle uNTx/Cr values BLQ, the estimates of $I_{\text{Max}}$ and $I_{C_{50}}$ are different from the values previously reported that did not use such methods [22]. The strategy of assigning uNTx/Cr values BLQ to the LOQ led to a model misspecification and suggested that an indirect response model, instead of a direct effect model, was the best structural model to describe uNTx/Cr suppression following denosumab treatment [22]. The high incidence of uNTx/Cr values BLQ in the current dataset and its correlation with the predicted denosumab concentration (Figure 1B) precludes assigning uNTx/Cr values BLQ to the LOQ and justifies the joint analysis of the continuous uNTx/Cr observations and the uNTx/Cr values BLQ conducted. In addition, instead of conditioning the uNTx/Cr observations that were greater than zero (M4 method), a log-transformation of the uNTx/Cr was used within the M3 method. This approach has been proven to provide unbiased estimates of model parameters in analyzing BLQ data [30, 33-36]. Upon incorporating the BLQ data using the M3 method, an indirect response model did not provide as good a fit as the direct effect model. In the previous analysis, the turnover rate of uNTx/Cr was estimated to be 1.26 days, which is significantly shorter than the absorption half-life for denosumab (2.71 days) and, therefore, denosumab absorption would be the rate-limiting step of suppressing the production rate of uNTx/Cr. In this situation, a direct effect model is expected to perform at least equally well, if not better, than an over-parameterized indirect
response model, in particular if one considers that only data from a sparse uNTx/Cr sampling schedule were available for these analyses.

The two methods used to handle uNTx/Cr values BLQ, acknowledging the uNTx concentrations BLQ versus the previously reported model fixing the uNTx concentrations BLQ to LOQ, allow us to compare the differences in $I_{Max}$ and $IC_{50}$ estimates between the two models [22]. Previous results suggest denosumab would have a 17% lower intrinsic efficacy and 2.6-fold higher potency with respect to the values reported here. In the current analysis, the RSE of $I_{Max}$ and $IC_{50}$ estimates were reduced by 64.6% and 42.7%, respectively, compared to the analysis performed by Lipton et al. In contrast, the magnitude of the BSV and the residual variability were similar or slightly lower to that previously reported [22]. These differences led to inaccuracies in the predicted proportions of patients with trough serum denosumab concentrations producing 90% of maximal uNTx/Cr suppression, and therefore discrepancy with the results of the current simulations. For example, since the previous estimate of $I_{Max}$ was 17% lower than the current estimate, the predicted proportions of patients with >90% of maximal uNTx/Cr suppression in the previous analysis is approximately equivalent to the proportions of patients with >~70% uNTx/Cr suppression in the current analysis, which is less likely to reflect an effective and maximal suppression of the bone resorption marker. In addition, for a given range of denosumab serum concentrations, a 2.6-fold higher potency estimate in the previous analysis leads artifactually to a higher proportion of patients achieving a certain target and explains the apparent lack of dose response reported previously [22].

Taken together, the results of the comparisons between different methodological approaches in handling uNTx/Cr values BLQ highlight the importance of analyzing the uNTx/Cr values BLQ as censored data and maximizing the likelihood for BLQ data with respect to the
model parameters. Therefore, the implications derived from the previous analysis should be interpreted with caution. Notably, the results of the current analysis confirm the high efficacy and potency of denosumab in suppressing uNTx/Cr. However, the high BSV in denosumab efficacy and potency suggests that the serum concentrations required to effectively suppress uNTx/Cr levels vary substantially among patients with bone metastases from solid tumors. These empirical observations indicating that denosumab 120 mg Q4W dosing suppresses bone resorption (uNTx/Cr) maximally in patients with bone metastases from solid tumor patients are supported by model-based simulations displayed in Figure 2A and 2B. Consistent with the population PK analysis, the larger fluctuations in denosumab serum concentrations following Q12W dosing resulted in larger fluctuations of uNTx/Cr levels during the dosing interval as compared with Q4W dosing, which translated to less effective uNTx/Cr suppression and higher probability of “escape” from bone resorption suppression with extended dosing intervals. Moreover, while Q4W dosing regimens with doses higher than 120 mg provide limited additional benefit in terms of bone resorption suppression, the 120 mg Q4W dosing regimen provides higher increases in the proportion of patients achieving the target inhibition as compared to the other dosing regimens evaluated, such as 30 mg Q4W and 180 mg Q12W.

Notably, age, sex, weight and race had no discernable effect on denosumab efficacy and potency. Given the limited magnitude of the effect, none of the covariates associated with statistically significant differences in denosumab PK parameters in cancer patients (namely, race and cancer type) are expected to manifest clinically relevant variations in uNTx/Cr suppression. In fact, the population PK analysis indicated that a denosumab dosing regimen of 120 mg Q4W provides at least 98% reduction of free RANKL in the vast majority of patients, regardless of the covariates affecting denosumab PK. Moreover, as body weight represents the covariate with the
highest effect on denosumab PK parameters, simulations were undertaken to compare the time course of uNTx/Cr following a denosumab weight-based dose of 2 mg/kg and a fixed dose of 120 mg. Both regimens provide comparable uNTx/Cr suppression over the Q4W dosing interval with respect to both the median and distribution of uNTx/Cr model-predictions (Figure 2C), regardless of patient body weight. Therefore, dose adjustments on the basis of body weight and the covariates affecting the PK of denosumab are not warranted.

In summary, this analysis indicates that the denosumab 120 mg Q4W dose regimen results in 1) a greater proportion of patients with normalized uNTx/Cr levels (<50 nM BCE/mM) relative to 30 mg Q4W and Q12W dosing regimens; 2) a greater proportion of patients with uNTx/Cr suppression >90% relative to Q12W dosing and 3) the lowest Q4W dose with the maximal proportion of patients with uNTx/Cr suppression >90%. Collectively, these results indicate that the denosumab dosing regimen of 120 mg SC Q4W is the optimal dosing regimen to maximally suppress bone resorption (uNTx/Cr) during the entire dosing interval, to minimize uNTx/Cr variability, and to avoid “escape” from suppression of bone resorption associated with longer dosing intervals.

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References


Table 1. Summary of Clinical Studies

<table>
<thead>
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<th>Study</th>
<th>N(^a) (% male)</th>
<th>Study Population</th>
<th>Doses (regimen)</th>
<th>Sampling</th>
<th>Age (years)(^b)</th>
<th>Weight (kg)(^b)</th>
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<td>20010123</td>
<td>22 (0)</td>
<td>Breast Cancer with Bone Metastases</td>
<td>0.1, 0.3, 1, 3 mg/kg (single dose)</td>
<td>Intensive for 12 weeks</td>
<td>55 (10)</td>
<td>73 (15)</td>
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<td>Intensive for 12 weeks</td>
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<td>At 1 week and monthly for 24 weeks</td>
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<td>73 (0)</td>
<td>Breast Cancer with Bone Metastases</td>
<td>120 mg (Q4W)</td>
<td>Week 13 and EOS</td>
<td>56 (11)</td>
<td>64 (15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>27-79</td>
<td>40-121</td>
</tr>
<tr>
<td>20050244</td>
<td>37 (70.3)</td>
<td>Solid Tumors(^c) with Bone Metastases</td>
<td>120 mg (Q4W)</td>
<td>Week 13 and EOS</td>
<td>61 (12)</td>
<td>76 (16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>35-82</td>
<td>40-113</td>
</tr>
<tr>
<td>Total</td>
<td>373 (24.7)</td>
<td></td>
<td></td>
<td></td>
<td>60 (12)</td>
<td>72 (17)</td>
</tr>
</tbody>
</table>

\(^a\) Number of patients with both pharmacokinetic and pharmacodynamic sampling
\(^b\) Numbers are mean (SD), range.
\(^c\) Excluding breast and prostate cancer.

Adv.: Advanced EOS; End of Study; Jap.: Japanese; Q4W: every 4 weeks, Q12W: every 12 weeks
Total uNTx/Cr values (including baseline measurements): 2013 from 373 patients. 1615 were post-dose samples. 840 (52%) out of 1615 post-dose samples were BQL.
Table 2. Estimated Population Pharmacodynamic Parameters of Denosumab

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate (%RSE)</th>
<th>Variability (%RSE)</th>
<th>Shrinkage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_{\text{Max}} ) (%)</td>
<td>93.7 (4.28)(^a)</td>
<td>127 (14.1)</td>
<td>24.4</td>
</tr>
<tr>
<td>( IC_{50} ) (ng/mL)</td>
<td>31.8 (37.5)</td>
<td>287 (30.0)</td>
<td>40.8</td>
</tr>
<tr>
<td>( \lambda )</td>
<td>0.806 (2.92)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) RSE value is for the logit-transformed value.

Correlation between \( I_{\text{Max}} \) and \( IC_{50} \) is 0.675. Residual variability: 70.1% (RSE: 1.05)
FIGURE LEGENDS

Figure 1.

A) **Relationship between uNTx/Cr and denosumab concentration.** The circles show the observed uNTx/Cr versus predicted denosumab concentration. The triangles show the baseline uNTx/Cr and the plus signs present the uNTx/Cr data BLQ. Baseline values and BLQ values were conditioned with the jitter functionality in S-Plus to more clearly display the values. The solid grey lines and blue shaded area represents the median and 90% prediction interval of uNTx/Cr. Solid and dotted vertical lines show 5th and 95th percentiles of trough denosumab concentration at week 25 after 120 mg Q4W and 30 mg Q4W, respectively. The horizontal dashed line is uNTx/Cr = 50 nM/mM as reference.

B) **Relationship between the proportion of uNTx/Cr values BLQ and denosumab concentration.** Black dots show the observed proportion of uNTx/Cr values BLQ and the segment lines shows the corresponding 90% confidence interval, grouped by denosumab concentration deciles. The solid grey lines and blue shaded area represents the model-based prediction of the proportion of uNTx/Cr values BLQ and the 90% confidence interval. The tick marks on the upper and lower axis reflect the denosumab concentration at the time the corresponding uNTx/Cr value was BLQ or above the limit of quantification, respectively.

C) **Time course of uNTx/Cr following denosumab 120 mg SC Q4W.** The circles show the observed uNTx/Cr versus predicted denosumab concentration. The solid grey lines and blue shaded area represents the median and 90% prediction interval of uNTx/Cr.

D) **Time course of proportion of uNTx/Cr values BLQ following denosumab 120 mg SC Q4W.** Black dots show the observed proportion of uNTx/Cr values BLQ and the segment lines shows the corresponding 90% confidence interval at each visit. The solid grey lines and blue shaded area represents the model-based prediction of the proportion of uNTx/Cr values BLQ and the 90% confidence interval.
Figure 2.

A) Model-based proportion of patients with higher than 90% uNTx/Cr reduction at week 25 as a function of denosumab trough concentration. Solid and dotted vertical lines show 5th and 95th percentiles of the trough denosumab concentration at week 25 after 120 mg Q4W and 30 mg Q4W, respectively.

B) Model-based proportion of patients with higher than 90% uNTx/Cr reduction at week 25 as a function of denosumab dose and schedule. The vertical dashed line marks the dose of 120 mg.

C) Model-based prediction of time course of uNTx/Cr and 90% prediction interval after denosumab 2 mg/kg (solid red lines) or 120 mg (dashed black lines) administered monthly.
Figure 1.

A

B

C

D

Denosumab Concentration (ng/mL)

$\text{uNTx/Cr (nM/mL)}$

$\text{Denosumab Concentration (ng/mL)}$

$\text{Probability of uNTx/Cr BLQ}$

$\text{Proportion uNTx/Cr BLQ}$

$\text{Time (weeks)}$

$\text{uNTx/Cr (nM/mL)}$

$\text{nObs}$

$\text{Time (weeks)}$

$\text{nTotal}$

$\text{nBLQ}$
Figure 2.

A

B

C

Denosumab Concentration (ng/mL)

Proportion of Subjects with >90% uNTx/Cr Reduction

Dose (mg)

Proportion of Subjects with >90% uNTx/Cr Reduction

Time (week)

Q4W

Q12W

2 mg/kg Q4W

120 mg Q4W

Research.
Denosumab Dose Selection for Patients with Bone Metastases from Solid Tumors

Sameer Doshi, Liviawati Sutjandra, Jenny Zheng, et al.

Clin Cancer Res  Published OnlineFirst March 6, 2012.

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