

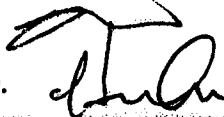

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

125320

**CLINICAL PHARMACOLOGY AND
BIOPHARMACEUTICS REVIEW(S)**

CLINICAL PHARMACOLOGY REVIEW

BLA:	125320/0047
Type/Category:	Resubmission
Brand Name:	Prolia
Generic Name:	Denosumab
Indication:	Treatment of osteoporosis in postmenopausal women
Dosage Forms:	Single-use prefilled syringe Single-use vials
Route of Administration:	Subcutaneous Injection
Dosing Regimen and Strength:	60 mg/mL every 6 months
Sponsor:	Amgen
OCP Division:	Division of Clinical Pharmacology 3
OND Division:	Division of Reproductive and Urologic Products (DRUP)
Submission Date:	1/25/2010
PDUFA Date:	7/23/2010
Primary Reviewer:	Jee Eun Lee, Ph.D.  4/30/10
Secondary Reviewer:	Hae-Young Ahn, Ph.D.  4/30/10

The sponsor has provided a complete response to the Action Letter dated 16 October 2009 which was issued to the denosumab biologic license application for the treatment of postmenopausal women with osteoporosis (STN BL 125320/0). The submission contains a proposed product labeling, safety update and plans/protocols for Postmarketing Observation Study, Risk Evaluation and Mitigation Strategy Requirements, Safety Postmarketing Requirement for a Long-term Observational Study, Postmarketing Requirement for a Long-term Safety Surveillance Program and Safety Post-Marketing Requirement for a Long-Term Pregnancy Exposure Study.

The sponsor states that the response to the request regarding Clinical/Clinical Pharmacology Deficiency for STN BL 125331/0 (for the prevention of osteoporosis) was not included in this submission since the submission is limited to STN BL 125320/0 (treatment of osteoporosis).

The sponsor requested reconsideration of the recommended revisions to the Drug Interaction section (Section 7) of the physician's package insert together with the scientific rationale for the request. The sponsor provided several literature articles as scientific rationale (Justification #9 in Response to Red-line Prescribing Information), and this review is focused on the potential drug interaction issue.

1. Background

The Clinical Pharmacology Review Team recommended the following statements in the Drug Interaction Section of the physician's package insert:

7. DRUG INTERACTIONS

No drug-drug interaction studies have been conducted with [TRADENAME]. The

(b) (4)

The sponsor provided background information asserting that drug-interaction of denosumab on cytochrome P450 (CYP) enzyme(s) would be unlikely.

"A role of RANKL in cytochrome P450 (CYP) regulation has not been demonstrated and is unlikely, given lack of expression of its receptor RANK on adult human hepatocytes. A RANKL inhibitor is thus unlikely to directly impact CYP expression or activity.

Denosumab is specific for RANKL and dose not appear to affect the levels or activities of inflammatory cytokines, therefore an indirect effect on CYP expression is also unlikely:

- 1. Treatment of subjects with rheumatoid arthritis (RA) with 60 mg or 180 mg Q6M denosumab did not affect C-reactive protein(CRP) levels, even in subjects with elevated CRP at baseline*
- 2. Data from several studies in rodents demonstrate that RANKL inhibition does not impact circulating levels of inflammatory cytokines"*

2. Potential drug-interaction of denosumab on CYP450 enzymes

Denosumab is a monoclonal antibody and is not eliminated via hepatic metabolism. The sponsor did not conduct any drug-drug interaction study via CYP isoforms of enzyme.

Major proinflammatory cytokines such as TNF- α , IL-1 α , IL-6 and interferons are known to be involved with modulation of CYP450 enzymes but the mechanisms are still unclear. The proposed mechanisms for these cytokines' effects on CYP450 gene expression so far are: (1) destruction of

enzymes via a free radical mechanism, (2) inhibition of translation of CYP450 mRNA (3) direct reduction of CYP450 mRNA levels and (4) inhibition of nitric oxide, etc.

We recognize the sponsor's position that denosumab does not necessarily behave like therapeutic proteins targeting inflammatory cytokines that have demonstrated roles in CYP regulation. However, it is still uncertain and premature to conclude that a RANKL antagonist will not impact CYP expression.

First, the sponsor asserts that RANK and RANKL are not constitutively expressed in the adult human liver, referencing a literature where thousands of gene patterns were predicted by microarray analysis using 79 human and 61 mouse tissues (Su et al., 2004). However, it is hard to conclude that they are not expressed constitutively in the adult human liver based on the referenced literature. As the author discussed in the paper, the negative detection does not necessarily mean its non-existence. It could be because the appropriate tissue for a given gene may have not been profiled, the gene may be present in a small number of copies (e.g., in a small subset of cells within a tissue), or the probe set may not properly interrogate the expression of the gene. Therefore, it could not be confirmed that either RANK gene or RANKL gene was properly profiled in the microarray analysis.

RANKL mRNA is highly expressed in fetal liver and exists in a biologically active soluble form (in circulation) either secreted from T cells or proteolytically cleaved from cell surfaces. Furthermore, serum RANKL shows high correlation with local changes in BMD. RANKL is frequently undetectable in normal vasculature. However, significant amount of RANKL is observed from patients with vascular disease (Collin-Osdoby P (2004), "Regulation of vascular calcification by osteoclast regulatory factor RANKL and osteoprotegerin", *Circ. Res.* 95: 1046~1057). Nonetheless, there is still uncertainty in the distribution of RANK and RANKL

Second, the sponsor states that denosumab does not decrease C-Reactive Protein (CRP) levels, claiming lack of potential for drug interaction with CYP450 enzymes for that reason. However, lack of effect of denosumab on CRP does not abolish its potential drug interaction with CYP450 enzymes. The sponsor also states that anti-RANKL therapy including denosumab consistently fails to influence inflammatory parameters within arthritic joints, although the cytokines such as TNF α , IL-1 and RANKL are commonly elevated locally and/or systemically in inflammatory arthritis. The effect of anti-RANKL might be more exclusive to bone resorption. However, there are minimal data on the effects of RANKL inhibitors on systemic parameters of inflammation in disease models (Stolina et al., 2009, a literature the sponsor provided). In addition to inflammatory mediator such as CRP, oxidative stress and subsequent NF- κ B activation have shown the correlation with CYP regulation.

Denosumab is a receptor activator for nuclear factor (NF)- κ B ligand (RANKL). NF- κ B has pleiotropic functions and has been shown to down-regulate the transcriptional activity of multiple steroid/nuclear receptors. The important role of NF- κ B in the suppression of *cyp3a4* has been demonstrated: NF- κ B may regulate PXR transcriptional activity by disrupting the association between PXR-RXR α complex and DNA sequences (Gu X et al. (2006), "Role of NF- κ B in regulation of PXR-mediated gene expression", *Journal of Biological Chemistry*, 281(26):17882-17889), NF- κ B activation may suppress oxidative stress thereby destabilize CYP3A4 (Zangar RC et al. (2008), "The nuclear factor- κ B pathway regulates cytochrome P450 3A4 protein stability", *Cell Biology and Biochemistry* 73(6): 1652-1658).

Although drug-interactions of cytokines with CYP have shown to be not tremendously significant, there are still several cases of significant drug-interaction where the safety of patients becomes a

concern: e.g., Baxiliximab increased tacrolimus concentration by 63%, muromonab doubled cyclosporine concentration in renal transplant recipients who received antilymphocyte globulin.

As the mechanism of other cytokines' effects on CYP450 is unclear, the possibility of anti-RANKL on CYP450 is unknown. Since denosumab is the first drug that belongs to this class for the treatment of osteoporosis, and the target patient population has a potential of polypharmacy, it would be informative and beneficial in terms of public health perspective if the sponsor conducts a drug-drug interaction study with a CYP3A4 substrate.

Recommendations:

The Clinical Pharmacology Review Team recommends the sponsor conduct an *in vivo* drug-drug interaction study with CYP3A4 substrate (e.g., midazolam) in postmenopausal female patients with osteoporosis as a postmarketing requirement.



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Memorandum

Date: 9-16-09
From: Sarah Kennett, Ph.D.
Through: Patrick Swann, Ph.D., Deputy Director, Division of Monoclonal Antibodies
To: File – BLA STN:125320, 125331, 125332, 125333
Sponsor: Amgen
Drug: Denosumab (AMG 162)
Subject: Bioanalytical Method Validation for Assays used to Detect Denosumab and CTx.

DMA received the following consult request from Chongwoo Yu (Clinical Pharmacology) on March 18, 2009.

Please evaluate the biochemical validity of the ELISA assay used in measuring denosumab (for PK) and CTX1 (for PD) levels.

Assay validation reports can be found as stated below:

Denosumab: Study 107381 in Module 5, Section 5.3.1.4

CTX1: Study 105941 in Module 5, Section 5.3.1.4

Reviews of the complete repertoires of PK assays and CTX1 assays were requested at later dates.

PK- detection of denosumab

Method validation report 107381 was submitted as part of the BLA. However, this method was not used for all assessments of denosumab levels in human serum. Amgen's response to CMC IR #2 included the assay protocols and validation reports for the original method (PK0106G2OPHuSe, validation report 101782), which was developed and validated at Amgen, the transfer to (b) (4) (ICD 171, validation report 102110), and the new method (MET-001831, originally named PK0608AMG162HuSe, validation report 107381).

Method PK0106G2OPHuSe and validation report 101782

This assay is based on an (b) (4) and is a solid phase sandwich ELISA. (b) (4)
(b) (4)
A human serum derived diluent (b) (4) is used for the assay. Standards and quality controls (QC) are generated from denosumab lot 49A007661. The calibration curve was obtained by plotting the OD versus the concentration of denosumab standards, using a Log-Log curve fit ($\text{Log}(y) = A + B * \text{Log}(x)$).

Assay Range/Dose Response:

An 8 point standard curve and 5 independent levels of QCs were used. Based on all assays performed during the validation for accuracy and precision, the assay range was determined to be 0.625 ng/ml to 40.0 ng/ml in 100% serum. The analytical assay range, defined by the LOQ to ULOQ was determined to be 0.8 to 35.0 mg/ml.

Accuracy and Precision:

Three independent spikes of an 8 point standard curve and 3 independent spikes of 5 levels of QCs were used. One spike for both the standards and QCs was divided into 3 aliquots and assayed over 3 days. Intra-spike was defined as one spike assayed on different days, and inter-spike (n=10) was defined as a minimum of 3 separate spikes performed within-day and between days. The standard curve intra-spike %CV was 3.2 to 10.8% (n=6), and the inter-spike %CV was 3.3 to 9.2% (n=10); the largest %CVs were for the 0.0625 ng/ml standard. The mean correlation determinations were calculated to be 1.00. The QC intra-spike %CV was 6.8 to 13.2% (n=24), and the inter-spike %CV was 4.3 to 7.0% (n=40). The QC inter-spike percent recoveries ranged from 88 to 104%, and the inter-spike percent recoveries ranged from 92% to 106%.

Assay Specificity:

Specificity was assessed by examining cross-reactivity with OPG, Trail, RANK, and CD40. The concentrations of the proteins examined included a concentration equivalent to the highest denosumab standard and 10, 100, and 1000 times this concentration. No significant cross-reactivity was observed, except with native hOPG dimer.

Freeze-thaw Stability:

A set of QCs was prepared, and aliquots were subjected to 0-3 freeze-thaw cycles (-70°C and 37°C). There were no statistical differences in recovery.

Bench Top Stability:

A set of QCs was prepared, and aliquots were frozen at -70°C, then were left frozen, held on ice for 3 hours, held at RT for 2 hours, or held at 37°C for 1 hour. There were no statistical differences in recovery.

Linearity (Dilution Parallelism):

An 8 point standard curve and 5 QCs were prepared. The highest QC was diluted with 100% serum to the mid and low range of the curve, and 4 independent spikes representing estimated concentrations of samples ("in vivo" samples) were made. The in vivo samples were further diluted within the assay range. The %CVs for the dilution of the highest QC were 5.6 to 6.6%, and the %CVs for the in vivo samples were 3.7 to 13.6%. The recoveries ranged from 90-109%.

Long Term Stability:

The validation report states that QCs prepared on Day 0 will be assayed in the future to assess the stability of denosumab in human serum maintained at -70°C and -20°C and that data will be evaluated and reported in a memo to the validation report. According to the validation report 102110, this was determined to be 25 months at -70°C.

Method ICD171 and validation report 102110

This method is the same as Method PK0106G2OPHuSe, above, but has been transferred to (b) (4) and the denosumab reference standard are supplied by Amgen. Human serum is used for dilution of denosumab; all standards and QC samples were subjected to 1 freeze-thaw before use in an assay. New lots of denosumab are qualified against a previously validated lot.

The LLOQ is the concentration of the lowest standard with a %Diff (difference from theoretical) $\pm 20\%$ and a %CV $\leq 20\%$. The ULOQ is the concentration of the highest standard with a %Diff $\pm 15\%$ and a %CV $\leq 15\%$. Assay acceptance criteria include a minimum of 5 acceptable standards, plus the blank; the average %Diff of each level on the standard curve must be $\pm 15\%$ (except the LLOQ, where $\pm 20\%$ is acceptable) and the %CV of each level on the standard curve must be $\leq 15\%$ (except the LLOQ, where $\leq 20\%$ is acceptable). The assays include a minimum of 3 QC concentrations representing at least 3 regions of the assay range (high, mid, low). QC acceptance criteria include a minimum of 2/3 of the QC samples within the acceptance limits of %Diff ($\pm 15\%$; $\pm 20\%$ for low) and %CV ($\leq 15\%$; $\leq 20\%$ for low), and at least 1 result from each level must meet these criteria. Sample concentrations are reportable only when they fall between or are equal to the concentrations of the LLOQ and ULOQ, and the %CV between replicates must be $\leq 15\%$.

For validation, 3 sets of standards and QCs were prepared from 3 different vials of denosumab RS. Multiple analysts performed the assays. Accuracy and precision were validated for the range of 0.800 to 35.000 ng/ml.

Linearity:

Linearity was demonstrated by comparing the back-calculated concentration of each standard with the theoretical value. Over the working range of 0.625 to 40.0 ng/ml, the recovery ranged from 93-107%.

Reproducibility:

For both the intra-assay calculations (n=6 from 3 assays) and inter-assay calculations (n=10 from 5 assays), the %CVs over the working range were 3-10% and the mean correlation determinations were calculated to be 0.997.

Accuracy:

The accuracy was evaluated using QCs at 0.8, 2.0, 3.5, 15.0, and 35.0 ng/ml. The accuracy ranged from 93 to 104% for intra-spike data (n=6 from 3 assays) and from 93 to 101% for intra-spike data (n=10 from 5 assays).

Precision:

The intra-assay intra-spike and inter-spike %CVs ranged from 3-12%; the largest %CVs were for the lowest concentration. The pooled variability results for the 3 intra-spike assays ranged from 6-11% (n=18), and the pooled variability results for the 5 inter-spike assays ranged from 8-14% (n=30).

Dilutional Linearity:

Dilutional linearity was evaluated by assaying a set of standards serially diluted from a highly concentrated denosumab stock. Duplicate assays containing 6 dilutions demonstrate a correlation determination of 0.996.

Specificity:

Specificity was again shown by a lack of cross-reactivity with Trail, CD40L, and OPGL; as expected, there was cross-reactivity with OPG.

Stability:

Freeze-thaw stability was assessed using 0-4 cycles of -70°C and 37°C. Bench top stability was assessed using samples that were thawed at 37°C then held on wet ice for 4 hours or at RT for 1 hour. Samples held at RT for 24 and 48 hours were also examined. All assays had recoveries within 15% and %CV from 0-10%. This validation report includes an addendum showing long term (25 month) stability of samples stored at -70 to -90°C and long term (13 month) stability of samples at -15 to -30°C.

Accuracy and Precision Addendum:

Additional experiments were performed to compare the performance of human serum derived diluent (b) (4) and normal human serum. Experiments were performed over 3 days in 3 separate assays; the standard curve was assayed in duplicate and the QCs in 6 wells. The QC intra-assay precision ranged from 1-8% for controls diluted in normal human serum or in (b) (4) calculated against both (b) (4) and normal human serum standard curves, the inter-assay precision ranged from 6-7% for controls evaluated against normal human serum and from 4-6% for controls evaluated against (b) (4). The intra-assay percent recoveries were 82-109% for the human serum curve and 77-99% for the (b) (4) curve, and the inter-assay percent recoveries were 91-101% and 84-95%, respectively. The precision between QCs prepared in normal human serum and (b) (4) ranged from 1-7% when evaluated against both the normal human serum curve and the (b) (4) curve. These data indicate that quantification of denosumab in normal human serum and (b) (4) is comparable.

Japanese Human Serum Addendum:

Selectivity assays were performed using serum from Japanese male non-smokers, female non-smokers, male smokers, female smokers, and postmenopausal females. The initial assays were done with spikes of 0.8 ng/ml denosumab (assay LLOQ). Recoveries for all but 1 lot ranged from 110-255%. Those spikes had been prepared using the matrix of the individuals for each dilution. A second assay was performed using (b) (4) for all dilutions except the final dilution, and spikes of 8 ng/ml were also prepared for the second assay. The results of the second assay are within an acceptable range for recovery.

Rheumatoid Factor Positive Serum Addendum and Chronic Kidney Disease:

This study was performed to assess the potential difference in quantitation levels of denosumab when spiked into normal human serum or RF+ human serum. For 18 lots of RF+ serum and 20 lots of normal serum, the %Diff for each spike level ranged from -8 to 5%, indicating that the recovery in the 2 matrices is comparable. Part of one addendum also assessed recovery in

individuals with 5 different levels of chronic kidney disease; the results show acceptable levels of recovery of spiked denosumab.

MET-001831 and validation report 107381 (current assay/BE study assay)

This assay is intended to quantify the concentration of denosumab in human serum over a concentration range of 20 ng/ml to 2000 ng/ml. Standards and quality controls are prepared by spiking denosumab into 100% human serum. (b) (4)

[REDACTED]

(b) (4) Conversion of color OD units to concentration is achieved through comparison to a standard curve assayed on the same plate, according to a Logistic regression model with a weighting factor of 1/Y using a current validated Watson data reduction package.

Critical reagents include recombinant human RANKL, biotinylated rabbit anti-denosumab polyclonal antibody, denosumab (all manufactured at Amgen), and human serum. Qualification of new lots of critical reagents is performed by parallel assays with old lots or by performing accuracy and precision suitability assays.

The standard curve consists of 10 standards, 2 of which are anchor points, plus a blank. Two sets of quality controls at three levels (low, middle, high) are also used. All samples are plated in replicates.

Assay acceptance criteria include at least 6 standard levels with %bias -15 to 15 (equivalent to %RE) and %CV \leq 15%, a minimum of 4 QCs and at least one at each level with total error of measurement \leq 15% (systematic bias and random error) and %CV \leq 15%, and study samples with a %CV \leq 15%.

The majority of the validation was performed using a total of 8 assays performed by 4 analysts over a 2 week period. For each assay, 2 lots of denosumab were used to generate independent standard curves and sets of validation samples. Validation included determination of inter- and intra-assay accuracy and precision, selectivity, specificity, stability, dilutional linearity, and hook effect.

Accuracy and Precision:

Assays were performed with a standard curve from 20 to 2000 ng/ml denosumab plus two anchor points outside the range of quantification to facilitate curve fitting (10 and 3000 ng/ml). Validation samples were prepared for the LLOQ, the ULOQ, and low, mid, and high points within the range. Validation samples were calculated from both their respective standard curve and the standard curve generated from a separate lot of denosumab. For the standard curves, inter-assay %CV ranged from 1-5% (n=16), and individual replicate %CV ranged from 0-21% (0-9% with 19% and 21% seen once each at the ULOQ). For QC samples, the individual

replicate CVs ranged from 0-13% (plus one sample at 48% that was removed as an outlier; n=32). Overall intra-assay %CV ranged from 3-12% and inter-assay %CV ranged from 3-13%. The %RE was 1-3%, and the maximum total error was 15%. Based on the back calculated concentrations of the standard curves, the 4 parameter (Logistic Auto-Estimate) model with a weighting factor of 1/Y was confirmed to be the regression model.

Ruggedness and Robustness:

Ruggedness and robustness were evaluated by having different analysts perform the assay using different instruments over multiple days during the accuracy and precision testing. The tolerance limits for plate coating, plate blocking, sample, detection, HRP, and substrate incubation times were also evaluated during those assessments. Extended coating resulted in accuracy ranging from 96-103% for the standards and 100-115% for the QCs (115% at LLOQ).

Selectivity (Matrix Effect):

A total of 6 normal human serum lots (equal male and female) were tested at the LLOQ and at 60 mg/ml; no matrix effect was observed (100% of spiked samples were within the acceptable range, based on %Diff ± 15). Selectivity testing was also performed using 33 individual serum lots from cancer, rheumatoid arthritis, and osteoporosis patients; no significant matrix effect was observed (88% of spiked samples were within the acceptable range).

Specificity:

Denosumab spikes of 60 and 1200 ng/ml (low and high QC levels) were added into 1X, 10X, and 100X the method ULOQ (2000 ng/ml) AMG 102 or AMG 108. All samples were within %Diff ± 15 .

Freeze-thaw Stability:

A 4 cycle freeze-thaw (-70°C and RT) study was performed with denosumab spiked at 60 and 1200 ng/ml. No statistical differences were seen in recovery.

Process Temperature Stability:

Stability at RT (3-5 hours) and 2-8°C (24 hours) was assessed on samples spiked at 60 and 1200 ng/ml. No statistical differences were seen in recovery.

Long Term Stability:

Samples prepared at 60 and 1200 ng/ml were assessed after storage at -70°C and at -20°C. There are no statistical differences in samples stored up to 754 days at -70°C or in samples stored for up to 11 days at -20°C.

Dilutional Linearity:

Samples were diluted in 100% normal human serum using dilution factors of 1000, 100, and 10. The maximum dilution tested demonstrated acceptable recovery and %CV of 3-4%. In addition, a hook effect was also evaluated, using concentrations of 10,000 and 100,000 ng/ml; all samples were above the quantification limit.

Sample Reanalysis:

To assess the reproducibility of the method, a total of 86 samples that had been tested were reanalyzed; the calculated % difference was <30% for all samples.

Method Comparison:

The concentrations of denosumab generated from both the new method and the kit method for set of clinical samples and a set of conformance samples was compared. For the spiked samples, the estimate of the mean of the ratio is 0.973 with a two sided 90% confidence interval of the ratio (0.953, 0.994), and for the incurred samples, the estimated mean of the ratio is 1.00 with a two sided 90% confidence interval (.986, 1.019). These confidence intervals are completely contained within the acceptance interval (0.8, 1.25).

PD – Detection of CTx

Method Validation Report 105941 (12 April 2006- Amgen DraftPK0504CTHuSe)

The CTx (C-terminal telopeptide) human serum assay (b) (4) and modified by Amgen. Amgen uses a customized Standard F (b) (4) to make standards and QCs, and Amgen replaced the kit substrate solution and stop solution. New lots of critical reagents, including standards, antibodies, and serum, will be qualified by assay in parallel with old lots. Samples, standards, and controls are prepared day of use.

(b) (4) ELISA uses 2 specific monoclonal antibodies against a CTx sequence; one antibody is biotinylated, and the other is HRP-conjugated. The samples, standards, QCs, and antibodies are (b) (4). An HRP-TMB colorimetric signal is measured and converted to concentration through comparison to a standard curve assayed on the same plate; regression is done according to a Logistics-Auto Estimate regression model with a weighting factor of $1/Y^2$.

Assay acceptance criteria include a minimum of 6 acceptable standards for the standard curve; the %Diff for each level must be within $\pm 20\%$ ($\pm 25\%$ at LLOQ) and the %CV must be $\leq 20\%$. Three QC levels are run; 67% of the 3 levels must meet the acceptance criteria, and 2 replicates at the same level cannot fail. The QC %CV must be $\leq 20\%$, and the %Diff must be within $\pm 20\%$. Sample values are reportable only when the concentration falls within the LLOQ to ULOQ range and the %CV is $\leq 20\%$. Each Sample Control level must recover within the pre-determined range, and the %CV must be $\leq 20\%$.

Standard Curve:

Standard curves (0.026 to 2.5 ng/ml) are prepared by diluting Standard F in Standard A (blank). Eight standard curves, consisting of 7 standards plus 2 anchor points, were assessed in 8 different assays. The %Diff was -2 to 2%, and the mean %CV was 1-8% (0-20% for individual replicates; 19% and 20% only at LLOQ).

Accuracy and Precision (and robustness/ruggedness):

Twelve independent spikes of validation samples (0.049-2.3 ng/ml) prepared by dilution of Standard F in either Standard A or in normal human serum were assessed in 8 different assays in replicates of 3. The assay range was determined to be 0.049 to 2.3 (LLOQ to ULOQ). The intra-assay variability (%CV) range was 3-13% in Standard A and 3-17% in human serum with intra-assay accuracy (RE) of -31 to 22 (highest at LLOQ). The inter-assay %CV ranged from 3-20% (>15% only at LLOQ) with RE of -7 to 5. The total error ranged from 4-27% (>20% at LLOQ). Assays were run on at least 4 different days, and at least 2 different work stations were used.

For this validation report, inter-analyst variation is not discussed; however, this assay was developed for use in a clinical lab setting, and is essentially the same as the method assessed in the validation report below, where inter-analyst variation was shown to be acceptable.

Sample Controls:

Two levels of sample controls (normal human serum pools) were analyzed in a total of 27 assays. The range for sample control 1 was 0.116 to 0.159 ng/ml, and the range for sample control 2 was 0.566 to 0.701 ng/ml.

Selectivity:

Forty lots of normal human serum (equal male and female) were tested with and without 0.15 ng/ml CTx spiking. In 98% of lots, recovery of spiked CTx was 80-120%. The CTx concentrations for the normal human population ranged from 0.058 to 0.48 ng/ml, with 3% of serum lots having values below the LLOQ. 58 lots of diseased state serum (osteoporosis, cancer, and rheumatoid arthritis) were also tested; 80-120% of the CTx spike was recovered in 74% of the lots. The majority of the osteoporosis and breast cancer lots (50% and 60%) had values below the LLOQ; the upper value for the osteoporosis range was 0.54 ng/ml.

Specificity:

Human CTx II and NTx were evaluated in the assay. No measurable CTx II was detected, but CTxII at levels of 45.5 ng/ml appear to interfere with detection of spiked CTx. NTx was detected at a concentration of 30 nM/BCE or higher; an additive effect of spiked CTx plus NTx is observed at this level.

Note: What are the endogenous levels of NTx and CTxII?

Serum NTx levels appear to be <24.2 nM BCE in normal women and men, but in cancer patients with bone metastases, an average of 46.8 nM BCE has been detected (Jablonka, et al., Sao Paulo Med J 127:19, 2009).

CTx II appears to typically be measured in urine, not in serum.

Bench Top Stability:

Samples were held at 2-8°C for 22 hours. The %Diff ranged from 1-6% when compared to frozen samples.

Long-term Stability:

Long-term stability is ongoing, and 1 year of long-term stability at -70°C and at -20°C was previously demonstrated (b) (4) # 102624, see below).

Freeze- thaw Stability:

The stability of 4 freeze-thaw cycles was demonstrated in the previous method verification

(b) (4) # 102624, see below).

Dilutional Linearity:

Three sets of sample control 2 were prepared at dilutions of 1:2, 1:5, and 1:10; the %Diff ranged from -1 to 0%.

Note: It appears that the potential for a hook effect was not evaluated. However, it is unlikely that any samples would be above the 2.3 ng/ml that was established as the ULOQ for this assay (osteoporosis sample high was 0.54 ng/ml), and the standard, clinically-used (b) (4) assay is linear to 3.38 ng/ml.

Partial Validation Report (b) (4) #107085 (14 November 2006- (b) (4) Method ICD 244)

This method is (b) (4)s ELISA (see above for method). A minimum of 6 acceptable calibration standard levels are required for curve fitting. The %CV for each level must be $\leq 20\%$ and the %Diff must be within 20% of nominal value, except at LLOQ (25%).

Standard Curve Accuracy and Precision:

Standard curves (0.026 to 2.791 ng/ml) were assessed. The standard curves produced an inter-assay %CV range of 2-5%; recoveries ranged from 97-103%. The %CV of individual replicates ranged from 0-18%. The normal reference range was evaluated using at least 20 individual samples for both male and female. The range for females was 0.068-0.661 ng/ml, and the range for males was 0.060-0.539 ng/ml.

Accuracy and Precision (also robustness):

Accuracy and precision were evaluated using 7 assays with 5 sets of validation samples performed on at least 3 days by 2 analysts using both manual and TECAN automated pipetting. Intra-assay %CVs ranged from 1-20% (20% for LLOQ; all others $\leq 6\%$), and %RE ranged from -26 to 2% (-26% for LLOQ; all others $\leq 17\%$). Repeatability %CVs were between 3.2 and 10.7%. Inter-assay %CVs ranged from 4-13% and %RE ranged from -5 to -10%. The total error ranged from 10-23% (23% is LLOQ).

LLOQ and ULOQ:

LLOQ was established as 0.049 ng/ml; inter-assay %RE was -10%, %CV was 13%, and total error was 23%. ULOQ was established as 2.343 ng/ml; inter-assay %RE was -5%, %CV was 8%, and total error was 13%.

Selectivity:

CTx (0.13 ng/ml) was spiked into 10 individual lots of prostate cancer specific human serum. All unspiked lots had detectable levels of CTx; 1 was below the assay LLOQ. % Differences within $\pm 25\%$ were seen for 8/10 spiked lots (72% and 74% for the other 2 lots).

Bench Top Stability:

QCs were examined for stability at RT and 4°C for 5 and 25 hours. RT stability of low and mid concentrations was shown for both time points (% Diff range of -3 to -19%); high concentration was stable at the 5 hour time point, but the % Diff at the 25 hour time point was -45%. The refrigerated stability study showed % Diff ranging from -8 to 5% for up to 24 hours. An additional study was done to examine stability samples (low, mid, and high concentrations) thawed for 1 hour at 37°C, placed on ice for 4 hours and then stored frozen overnight. All samples were stable (%Diff -4 to 1%).

Dilutional Linearity:

Dilutional linearity was determined by analyzing the mid and high QC at dilutions of 1:2, 1:5, and 1:10. The mid QC was below LLOQ at dilutions of 1:5 and 1:10; other dilutions show linearity.

Method Verification Report (b) (4) #102624 (30 July 2002- Amgen PK0302TCNCTHuSe)

The analytical method is the (b) (4) serum CTx kit ELISA (b) (4), a solid phase sandwich assay, and is being verified for use with the TECAN automated system (see above for (b) (4) reagents were used, but the assay is modified by utilizing vendor supplied calibrator F (high concentration) and calibrator A (diluent) to make standards and quality controls. For this assay, a 4-parameter curve fit is used, and calibrators C-F (standard curve) must have a concentration %CV \leq 20% and recovery of 80-120%. The concentration %CV between duplicate QC and serum samples must be \leq 20%.

Standard Curve Accuracy and Precision:

Standard curves (0.049 to 2.428 ng/ml) were assessed (n=20; 10 assays). The standard curves produced an inter-assay %CV range of 2.0-4.3%; recoveries ranged from 94% to 107%.

Accuracy and Precision:

The quality controls (0.08 to 2.00 ng/ml) and human serum samples were assessed for inter-assay and intra-assay variability, and precision (n=20; 10 assays). The QC inter-assay %CV ranged from 3.1-7.4%, and the sample (3) %CVs were 3.9%, 9.6%, and 23.2%. Human serum samples (8; 4 replicates in 1 assay) produced an intra-assay variability range of 2.5-10.6%. The recovery of the quality controls ranged from 93-105%.

LLOQ:

LLOQ samples (Calibrator F diluted in A) were prepared at a concentration of 0.049 ng/ml; a total of 6 samples were prepared, and these samples were divided into 3 aliquots for testing. The overall %CV was 10.1%, and the % recovery was 104%. This assay was performed at a different time from the original standard curve and QC accuracy and precision assays. In these assays, the standard curve inter-assay %CVs ranged from 2.1-8.1% with % recovery from 95-106%, and the QC sample inter-assay %CVs ranged from 3.6-6.1% with % recovery from 93-103%.

Selectivity:

A standard (Calibrator F; 16739 pM) was spiked into 5 normal human serum samples and 5 samples from osteoporosis subjects and assessed for recovery. Recovery ranged from 95-98%

for normal serum and 93-102% for serum from individuals with osteoporosis. In addition, standards and quality controls were spiked into either pooled normal human serum (8) or dilution buffer; the %CVs between serum and buffer samples were <10%.

Specificity:

No significant cross-reactivity was observed with AMG162, OPGL, and OPG. The (b) (4) (b) (4) CTx Immunoassay Kit insert is referenced for additional specificity information.

Bench Top Stability:

There was no statistical difference in recovery when serum samples were subjected to storage at RT for 2 hours, 4°C for 3 hours, or 37°C for 1 hour.

Long-term Stability:

Serum samples with values near the high, mid, and low portion of the assay range were selected. Stability at -70°C and at -20°C over a period of 1 year was examined. There is no change in recovery after a year of storage at -70°C. There may be a small decrease in recovery after a year of storage at -20°C; however the recovery range at 370 days is 78-95%, and the difference between the lower end of the range and 100% may be due to assay variability. Long-term stability studies are ongoing.

Freeze/thaw Stability:

There was no statistical difference in recovery when diluted standards and serum samples were subjected to 1-4 freeze/thaw cycles.

Dilutional Linearity:

Samples diluted 1:2 had recoveries <90%; however samples and standards diluted further (1:10 or more) show no dilutional linearity. Some lack of dilutional linearity in this case is due to dilution below the LLOQ.

Ruggedness and robustness are not addressed. However, it is not clear that this assay was performed in more than 1 location or with more than 1 instrument at this time. Given the number of assays used for determination of specificity, precision, etc., they may have been performed by multiple analysts and on different days. The specificity and precision for the standards and quality controls run during the separate LLOQ assessment are acceptable and similar to the results obtained during the original specificity and precision determinations.

Conclusions:

The PK assays (Method PK0106G2OPHuSe, Method ICD171, and MET-001831) appear to be appropriate methods for the detection of denosumab in human serum. The assay acceptance criteria and validation study results are within the recommendations discussed in the FDA Guidance for Industry: Bioanalytical Method Validation (2001) and in DeSilva, et al., Recommendations for the Bioanalytical Method Validation of Ligand-binding Assays to Support Pharmacokinetic Assessments of Macromolecules (Pharm Res 20:1885, 2003).

The PD assays (PK0505CTHuSe, ICD244, and PK0302TCNCTHuSe) are also generally within the guidelines of these documents, and the methods themselves represent an acceptable approach to the detection of CTx in human serum. There is a question regarding the possibility of interference of physiological levels of serum CTx II or NTx. The assay is validated through CTx II and NTx serum levels of 9.1 ng/ml and 30 nM, respectively. CTx II at levels of 45.5 ng/ml appear to interfere with detection of spiked CTx (interference begins between the 9.1 and 45.5 ng/ml test points); however, I have found CTx II in the literature measured only as urine levels, not serum levels, so I do not know if endogenous serum CTx II levels above 9.1 ng/ml would exist. From the literature, it appears that normal serum NTx levels are below 25 nM BCE but that levels averaging approximately 46 nM BCE have been detected in serum from cancer patients with bone metastases. This CTx assay will detect NTx at concentrations of 30 nM BCE or higher, and an additive effect of spiked CTx plus NTx is observed at this level. Therefore, it is possible for NTx levels to have an effect on the CTx assay for samples from subjects in some of the clinical studies for cancer indications. The clinical pharmacology reviewer (Chongwoo Yu) does not have a concern with the potential interference with respect to this BLA.

CLINICAL PHARMACOLOGY REVIEW

BLA:	125320, 125331, 125332, and 125333
Type/Category:	New molecular entity, First in class
Brand Name:	Prolia TM
Generic Name:	Denosumab
Proposed Indication:	Treatment (BLA 125320) and prevention (BLA 125331) of osteoporosis; Treatment and prevention of bone loss in patients undergoing hormone ablation therapy (HALT) for breast (BLA 125332) or prostate (BLA 125333) cancer
Dosage Form:	Injection
Route of Administration:	Subcutaneous
Dosing Regimen and Strength:	60 mg/ml every 6 months
Sponsor:	Amgen Inc.
OCP Division:	Division of Clinical Pharmacology 3 (DCP3); Division of Clinical Pharmacology 5 (DCP5)
OND Division:	Division of Reproductive and Urologic Products (DRUP); Division of Biologics and Oncology Products (DBOP)
Submission Date:	December 19, 2008 (original)
Primary Reviewers:	Chongwoo Yu, Ph.D.; Sarah Schrieber, Ph.D.
Secondary Reviewers:	Jang-Ik Lee, Pharm.D., Ph.D.; Hong Zhao, Ph.D.
Pharmacometrics Primary Reviewer:	Ping Ji, Ph.D.
Pharmacometrics Secondary Reviewer:	Pravin Jadhav, Ph.D.

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1 EXECUTIVE SUMMARY

Denosumab is a fully human IgG2 monoclonal antibody that inhibits receptor activator of nuclear factor kappa B (RANK) ligand, proposed for the treatment (BLA 125320) and prevention (BLA 125331) of osteoporosis in postmenopausal women (PMO) and for the treatment and prevention of bone loss in patients undergoing hormone ablation therapy (HALT) for breast (BLA 125332) or prostate (BLA 125333) cancer. Denosumab is considered to be a new molecular entity (NME) and the first monoclonal antibody proposed for the indications above (i.e., first in class). The proposed proprietary name for denosumab, Prolia™ was found to be acceptable to the Division of Medication Error Prevention and Analysis (DMEPA) and the Sponsor was notified via a letter on May 20, 2009.

This application includes 30 clinical studies including 13 Clinical Pharmacology and Biopharmaceutics studies conducted in healthy volunteers and patients performed from June 2001 to September 2008. Among the 13 Clinical Pharmacology and Biopharmaceutics studies subject to review, there were 4 pharmacokinetics (PK) and tolerability studies conducted in healthy volunteers, 2 PK and tolerability studies conducted in cancer patients, 2 dose-ranging studies conducted in PMO patients, 1 intrinsic factor PK study conducted in patients with renal impairment, and 1 extrinsic factor PK study conducted in patients with prior exposure to bisphosphonates.

The Division of Reproductive and Urologic Products (DRUP) has conducted review for the PMO related indications (BLAs 125320 and 125331) while the Division of Biologics and Oncology Products (DBOP) has conducted review for the cancer related indications (BLAs 125332 and 125333).

A required office-level Clinical Pharmacology briefing was held on Tuesday, August 11, 2009 with approximately 40 attendees. An Advisory Committee (AC) meeting was held on Thursday, August 13, 2009 to discuss about this product.

1.1 RECOMMENDATIONS

The Division of Clinical Pharmacology 3 (DCP-3) has reviewed BLA 125320 and 125331 and the Division of Clinical Pharmacology 5 (DCP-5) has reviewed BLA 125332 and 125333. The overall Clinical Pharmacology and Biopharmaceutics data submitted to support the approval of BLAs 125320, 125332, and 125333 are acceptable provided that a satisfactory agreement is reached regarding the labeling language.

With regards to BLA 125331(prevention of PMO), while the general pharmacokinetics of denosumab should be unchanged in this indication, (b) (4)

[REDACTED]

1.2 PHASE IV COMMITMENTS

The Clinical Pharmacology Review Team recommends the following post marketing commitments (PMC):

Anti-cytokine antibodies such as tocilizumab, an anti-IL-6 monoclonal antibody, showed the alteration of CYP substrate drug exposure by affecting the effect of cytokine on drug metabolism. Thus, denosumab may affect the exposure to CYP substrate drugs by altering the concentration of RANKL, a cytokine that affects B- and T-cell differentiation, and dendritic cell maturation.

Therefore, the Clinical Pharmacology Review Team recommends the sponsor conduct an in vitro study to assess whether RANKL modulate expression of major CYP enzymes (e.g., CYP 3A4, CYP 1A2, CYP 2C9, CYP 2C19, and CYP 2D6). If, upon review, there is no significant modulation of any of the major CYP enzyme(s) observed, further exploration would not be necessary. If the results of the in vitro study are positive, a drug interaction study or studies will be needed to further characterize the effect of denosumab on the metabolism of CYP probe drugs in PMO patients.

As an alternative to the in vitro study and the subsequent drug interaction study above, the sponsor may conduct a drug interaction study to determine the potential of denosumab to alter CYP substrate metabolism in PMO patients (e.g., using a cocktail of the major CYP probe drugs).

1.3 SUMMARY OF CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FINDINGS

Pharmacokinetics

It should be noted that early clinical studies (Studies 20010124, 20030180, 20030148, 20030164, 20010123, and parts of 20010223) were conducted using denosumab drug substance manufactured using an initial version of the commercial process, designated CP1 at Amgen Thousand Oaks (ATO) (see more information under *Comparability* section below).

In an open-label, randomized, single-dose, parallel-group study (Study 20050146) in healthy men and women volunteers (n=73) having a mean age of 33.6 years (range 18 to 64 years), the mean maximum serum denosumab concentrations (C_{max}) of 6.75 µg/ml (standard deviation [SD]: 1.89 µg/ml) was reached in the median time of 10 days (range: 3 to 21 days) following a 60 mg SC dose after at least 12 hrs of fasting prior to

denosumab administration. After C_{max} , serum denosumab concentrations decline over a period of 4 to 5 months with a mean half-life of 25.4 days (SD: 8.5 days; $n=46$; Study 20010223). No accumulation in serum denosumab concentrations was observed with repeated doses of 60 mg once every 6 month (Q6M), and denosumab PK did not appear to change with time (up to 4 years exposure). Denosumab PK was not affected by the formation of binding antibodies to denosumab and was similar in men and women.

The serum concentration time profiles of denosumab were best characterized as a two-compartment model with first-order absorption and a parallel linear and non-linear elimination. Approximately dose-proportional increases in exposure (based on $AUC_{0-\tau}$) were observed for doses ≥ 60 mg (i.e., in the range of fixed doses of 60 to 210 mg in Study 20010223 in the PMO population). Across the range of doses tested, denosumab plasma concentrations declined at a faster rate when serum denosumab concentration dropped below approximately 1 $\mu\text{g/ml}$. The mechanism behind this change in elimination rate is likely related to denosumab binding to RANKL (i.e target-mediated disposition). This non-linear elimination mechanism predominates at low serum denosumab concentrations (i.e., < 1 $\mu\text{g/ml}$ in this case) and becomes saturated as serum denosumab concentration increases.

Because denosumab is a monoclonal antibody and is not eliminated via hepatic metabolic mechanisms (e.g., by cytochrome P450 [CYP] enzymes), hepatic impairment and drug interaction studies (e.g., with CYP inhibitors or inducers) were not considered appropriate by the Sponsor and have therefore not been conducted. However, considering that the effect of denosumab, an anti-cytokine antibody, on CYP activities is unknown, a post-marketing commitment (PMC) recommendation is being made to the Sponsor to address denosumab's effect on CYP activities and drug interaction potential.

A study including transition from a bisphosphonate to denosumab was conducted (Study 20050241) and showed that the PK of denosumab was not altered in subjects who transitioned from bisphosphonates to denosumab.

A renal impairment study (Study 20040245) was conducted and included 55 patients with normal, mild, moderate, severe, and end-stage renal disease, defined by creatinine clearance (CrCL). Overlap was observed in denosumab exposure across renal impairment cohorts, and no notable relationship was apparent between denosumab PK and renal impairment. No dose adjustment is necessary in patients with renal impairment.

Pharmacodynamics

Denosumab administration resulted in significant inhibition of bone resorption, as assessed by reductions in serum levels of Type 1 C-telopeptide (CTX1). In clinical studies, treatment with 60 mg of denosumab resulted in rapid reduction in the bone resorption marker serum CTX1 (sCTX1) within 6 hours of SC administration by approximately 70% (Studies 20030216 and 20040132), with reductions of approximately 85% occurring by 3 days (Study 20010223). sCTX1 reductions in bone turnover appeared to be maintained throughout the dosing interval (6 months). At the end of the

dosing cycle, some attenuation of bone resorption inhibition was observed, indicating that reduction of bone turnover associated with denosumab administration is reversible when serum concentrations of denosumab diminish. Bone mineral density (BMD) continuously increased during treatment. The effects of denosumab on BMD were rapid, with significant increases in lumbar spine BMD and hip BMD observed as early as 1 month following initiation of treatment, and sustained, with greater increases compared with placebo observed at each time point through the end of treatment (up to 3 years, depending on the study) in these subject populations.

No relationship between denosumab exposure and changes in serum calcium following denosumab administration was observed.

Exposure-Response Relationship

In Study 20010223, the percent change from baseline in sCTX1 and lumbar spine BMD following 2 consecutive doses of various denosumab concentrations given every 3 or 6 months to postmenopausal women with low BMD was assessed. Denosumab treatment was associated with an increase in BMD of the lumbar spine. The gain in lumbar spine BMD of all active treatment groups was significantly greater compared to placebo. The gain in lumbar spine BMD following 60 mg Q6M dosing is comparable to that observed following 100 and 210 mg Q6M dosing and greater than that observed following 14 mg Q6M dosing. However, all dose groups achieved similar increases in BMD after 48 months. There was no dose-response relationship for safety established.

Intrinsic Factors

Based on the pharmacometrics review (attached), age and gender were not significant covariates in the population PK analysis. In the population PK analysis both race (as Black and Hispanic) and solid tumor were identified as covariates for clearance. The PK of denosumab did not appear to be affected by these covariates as shown in the simulation results.

The appropriateness of fixed dosing regimen of 60 mg Q6M was evaluated through the effect of body weight on new vertebral fractures and BMD levels. Although body weight was identified as a covariate for clearance, body weight did not appear to affect the incidence of new vertebral fracture over the 36 months period and change in the BMD levels. While denosumab PK parameters are dependent on body weight, these differences in exposure did not affect the response to denosumab. Therefore, the proposed dosing regimen was found to be appropriate for all patients recommended for use.

Comparability

The Biopharmaceutics program demonstrated the comparability between denosumab drug substance and drug product produced for the pivotal Phase 3 clinical trials and those intended for commercial use.

Drug substance: During the development of denosumab, there were process changes of manufacturing the drug substance. Denosumab drug substance used in the nonclinical and early clinical studies (Studies 20010124, 20030180, 20030148, 20030164, 20010123,

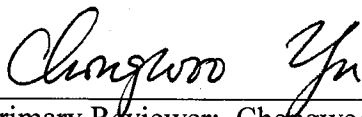
and parts of 20010223) was manufactured using an initial version of the commercial process, designated CP1 at Amgen Thousand Oaks (ATO). Denosumab drug substance manufactured at ATO using an optimized process with increased product yields and improved process robustness, designated as CP2 has been used in all of the later clinical trials. Although the Sponsor has assessed the comparability of denosumab produced using the CP1 and CP2 processes in a nonclinical study with cynomolgus monkeys, the PK comparability of denosumab produced using CP1 and CP2 processes in human is unknown. It should be noted that the Sponsor is proposing to use PK data obtained from studies conducted using CP2 process drug substance for labeling purpose.

Manufacturing site change and product preparation: The drug substance used in the pivotal Phase 3 trials was manufactured at ATO while the to-be-marketed drug substance was manufactured at Amgen Colorado (ACO) and Boehringer-Ingelheim Pharma (BIP). The Sponsor is proposing to have both a vial and a prefilled syringe (PFS) product preparation. PK and PD comparability assessments were conducted by the Sponsor to establish comparability across the different manufacturing sites as well and the different product preparations. Assessments of PK and PD comparability were based on the rate (maximum observed serum denosumab concentration [C_{max}]) and extent (area under the serum denosumab concentration-time curve from time zero to 16 weeks [$AUC_{0-16 \text{ weeks}}$]) of denosumab exposure and were supported by PD parameters (e.g., area under the effect curve from time zero to 16 weeks [$AUEC_{0-16 \text{ weeks}}$] for reductions in sCTX1). In addition, the safety profiles including immunogenicity were also compared and appeared to be consistent between the drug substances from the three different manufacturing sites. In the Clinical Pharmacology studies performed in healthy volunteers (Studies 20050227, 20060286, and 20050146), the PK and PD comparability of denosumab drug substances manufactured at 3 manufacturing sites (ATO, ACO, and BIP) and using 2 drug product preparations (PFS and vial) was established.

Immunogenicity

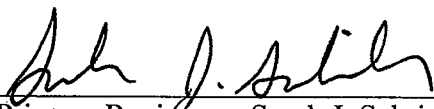
Immunogenicity testing (using a validated electrochemiluminexcent bridging immunoassay) has been performed in all denosumab clinical studies. The immunogenicity potential with denosumab is low. Less than 1% (43 out of 8113) of patients treated with denosumab tested positive for binding antibodies. No patients tested positive for neutralizing antibodies. No evidence of altered PK, PD, safety profile, or clinical response has been observed in patients who tested positive for binding antibodies.

1.4 SIGNATURES



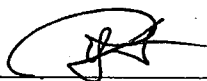
Primary Reviewer: Chongwoo Yu, Ph.D.
Division of Clinical Pharmacology 3

Date: 8/21/2009



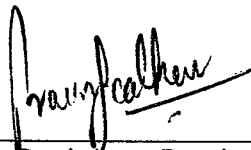
Primary Reviewer: Sarah J. Schrieber, Pharm.D.
Division of Clinical Pharmacology 5

Date: 8/21/09



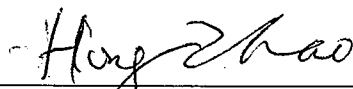
Primary Reviewer: Ping Ji, Ph.D.
Division of Pharmacometrics

Date: 8/21/2009



Secondary Reviewer: Pravin Jadhav, Ph.D.
Division of Pharmacometrics

Date: 8/21/2009



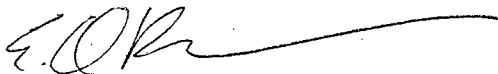
Secondary Reviewer: Hong Zhao, Ph.D.
Division of Clinical Pharmacology 5

Date: 8/21/2009



Secondary Reviewer: Jang-Ik Lee, Pharm.D., Ph.D.
Division of Clinical Pharmacology 3

Date: 8/21/2009



Division Director: Edward D. Bashaw, Pharm.D.
Division of Clinical Pharmacology 3

Date: 8/25/09

QUESTION BASED REVIEW

2.1 General Attributes

2.1.1 What are the highlights of the chemistry and physical-chemical properties of the drug substance and the formulation of the drug product?

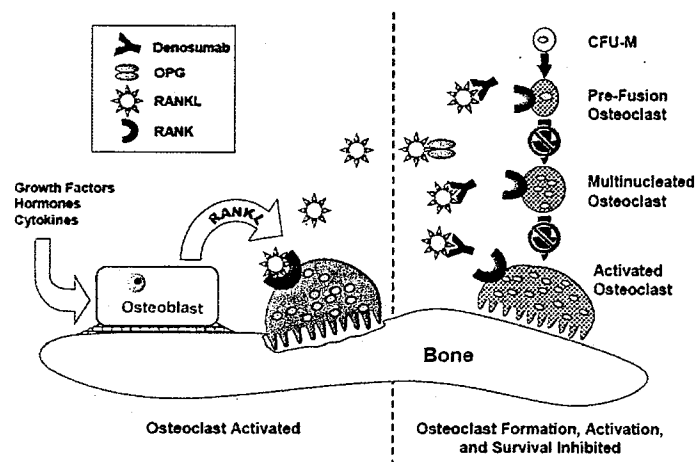
Denosumab (molecular weight: 147 kDa) is a fully human IgG2 κ -type monoclonal antibody to receptor activator of nuclear factor- κ B (RANK) ligand (RANKL; a member of the tumor necrosis factor [TNF] family of proteins) that binds with high affinity and specificity to RANKL (K_d 3×10^{-12} M). Denosumab binds only to RANKL and does not bind to other members of the TNF family of proteins, including TNF α , TNF β , TNF-related apoptosis-inducing ligand, or CD40 ligand (Elliott *et al*, 2006).

Denosumab drug product is supplied as a single-use, sterile, preservative-free solution intended for delivery by subcutaneous injection, supplied in either a 60 mg/ml prefilled syringe (PFS) or 60 mg/ml vial presentation with a 1.0 ml deliverable volume to support dosing of 60 mg every 6 months (Q6M).

2.1.2 What are the proposed mechanisms of action and therapeutic indications?

Denosumab is a fully human IgG2 monoclonal antibody binding to RANKL. This binding prevents the activation of RANK and inhibits the formation, activation, and survival of osteoclasts (Figure 1), the result of which is a reduction in the number and function of osteoclasts and, consequently, a decrease in bone resorption and an increase in cortical and trabecular bone mass, volume, and strength (Kostenuik, 2005). As a result of its mechanism of action, denosumab is being investigated as a therapy for postmenopausal osteoporosis (PMO) and bone loss associated with hormone ablation therapy (HALT).

Figure 1: Mechanisms of Action for Denosumab, Osteoprotegerin, and RANKL



CFU-M - colony-forming unit-macrophage; OPG = Osteoprotegerin; RANK = receptor activator of nuclear factor- κ B; RANKL = RANK ligand
Adapted from Boyle *et al*, 2003

2.1.3 What are the proposed dosage and route of administration?

The Sponsor's proposed dose of denosumab is 60 mg subcutaneously once every six months (Q6M). The proposed dosing regimen of denosumab was evaluated up to a length of 4 years and was generally well tolerated.

2.2 General Clinical Pharmacology and Biopharmaceutics

2.2.1 What are the design features of the clinical pharmacology and clinical studies used to support dosing or claims?

The Clinical Pharmacology program was designed to: characterize the initial safety, tolerability, PK, PD, and exposure-response properties of denosumab in healthy volunteers (men ≥ 50 yrs old] and postmenopausal women ≥ 40 yrs old]) and in patients with BMD or osteoporosis or bone loss associated with hormone ablation therapy (HALT); guide the selection of the dose regimens for Phase 2 and 3 studies; evaluate the effect of covariates (gender, body weight, body mass index (BMI), race, and age) on the PK and PD of denosumab; explore possible relationships between the presence of denosumab binding antibodies and denosumab PK and PD properties; and explore the relationship between serum concentrations of denosumab and calcium. Furthermore, the Clinical Pharmacology program was designed to assess the impact of renal impairment on the PK, PD, and safety profiles of denosumab, and to explore the effect of transitioning from a bisphosphonate on the PK, PD, and safety profiles of denosumab.

The Biopharmaceutics program demonstrated the PK and PD comparability between denosumab drug substance and drug product produced for the pivotal Phase 3 clinical trials and those intended for commercial use.

The Clinical Pharmacology and Biopharmaceutics studies are outlined in Section 5.1 of the Appendix.

The PMO clinical development program is supported by 2 pivotal Phase 3 studies (Studies 20030216 and 20040132). Study 20030216 was a 3-year randomized, double-blind, placebo-controlled study in postmenopausal women with osteoporosis to determine whether denosumab treatment can reduce the incidence of new vertebral (primary endpoint), and nonvertebral and hip fractures (secondary endpoints) as compared with control. Denosumab decreased the incidence of new vertebral fractures (primary endpoint) by approximately two thirds, from 7.2% in the placebo group to 2.2% (risk ratio: 0.32; $p < 0.0001$).

Study 20040132 is a randomized, double-blind, placebo-controlled study in postmenopausal women with low bone mass to determine whether denosumab treatment can prevent lumbar spine bone loss. Denosumab statistically significantly increased BMD (based on dual energy x-ray absorptiometry [DXA]) at the lumbar spine, total hip, femoral neck, trochanter, distal 1/3 radius, and total body at month 24 ($p < 0.0001$ after

multiplicity adjustment) for both early and late postmenopausal women and for both strata combined.

To support the approval in breast cancer, the Sponsor conducted two Phase 1 studies, one Phase 2 study, and one Phase 3 study. Patients in the Phase 1 studies and Phase 2 study were assigned to receive various doses (0.1-3 mg/kg and 30-180 mg, respectively) of denosumab administered at various intervals (single, or every 4 or 12 weeks). The dose and dosing regimen for the Phase 3 study was based on data obtained from a dose ranging Phase 2 study in post-menopausal women. The Phase 1 and Phase 2 breast cancer studies provided supportive data for the 60 mg dose used in the Phase 3 study. In the Phase 3 study, 252 patients were randomly assigned to receive placebo or denosumab 60 mg subcutaneously every 6 months for 18 months (i.e., 4 total doses). Results indicate that treatment with denosumab statistically significantly increased BMD, as assessed by DXA, at the lumbar spine, total hip, and femoral neck at Months 6 and 12 ($p < 0.0001$), as compared to placebo.

To support the approval in prostate cancer, the Sponsor conducted one Phase 3 study. In the Phase 3 study, 1,468 patients were randomly assigned to receive placebo or denosumab 60 mg subcutaneously every 6 months for 30 months (i.e. 6 total doses). Results indicate that treatment with denosumab statistically significantly increased BMD, as assessed by DXA, at the lumbar spine, total hip, and femoral neck at Month 24 and Month 36 (adjusted $p < 0.0001$), as compared to placebo.

2.2.2 What is the basis for selecting the response endpoints or biomarkers and how are they measured in clinical pharmacology and clinical studies?

Clinical Endpoints

Postmenopausal Osteoporosis

Bone fracture is considered to be the primary clinical endpoint for 'treatment' of osteoporosis. BMD will be considered as the primary clinical endpoint for the 'prevention' indication only if the Sponsor already has an approval for the indication of 'treatment' of osteoporosis. Per *Guidelines for Preclinical and Clinical Evaluation of Agents used in the Prevention or Treatment of Postmenopausal Osteoporosis* (Division of Metabolic and Endocrine Drug Products, April, 1994), if an agent has already been approved to treat osteoporosis, increased BMD may be an appropriate efficacy endpoint for prevention since fracture protection has already been shown in the treatment approval.

Because PMO is characterized by increases in fracture risk and reductions in BMD, the efficacy endpoints in the denosumab clinical studies included vertebral fractures, nonvertebral fractures, hip fractures, and BMD at several anatomical sites (lumbar spine, total hip, femoral neck, trochanter, 1/3 distal radius, and total body) (Table 1). In order to further characterize the mechanism of action of denosumab, serum biochemical markers of bone turnover were also evaluated as endpoints.

Table 1: Efficacy Endpoints in the Pivotal and Supportive Phase 3 Studies in Postmenopausal Women with Low Bone Mass or Osteoporosis

Endpoint	Pivotal Phase 3 Efficacy Studies		Supportive Efficacy Studies			Other Studies
	Study 20030218	Study 20040132	Study 20050141 (phase 3)	Study 20050234 (phase 3)	Study 20050173 (phase 2)	Study 20050233 (phase 3)*
Incidence of new vertebral fractures	Primary	Tertiary	--	--	--	--
Time to first non-vertebral fracture	Secondary	--	--	--	--	--
Time to first hip fracture	Secondary	--	--	--	--	--
Percent change from baseline in lumbar spine BMD	Tertiary	Primary	Secondary	Secondary	--	Secondary
Percent change from baseline in total hip BMD	Tertiary	Secondary	Primary	Primary	Secondary	Secondary
Percent change from baseline in femoral neck, trochanter, 1/3 distal radius, and total body BMD	Tertiary	Secondary	--	--	Secondary	--
Percent change from baseline in femoral neck, trochanter, and 1/3 distal radius	--	--	Secondary	Tertiary	--	Secondary
Percent change from baseline in trabecular, cortical, and total volumetric BMD of the distal radius	Tertiary	Secondary	--	--	Secondary	--
Percent change from baseline in cortical thickness at the distal radius by XtremeCT	--	--	--	--	Primary	--
Percent change from baseline in bone markers (serum CTX1, P1NP, BSAP and TRAP 5b)	Tertiary	Tertiary	Tertiary (CTX1 and P1NP only)	Secondary (serum CTX1 only)	Secondary (CTX1, P1NP, TRAP 5b only)	Secondary (CTX1 and BSAP only)

* The primary objective was to evaluate the long-term safety of denosumab administration in postmenopausal women with low BMD who completed the parent Study 20010223.

Breast and Prostate Cancer

The endpoints for both of the pivotal Phase 3, multicenter, randomized, double-blinded, placebo-controlled studies were percentage change from baseline in lumbar spine BMD.

Bone Turnover Markers

sCTX1 is a product of the proteolytic process of bone resorption brought about by osteoclasts and is a recognized biochemical marker for bone resorption. Changes in sCTX1 concentrations have been shown to correlate with changes in BMD in elderly, osteopenic and osteoporotic, postmenopausal women treated with alendronate (Greenspan *et al*, 2000). The Sponsor believes that rapid and sustained effect of denosumab on bone turnover markers has been demonstrated and therefore, is proposing to include a summary of the effects of denosumab on bone turnover markers, notably sCTX1, in the PD section of the label.

2.2.3 Are the active moieties in the plasma (or other biological fluid) appropriately identified and measured to assess pharmacokinetic parameters and exposure response relationships?

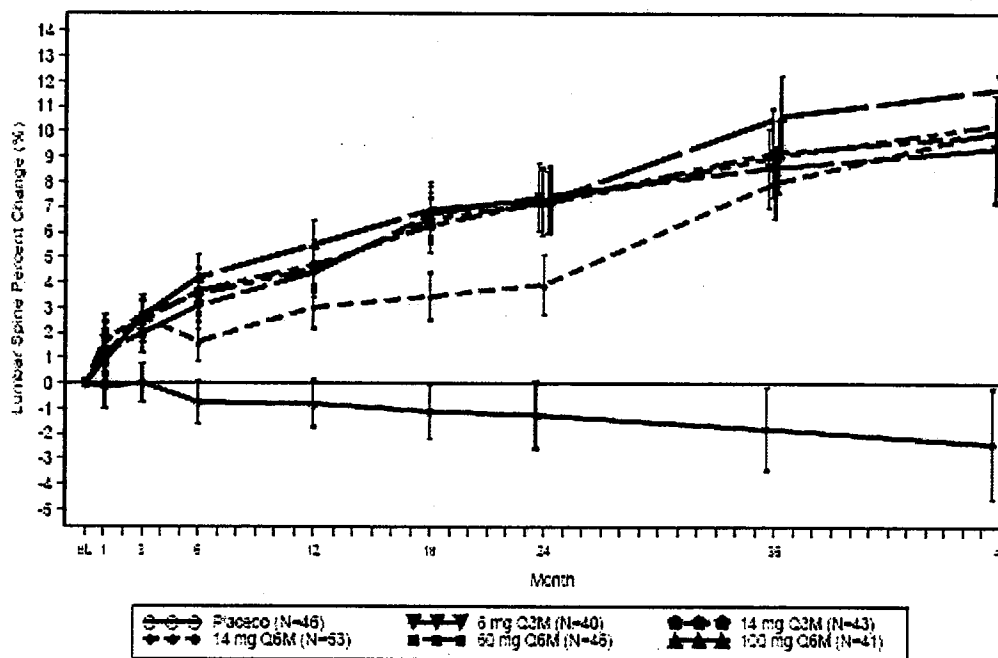
Yes. A validated, conventional sandwich enzyme-linked immunosorbent assay (ELISA) was used to quantify serum denosumab concentrations (see Section 2.6 for detail information).

2.2.4 Exposure-response

2.2.4.1 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for efficacy?

In Study 20010223, the percent change from baseline in sCTX1 and lumbar spine BMD following 2 consecutive doses of various denosumab concentrations given every 3 or 6 months to postmenopausal women with low BMD was assessed. Denosumab treatment was associated with increase in BMD of the lumbar spine and the effect was significant compared to placebo. The gain in lumbar spine BMD following 60 mg Q6M dosing is comparable to that observed following 100 and 210 mg Q6M dosing and greater than that observed following 14 mg Q6M dosing (Figure 2). This result indicates that longer durations of maximal reductions in bone resorption for doses ≥ 60 mg does not result in greater gains in BMD. There was no dose-response relationship for efficacy established.

Figure 2: Least Squares Mean (+ 95% Confidence Interval [CI]) Percent Change from Baseline in Lumbar Spine BMD for Subjects in the Continuous Treatment Cohorts (Study 20010223, 48-month Analysis)



Population includes all subjects who had at least one baseline and at least one postbaseline measurement.
Note: Least Squares Means and 95% Confidence Intervals are from a linear model with percent change from baseline value as the dependent variable and treatment, geographic location and baseline value as independent variables.

2.2.4.2 What are the characteristics of the exposure-response relationships (dose-response, concentration-response) for safety?

The overall incidence of adverse events, serious adverse events, and adverse events leading to treatment withdrawal were generally similar between denosumab and placebo groups. No relationship between denosumab exposure and changes in serum calcium following denosumab administration was observed. Refer to Section 1.1.3 of Dr. Ping

Ji's pharmacometrics review attached separately. There was no dose-response relationship for safety established.

2.2.4.3 Does this drug prolong the QT or QTc interval?

According to Dr. Vaishali Popat's (clinical primary reviewer) clinical review, denosumab is not anticipated to have a direct effect on ion channels. Therefore, a thorough QT study was not required.

In preclinical studies, no effect of denosumab on QTc interval was observed. The clinical development program included an intensive assessment of the effects of denosumab on electrocardiograms, with particular emphasis on the QTc interval. There were no significant differences in the changes from baseline in QTcF across the denosumab and placebo treatment groups, however, several outliers with QTcF value > 500 ms and QTcF change from baseline > 60 ms were noted more frequently in the denosumab group. A QT consult was requested from QT-IRT team to evaluate effect of denosumab on QT interval. The QT-IRT Team's opinion is that the sponsor's ECG evaluations appear adequate and there are no large effects on the QT interval due to denosumab. Outliers (patients with absolute post-dose QTcF over 500 ms or over 60 ms change from baseline) have been noted in several studies although underlying ECG abnormalities were noted in several of the studies except Study 20050172 and Study 20040114. It is important to note that subjects were not excluded because of baseline QTc prolongation. There was no imbalance in the reports of sudden death between the denosumab and comparator groups.

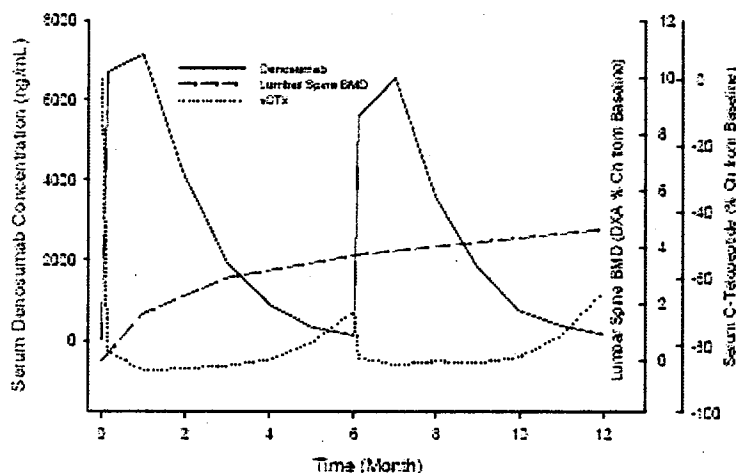
In addition, no relationship was observed between change from baseline in QTc and change from baseline in serum calcium concentration. There is no discernible relationship between denosumab exposure and changes in serum calcium. There was no relationship between denosumab serum concentration and change in QTcF. For Details, please refer to Dr. Suchitra Balakrishnan's QT-IRT consult review.

2.2.4.4 Is the dose and dosing regimen selected by the Sponsor consistent with the known relationship between dose-concentration-response, and are there any unresolved dosing or administration issues?

Figure 3 illustrates the mean serum denosumab concentrations and percent change from baseline in sCTX1 and lumbar spine BMD following 2 consecutive doses of 60-mg denosumab Q6M to postmenopausal women with low BMD in Study 20010223. Eligible subjects in this study were postmenopausal women with low BMD ($-4.0 \leq \text{T-score} \leq -1.8$ for the lumbar spine or $-3.5 \leq \text{T-score} \leq -1.8$ for the total hip or femoral neck) who were ≤ 80 years of age at the time of randomization, not receiving medication that affected bone metabolism, and free from any underlying condition that might have resulted in abnormal bone metabolism. C_{max} for denosumab is typically attained at 1 month post-dose, which coincides with a rapid, extensive, and sustained reduction in the bone resorption marker, sCTX1. Following C_{max} , serum denosumab concentrations decrease with a mean half-life of approximately 30 days. During the last 2 months (on average) of the Q6M dose interval, serum denosumab concentrations decrease to a level at which

binding to RANKL is no longer saturated. This level corresponds to more rapid elimination of denosumab and a lessening in denosumab's effect on sCTX1 levels, although significant suppression remains (\geq approximately 55%). This partial attenuation in denosumab's effect on sCTX1 does not impact denosumab's effect on lumbar spine BMD

Figure 3: Mean Serum Denosumab Concentration and Mean Percent Change From Baseline for Serum CTX1 and Lumbar Spine BMD Following Two 60-mg Q6M Doses of Denosumab to Postmenopausal Women with Low BMD (Study 20010223)



As mentioned in Section 2.2.4.1, the 60 mg Q6M dose was selected by the sponsor because no additional efficacy was observed at higher doses. The Sponsor's PK based argument on the appropriateness of fixed dose (60 mg 6QM) was evaluated through the effect of body weight on new vertebral fractures and BMD levels and was found to be appropriate for all patients recommended for use in treatment of PMO (see Dr. Ping Ji's pharmacometrics review attached separately)

Reviewer's Comment to the Sponsor:

The proposed dosing regimen of 60 mg Q6M was based on a Phase 2 dose ranging study, Study 20010223. Eligible subjects in this study were postmenopausal women with low BMD ($-4.0 \leq T\text{-score} \leq -1.8$ for the lumbar spine or $-3.5 \leq T\text{-score} \leq -1.8$ for the total hip or femoral neck) who were ≤ 80 years of age at the time of randomization, not receiving medication that affected bone metabolism, and free from any underlying condition that might have resulted in abnormal bone metabolism. The proposed dosing regimen of 60 mg Q6M was found to be acceptable for the indication of treatment of PMO.

(b) (4)

(b) (4)

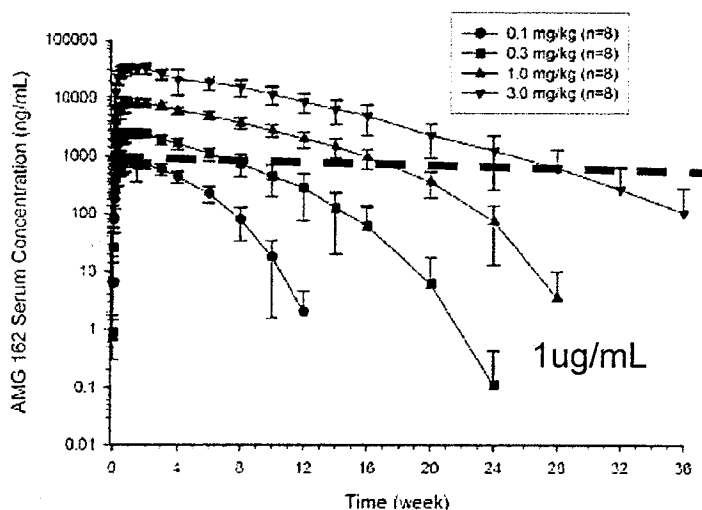
2.2.5 Pharmacokinetic characteristics of the drug and its major metabolites

2.2.5.1 What are the single dose (SD) and multiple dose (MD) PK parameters?

PK of Healthy Volunteers and PMO Patients

As shown in Figure 4 below, mean concentration-time profiles of the 1.0 and 3.0 mg/kg dose cohorts declined in parallel over a long duration (up to 20 weeks post-dose where the denosumab serum concentrations were $> 1 \mu\text{g/ml}$), which represented a large proportion of the total exposure ($\text{AUC}_{0-16 \text{ weeks}}$) to denosumab, due to the higher serum denosumab concentration maintained. Approximately dose-proportional increases in exposure (based on $\text{AUC}_{0-\text{tau}}$) were observed for doses $\geq 60 \text{ mg}$ (in the range of fixed doses of 60 to 210 mg in Study 20010223 in the PMO population).

Figure 4: Mean (\pm SD) Serum Concentrations of Denosumab After Single-dose SC Administration to Healthy Men 50 Years and Older (Study 20030148)



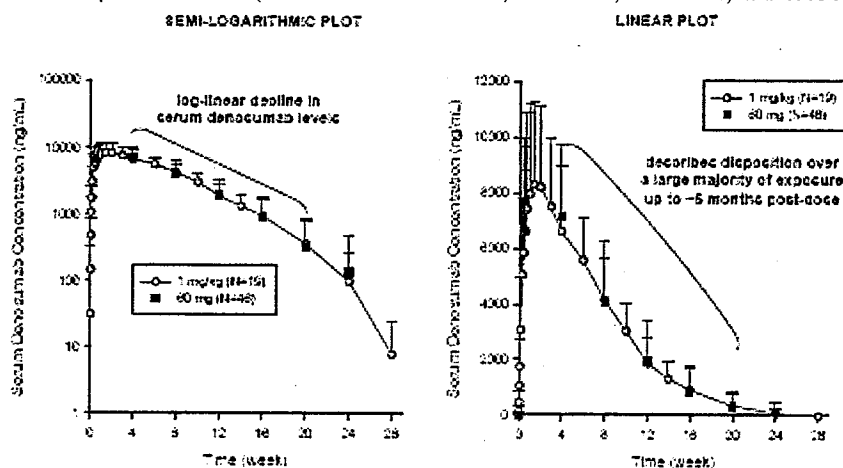
The serum concentration time profiles of denosumab were best characterized as a two-compartment model with first-order absorption and a parallel linear and non-linear elimination. Denosumab was eliminated with a faster rate when serum denosumab concentration dropped below approximately $1 \mu\text{g/ml}$. As shown in Figure 4, this phenomenon becomes apparent earlier in the lower dose groups (i.e., 0.1 and 0.3 mg/kg) compared to the higher dose groups (i.e., 1.0 and 3.0 mg/kg). The mechanism behind this change in elimination rate is likely related to denosumab binding to RANKL (i.e target-mediated disposition). This mechanism predominates at low serum denosumab concentrations (i.e., $< 1 \mu\text{g/ml}$ in this case) and becomes saturated as serum denosumab concentration increases.

Target-mediated disposition refers to a phenomenon wherein a significant proportion of a drug (relative to dose) is bound with high affinity to a pharmacological target, such as this interaction is reflected in the PK properties of the drug. Although a few small molecular weight compounds have been identified to exhibit target-mediated disposition, the incidence of target-mediated disposition is likely to increase particularly among

biologic drug products. Similar target-mediated drug disposition has been reported for several monoclonal antibodies (Hayashi *et al*, 2006; Ng *et al*, 2006; Ng *et al*, 2005).

Figure 5 shows the PK profile of the proposed 60 mg fixed dose and also compares it to the 1.0 mg/kg weight based dose showing comparable PK profiles.

Figure 5: Mean Serum Denosumab Concentration-Time Profiles following SC Administration of 60 mg or 1 mg/kg to Postmenopausal Women (From Studies 20010124, 20010223, 20030164, and 20030180)



In an open-label, randomized, single-dose, parallel-group study (Study 20050146) in healthy men and women volunteers (n=73) having a mean age of 33.6 years (range 18 to 64 years), the mean maximum serum denosumab concentrations (C_{max}) of 6.75 μ g/ml (standard deviation [SD]: 1.89 μ g/ml) was reached in the median time of 10 days (range: 3 to 21 days) following a 60 mg SC dose after at least 12 hrs of fasting prior to denosumab administration. After C_{max} , serum denosumab concentrations declined over a period of 4 to 5 months with a mean half-life of 25.4 days (SD: 8.5 days; n=46; Study 20010223). Mean (Standard Deviation) denosumab PK Parameters are summarized in Table 2.

It should be noted that the Phase 1 healthy volunteer PK studies (Studies 20010124, 20010223, 20030164, and 20030180) were conducted using denosumab drug substance manufactured using an initial version of the commercial process, designated CP1 at ATO while denosumab drug substance manufactured using an optimized process with increased product yields and improved process robustness, designated as CP2, has been used in all of the later clinical trials.

Table 2: Mean (Standard Deviation) Denosumab PK Parameters

Study Number	Denosumab treatment cohort	AUC (µg·day/ml)	C _{max} ^a (ng/ml)	T _{max} ^b (day)	t _{1/2β} (day)	CL/F ^c (ml/day/kg)
Healthy Volunteer PK and Tolerability Studies						
20010124	SD SC (n=3-7)	AUC _{0-∞}				(ml/hr/kg)
	0.01 mg/kg	0.77 (0.27)	49.9 (16.0)	7 (3, 7)	N/A	0.62 (0.17)
	0.03 mg/kg	3.77 (2.04)	157 (67.3)	7 (5, 14)	23.3 (2.44)	0.52 (0.40)
	0.1 mg/kg	20.4 (4.08)	721 (103)	7 (3, 14)	19.5 (3.15)	0.21 (0.04)
	0.3 mg/kg	115 (42.1)	2230 (873)	14 (7, 56)	33.2 (9.5)	0.13 (0.06)
	1.0 mg/kg	538 (224)	8990 (3340)	18 (7, 42)	30.2 (7.04)	0.09 (0.04)
	3.0 mg/kg	2070 (483)	36200 (7280)	10 (5, 42)	29.5 (5.46)	0.06 (0.01)
	MD SC (n=5-6)					
	0.1 mg/kg (1 st dose)	23.3 (9.04)	730 (243)	11 (7, 21)	5.3 (0.60)	0.24 (0.13)
	0.1 mg/kg (last dose)	22.2 (6.75)	724 (231)	14 (3, 28)	9.2 (2.0)	0.24 (0.13)
	SD IV (n=6)					
	0.01 mg/kg	2.16 (0.83)	506 (153)	0 (0, 0.2)	N/A	0.22 (0.06)
	0.03 mg/kg	6.00 (0.73)	1040 (36.5)	0 (0, 0.2)	8.3 (1.16)	0.19 (0.02)
	0.1 mg/kg	33.7 (5.71)	3830 (779)	0 (0, 0.0)	12.7 (3.30)	0.13 (0.02)
	0.3 mg/kg	180 (48.3)	10600 (1730)	0.0 (0, 0.0)	24.4 (5.83)	0.08 (0.02)
	1.0 mg/kg	688 (195)	35200 (10400)	0.0 (0, 0.0)	35.1 (4.96)	0.06 (0.02)
	3.0 mg/kg	2760 (738)	110000 (26600)	0.0 (0, 0.2)	37.3 (6.96)	0.05 (0.02)
20030148	SD SC (n=8)	AUC _{0-t}				
	0.1 mg/kg	24.9 (6.5)	0.85 (0.23)	7 (3, 14)	-	4.24 (1.05)
	0.3 mg/kg	105 (22)	2.60 (0.40)	10 (7, 14)	-	2.96 (0.60)
	1.0 mg/kg	479 (74)	8.85 (1.91)	7 (5, 14)	-	2.14 (0.40)
	3.0 mg/kg	1990 (540)	34.9 (6.6)	10 (4, 14)	-	1.60 (0.40)
20030164	SD SC (n=6)	AUC _{0-t}				
	0.03 mg/kg	2.06 (0.53)	99.6 (25.8)	7 (7, 10)	-	15.3 (4.2)
	0.1 mg/kg	15.2 (6.7)	492 (166)	12 (7, 21)	-	8.31 (4.97)
	0.3 mg/kg	84.3 (20.1)	1910 (658)	14 (7, 21)	-	3.72 (0.89)
	1.0 mg/kg	481 (131)	8690 (2170)	14 (10, 21)	-	2.20 (0.56)
	3.0 mg/kg	1790 (650)	27400 (7880)	14 (14, 42)	-	1.85 (0.58)
20030180	SD SC (n=6-8)	AUC _{0-t}				
	0.03 mg/kg	3.63 (2.59)	201 (129)	7 (3, 10)	-	13.2 (9.13)
	0.1 mg/kg	14.8 (6.0)	563 (149)	9 (7, 14)	-	7.84 (3.34)
	0.3 mg/kg	78.3 (44.6)	2050 (876)	10 (7, 14)	-	5.52 (3.69)
	1.0 mg/kg	476 (201)	9530 (4270)	7 (2, 14)	-	2.64 (1.68)
	3.0 mg/kg	1660 (227)	30800 (8510)	7 (1, 10)	-	1.84 (0.27)
Patients PK and Initial Tolerability Studies						
20010123	BC SD SC (n=3-7)	AUC _{0-t} (µg·hr/ml)				
	0.1 mg/kg	234 (101)	448 (282)	14 (3, 21)	N/E	-
	0.3 mg/kg	1400 (754)	1430 (758)	14 (7, 28)	20.7 (4.5)	-
	1.0 mg/kg	5870 (2940)	4850 (2550)	14 (7, 28)	29.7 (6.7)	-
	3.0 mg/kg	27200 (10500)	19800 (6520)	21 (14, 28)	46.3 (16.7)	-
	MM SD SC (n=3-9)	AUC _{0-t} (µg·hr/ml)				
	0.1 mg/kg	502 (270)	625 (314)	7 (7, 14)	N/E	-
	0.3 mg/kg	2460 (787)	2420 (572)	7 (7, 14)	19.5 (3.1)	-
	1.0 mg/kg	11300 (5430)	8260 (3590)	21 (7, 28)	38.6 (13.9)	-

	3.0 mg/kg	20800 (9780)	20000 (6420)	14 (3, 21)	33.3 (21.7)	-
20040176	SD SC (n=5-6)	AUC _{0-τ}				
	60 mg	351 (144)	7730 (3130)	8 (7, 28)	24.7 (2.44)	-
	180 mg	1320 (640)	31100 (14900)	10 (4, 28)	29.1 (7.15)	-
	MD SC (n=6)					
	180 mg (dose 1)	545 (123)	24100 (5130)	18 (7, 28)	N/A	-
	180 mg (dose 2)	1210 (240)	48000 (9340)	14 (7, 21)	N/A	-
Intrinsic Factor PK Study						
20040245	SD SC 60 mg	AUC _{0-16 weeks}				-
	Normal (n=9-11)	217 (76)	5160 (1770)	10 (3, 14)	-	-
	Mild (n=11-13)	266 (143)	6200 (2880)	10 (2, 28)	-	-
	Moderate (n=13)	322 (154)	7040 (3060)	10 (3, 28)	-	-
	Severe (n=9)	295 (120)	6020 (2320)	10 (7, 14)	-	-
	ESRD (n=8)	208 (107)	5370 (2590)	10 (5, 21)	-	-
Extrinsic Factor PK Studies						
20050241	SD SC	AUC _{0-τ}				
	15 mg (n=3)	41.5 (27.6)	1100 (610)	21 (14, 21)	-	-
	60 mg (n=10-12)	332 (176)	7570 (4410)	13 (3, 28)	34.1 (6.7)	-
PK and PD Comparability Studies						
20050227	SD SC	AUC _{0-16 weeks}				
	1 mg/kg ACO (n=58)	432 (150)	9420 (3090)	7.0 (2.0, 21)	-	-
	1 mg/kg ATO (n=59)	440 (110)	9070 (2310)	7.0 (2.0, 21)	-	-
20060286	SD SC	AUC _{0-16 weeks}				
	60 mg ATO (n=58)	258 (81)	5330 (1530)	10 (3.0, 21)	-	-
	60 mg BIP (n=58)	259 (91)	5430 (1820)	7 (3.0, 28)	-	-
20050146	SD SC	AUC _{0-16 weeks}				
	60 mg PFS (n=74)	331 (111)	7110 (2040)	10 (3.0, 21)	-	-
	60 mg GS (n=73)	316 (101)	6750 (1890)	10 (3.0, 21)	-	-
Dose-ranging Study						
20010223	MD SC	AUC _{0-τau}				
	14 mg dose 1 (n=53)	64.4 (39.0)	0 (0)	4 (2.0, 35)	N/A	-
	14 mg dose 2 (n=49)	59.8 (36.5)	0 (0)	21 (2.0, 37)	N/A	-
	60 mg dose 1 (n=46)	503 (239)	7930 (2950)	26 (2.9, 32)	25.4 (8.48)	-
	60 mg dose 2 (n=44)	448 (239)	6940 (3180)	29 (1.9, 42)	27.1 (8.99)	-
	100 mg dose 1 (n=40)	937 (341)	288 (397)	28 (2.9, 38)	30.1 (10.3)	-
	100 mg dose 2 (n=36)	863 (319)	262 (358)	22 (2.0, 37)	28.7 (7.39)	-
	210 mg dose 1 (n=46)	2230 (865)	1390 (1430)	26 (2.9, 35)	32.6 (8.84)	-
	210 mg dose 2 (n=42)	2140 (988)	1470 (1470)	27 (2.9, 39)	33.7 (11.9)	-

SD= Single Dose; MD= Multiple Dose; ESRD= End-state renal disease; IV= Intravenous; SC= Subcutaneous; N/A: Not Applicable; N/E: Not Accurately Estimable; AUC_{0-τau}: Area under the serum denosumab concentration-time curve over the dosing interval

^a C₀ (initial concentration) for IV doses

^b median (range)

^c clearances for intravenous doses

It should be noted that the Sponsor is proposing to use PK data from Studies 20040245, 20050241, 20050146, 20060286, and 20010223 for labeling purposes and these studies were conducted with drug substance manufactured with the CP2 process. While the Sponsor's proposal of using PK data generated with the CP2 process is appropriate, it

should be noted that PK parameters on the label should be from the most reliable single study rather than combining PK parameters from multiple studies.

PK of Cancer Patients

Phase 1 – Breast cancer: The Sponsor conducted a single dose study in breast cancer and multiple myeloma patients and a single and multiple dose study in Japanese breast cancer patients. The single dose study utilized weight-based dosing (0.1, 0.3, 1, and 3 mg/kg) while the other study used fixed doses of 60 and 180 mg; multiple dosing was only conducted at the 180 mg dose level. Additionally, this was the only multiple dosing Phase 1 study in the oncology setting. The sampling times are summarized below:

In the single dose study, samples were obtained at:

- pre-dose and post-dose at 1, 2, 4, 8, and 24h, Days 3, 4, 8, 15, 22, 29, 43, 57, 71, and 85.

In the single dose part of the single and multiple dose study, blood samples for were obtained at:

- pre-dose, and post-dose at 6h; Days 2, 3, 8, 15, 22, 29, 57, and 85.

In the 180 mg multiple dosing arm, samples were obtained at:

- pre-dose, and Days 8, 15, 22, 29, 57, 64, 71, 78, 85, 113, and 141.

Following a single dose, denosumab demonstrated nonlinear PK and a dose-dependent increase in half-life (Table 3, Figure 6). After SC administration, denosumab demonstrated rapid and prolonged absorption, with serum levels that were detectable as early as 1 hour post-dose and mean maximum serum concentrations of 448 ng/ml (0.1-mg/kg cohort) to 3,100 ng/ml (180 mg cohort) occurring 8 to 21 days post-dose. Mean half-lives increased with increased dose (0.1-3 mg/kg) from 20.7 to 46.3 days. Following multiple dosing, an approximate 2.2-fold accumulation was observed by the third dose relative to the first dose (Table 4).

Table 3: Mean (SD) Single Dose Denosumab Pharmacokinetic Parameters in Breast Cancer.

Study #	Dose	C _{max} (ng/ml)	C _{max} / D (ng/ml / mg/kg)	T _{max} * (day)	AUC _{0-t} (μg•h/ml)	t _{1/2} , β (day)
20010123	0.1 mg/kg, N=7	448 (282)	4480 (2820)	14 (3 – 21)	234 (101)	NA
	0.3 mg/kg, N=7	1430 (758)	4770 (2530)	14 (7 – 28)	1400 (754)	20.7 (4.5)
	1.0 mg/kg, N=7	4850 (2550)	4850 (2550)	14 (7 – 28)	5870 (2970)	29.7 (6.7)
	3.0 mg/kg, N=3	19800 (6520)	6610 (2170)	21 (14 – 28)	27200 (10500)	46.3 (16.7)
20040176	60 mg, N=6	7730 (3130)	-	8.0 (7 – 28)	8424 (3456)	24.7 (2.44)
	180 mg, N=6	31100 (14900)	-	10 (4 – 28)	31680 (15360)	29.1 (7.15)

*Data presented as: median (range)

NA= Not applicable

Figure 6: Mean (\pm SD) Denosumab Concentrations Time Profiles in Breast Cancer Patients.
Panel A. Study # 20010123: Single Dose. Panel B. Study # 20040176: Single and Multiple Dose.

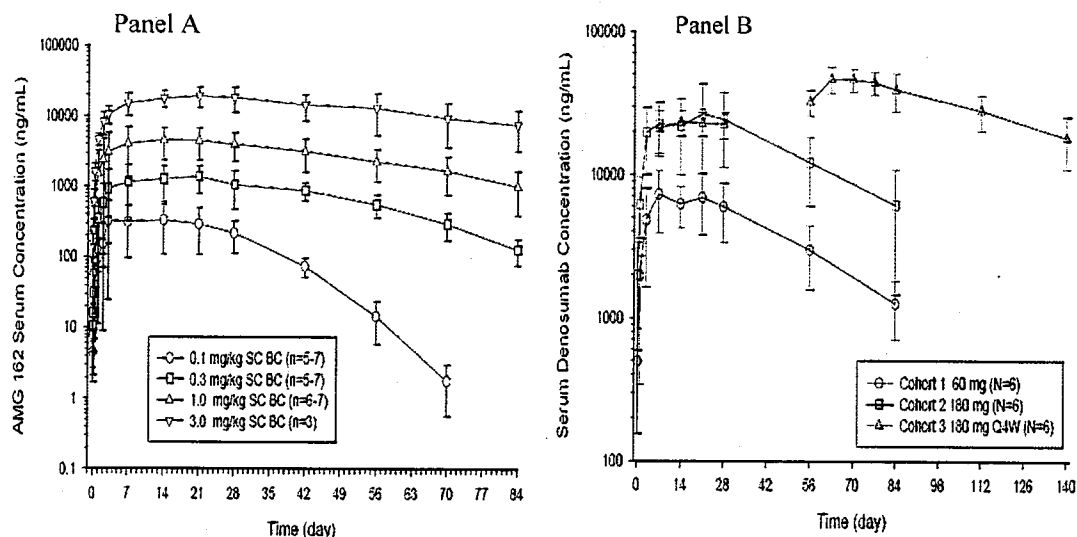


Table 4: Study # 20040176: Mean (SD) Denosumab Pharmacokinetic Parameters Following Multiple Dose Administration of 180 mg Denosumab Every 4 Weeks.

Dose (mg)	Dose	Cmax (ng/ml)	Tmax* (day)	AUC0-28 ($\mu\text{g}\cdot\text{h}/\text{ml}$)	AR
180 Q4W, N=6	1 st	24100 (5130)	18 (7.0 – 28)	13080 (2952)	2.2 (0.18)
	3 rd	48000 (9340)	14 (7.0 – 21)	29040 (5760)	

*Data presented as: median (range)

AR = Accumulation ratio, calculated as AUC0-28 (dose 3) / AUC0-28 (dose 1)

Phase 3 - Cancer (breast or prostate): In the two Phase 3 clinical studies in bone loss associated with hormone-ablative therapy, breast cancer: Study 20040135 and prostate cancer: Study 20040138, approximately 100 and 700 patients, respectively, had denosumab concentrations determined at each sampling time point; the Sponsor's results are listed in Table 5. Denosumab 60 mg was administered as a SC injection every 6 months.

Table 5: Serum Denosumab Concentrations (ng/ml) After SC Administration of 60 mg Denosumab Every 6 Months in Women with Breast Cancer (up to 24 months) and Men with Prostate Cancer (up to 36 months).

Summary Statistics	Month							
	1		3		6		12	
	Breast	Prostate	Breast	Prostate	Breast	Prostate	Breast	Prostate
N	117	701	112	695	112	673	101	648
Mean (SD)	5890 (2500)	4280 (1930)	1730 (1200)	942 (737)	63 (183)	40 (286)	50 (97)	26 (113)
Median	5560	4040	1520	798	1.1	BLQ	BQL	BLQ
Range	1430 - 12200	BLQ - 13800	79.2 - 6920	BLQ - 5000	BLQ - 1480	BLQ - 4340	BLQ - 486	BLQ - 2250
BLQ = Below the lower limit of quantification (LLOQ = 0.8 ng/ml)								

Table 5 cont.

Summary Statistics	Month							
	15		18		24		30	36
	Breast	Prostate	Breast	Prostate	Breast	Prostate	Prostate	Prostate
N	100	624	100	608	95	539	485	462
Mean (SD)	1430 (886)	1060 (832)	116 (413)	35 (104)	76 (196)	40 (174)	43 (130)	47 (142)
Median	1290	895	1.29	BLQ	1.5	BLQ	BLQ	BLQ
Range	40.2 - 5490	BLQ - 6680	BLQ - 3270	BLQ - 932	BLQ - 1510	BLQ - 2960	BLQ - 1310	BLQ - 1830
BLQ = Below the lower limit of quantification (LLOQ = 0.8 ng/ml)								

Within each study, mean and median concentrations at Months 3 and 15 (approximately 3 months post-dose) were similar; indicating that PK did not change with time and that there was no accumulation with repeated dosing (Table 5). When comparing across the 2 studies, the mean concentrations appear to be greater in women with breast cancer compared to men with prostate cancer at months 1, 3, 6, 12, 15, 18, and 24. However, upon population PK analysis, the respective patient populations did not affect denosumab PK, as summarized by Clearance (L/h). Refer to Dr. Ping Ji's Pharmacometrics Review attached separately.

2.2.5.2 How does the PK of the drug and its major active metabolites in healthy volunteers compare to that in patients?

Denosumab PK was similar between healthy volunteers, postmenopausal women with low BMD or osteoporosis, or patients with breast or prostate cancer receiving HALT. No metabolism studies were conducted. Refer to Section 1.1.3 of Dr. Ping Ji's pharmacometrics review attached separately.

2.2.5.3 What are the characteristics of drug absorption?

Following a 60 mg SC dose, bioavailability was 61% based on the population PK analysis. Maximum mean serum denosumab concentrations (C_{max}) of 6.75 µg/ml (SD 1.89 µg/ml) occurred in a median time of 10 days (range 3 to 21 days).

2.2.5.4 What are the characteristics of drug distribution?

Plasma protein binding has not been conducted. Based on non-compartmental analysis (1 mg/ml IV), the mean (SD) V_{ss} is 54.1 (5.67) ml/kg. Based on population PK analysis (2-compartment model), the volume of the central compartment and the volume of the peripheral compartment is 2,460 ml and 1,300 ml, respectively.

2.2.5.5 Does the mass balance study suggest renal or hepatic as the major route of elimination?

Denosumab is a monoclonal antibody product and no mass balance study was conducted.

2.2.5.6 What are the characteristics of drug metabolism?

Denosumab is a monoclonal antibody product and is not expected to be eliminated via hepatic metabolic mechanisms (e.g., CYP enzymes). Thus, no metabolism studies were conducted.

2.2.5.7 What are the characteristics of drug excretion?

Denosumab is a monoclonal antibody product and is not expected to be excreted via the kidney and/or bile as an intact form. Thus, no excretion studies were conducted.

2.2.5.8 Based on PK parameters, what is the degree of linearity or non-linearity based in the dose-concentration relationship?

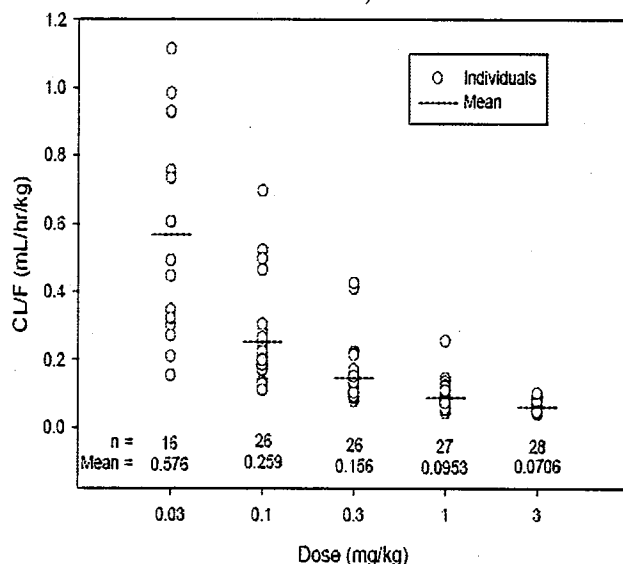
The early Phase 1 studies of denosumab conducted in healthy postmenopausal women and healthy men ≥ 50 years of age using CP1 as drug substance (Studies 20010124, 20030148, 20030164, and 20030180) explored a wide range of weight-based SC doses (0.01 to 3.0 mg/kg) with intensive PK sampling.

Denosumab displayed approximately dose-proportional increases in exposure (based on $AUC_{0-\tau}$) in the dose range of 1.0 mg/kg to 3.0 mg/kg.

As noted previously, denosumab was eliminated with a faster rate when serum denosumab concentration drops below approximately 1 $\mu\text{g/ml}$. The mechanism behind this change in elimination rate is likely related to denosumab binding to RANKL (i.e. target-mediated disposition). This mechanism predominates at low serum denosumab concentrations (i.e., $< 1 \mu\text{g/ml}$ in this case) and becomes saturated as serum denosumab concentration increases. As shown in Figure 4, this phenomenon becomes more apparent in the lower dose groups (i.e., 0.1 and 0.3 mg/kg) compared to the higher dose groups (i.e., 1.0 and 3.0 mg/kg). Consistent with this observation, mean apparent clearance (CL/F) was approximately 8-fold higher at a dose of 0.03 mg/kg (i.e., faster rate of elimination as the plasma levels are below saturation) compared to a higher dose of 3.0 mg/kg (where levels are above the saturation point for the majority of the dosing interval). In addition, mean CL/F values were similar ($< 35\%$ difference), in the dose range of 1.0

and 3.0 mg/kg (Figure 7), again the lack of difference between the doses being related to the amount of time plasma concentrations are above 1 µg/mL.

Figure 7: Apparent Clearance (CL/F) vs. Dose for Weight-Based SC Doses of Denosumab in Healthy Postmenopausal Women and Healthy Men ≥ 50 Years (Studies 20010124, 20030148, 20030164, and 20030180)



Dose linearity was also assessed in Study 20010223 where denosumab was given 6 to 30 mg every 3 months and 14 to 210 mg every 6 months. Mean $AUC_{0-\tau}$ values increased in an approximately dose-proportionately manner between the 60 and 210 mg every 6 months doses. Additionally, exposure based on mean C_{max} values increased approximately dose-proportionately across both the every 3 months and every 6 months dose ranges.

Table 6: Mean (SD) Denosumab PK Parameters Following Administration of 6, 14, or 30 mg Denosumab Every 3 Months

Dose (mg)	Dose number	T_{max} ^a (day)	C_{max} (ng/mL)	$AUC_{0-\tau}$ (day•µg/mL)	C_{min} (ng/mL)	AR
6	1	3.0 (2.9 – 32)	554 (244)	17.4 (8.54)	2.25 (9.80)	1.23
	3	3.9 (1.9 – 35)	638 (276)	20.6 (11.4)	2.36 (5.23)	
14	1	4.0 (2.8 – 39)	1450 (621)	60.3 (25.5)	58.2 (87.2)	1.27
	3	27 (2.0 – 93)	1550 (693)	68.3 (34.5)	97.2 (144)	
30	1	5.0 (2.9 – 34)	3540 (1590)	170 (87.5)	146 (360)	1.04
	3	4.0 (1.9 – 37)	3760 (1830)	193 (108)	799 (720)	

^a Median (range)

T_{max} = time of maximum observed serum denosumab concentration (C_{max})

$AUC_{0-\tau}$ = area under the serum denosumab concentration-time curve over the dosing interval

C_{min} = trough serum denosumab concentration

AR = accumulation ratio

Table 7: Mean (SD) Denosumab PK Parameters Following Administration of 14, 60, 100, and 210 mg Denosumab Every 6 Months

Dose (mg)	Dose number	T _{max} ^a (day)	C _{max} (ng/mL)	AUC _{0-τ} (day·ng/mL)	t _{1/2} (day)	C _{min} (ng/mL)	AR
14	1	4.0 (2.0 - 35)	1490 (681)	64.4 (39.0)	NA	0 (0)	1.74
	2	21 (2.0 - 37)	1390 (672)	59.8 (36.5)	NA	0 (0)	
60	1	28 (2.9 - 32)	7930 (2950)	503 (239)	25.4 (9.47)	137 (334)	0.910
	2	29 (1.9 - 42)	6940 (3180)	443 (239)	27.1 (9.99)	132 (334)	
100	1	28 (2.9 - 38)	14200 (5300)	937 (341)	30.1 (10.3)	288 (397)	1.02
	2	22 (2.0 - 37)	13200 (4550)	863 (319)	28.7 (7.39)	262 (358)	
210	1	28 (2.9 - 35)	32300 (11900)	2230 (865)	32.6 (8.84)	1390 (1430)	1.02
	2	27 (2.9 - 39)	30000 (12700)	2140 (988)	33.7 (11.9)	1470 (1470)	

^a Median (range)

T_{max} = time of maximum observed serum denosumab concentration (C_{max})

AUC_{0-τ} = area under the serum denosumab concentration-time curve over the dosing interval

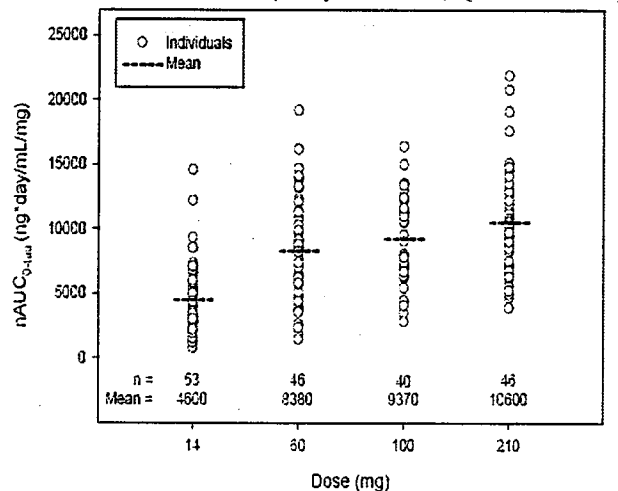
t_{1/2} = half-life describing a large majority of exposure

C_{min} = trough serum denosumab concentration

AR = accumulation ratio

Consistent with weight-based dosing, mean dose-normalized AUC_{0-τ} values increased approximately 2-fold for fixed doses of 14 to 60 mg Q6M (equivalent to approximately 0.3 to 1.0 mg/kg), but were *similar* (< 27% different) for fixed doses of 60 to 210 mg Q6M, with notable overlap observed in individual values (Study 20010223, Figure 8).

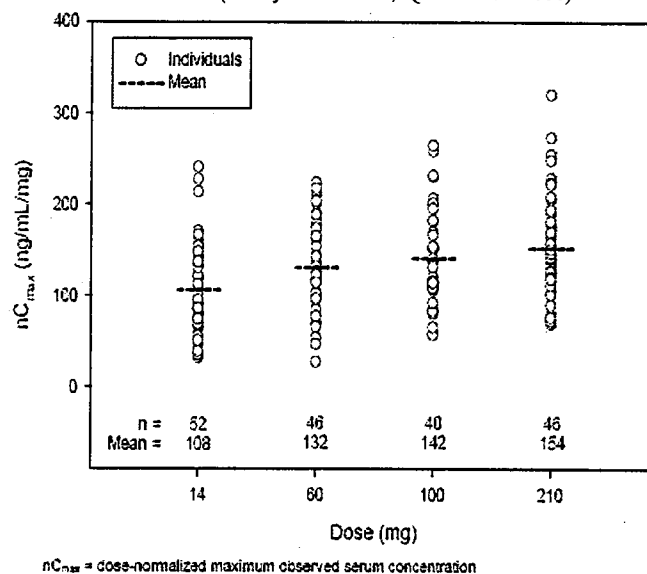
Figure 8: Mean and Individual Dose-normalized AUC_{0-τ} (nAUC_{0-τ}) Values Following SC Administration of 14 to 210 mg Denosumab to Postmenopausal Women with Low BMD (Study 20010223, Q6M First Dose)



nAUC_{0-τ} = dose-normalized area under the serum concentration curve during the dosing interval

Mean dose-normalized C_{max} (nC_{max}) values did not vary markedly (> 50%) across the entire 14 to 210 mg dose range, with differences of < 17% observed for doses ≥ 60 mg (Figure 9). Thus, denosumab exposure, based on both C_{max} and AUC, increases approximately dose-proportionally for a fixed dose range between 60 and 210 mg.

Figure 9: Mean and Individual Dose-normalized Cmax (nCmax) Values Following SC Administration of 14 to 210 mg Denosumab to Postmenopausal Women with Low BMD (Study 20010223, Q6M First Dose)



2.2.5.9 How do the PK parameters change with time following chronic dosing?

No accumulation in serum denosumab concentrations was observed with repeated doses of 60 mg once every 6 month (Q6M), nor would it be expected to with an observed plasma half-life of ~ 1 month. Denosumab PK did not appear to change following up to 4 years exposure.

2.2.5.10 What is the inter- and intra-subject variability of PK parameters in volunteers and patients, and what are the major causes of variability?

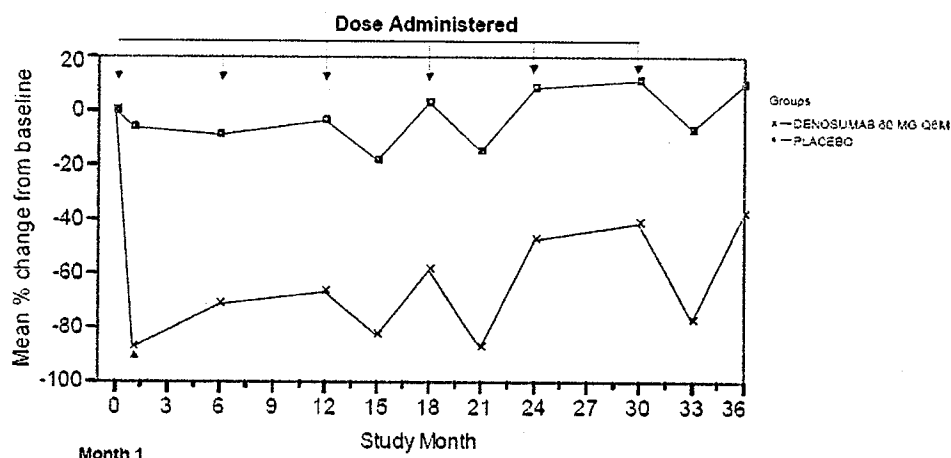
The serum concentration time profiles of denosumab were best characterized as a two-compartment model with first-order absorption and a parallel linear and non-linear elimination. The following parameters were allometrically scaled on the basis of body weight: linear clearance (CL), central volume of distribution (V_c), inter-compartmental clearance (Q), and peripheral volume of distribution (V_p), absorption rate constant (k_a), Michaelis-Menten rate constant (K_m) and maximal velocity (V_{max}). In addition, disease type as solid tumor and subject type as Black and Hispanic were also identified as categorical covariates of CL. After adjusting these covariates, the inter-subject variability (%CV) of CL, V_{max} , V_c , and k_a was 40, 51, 50, and 43, respectively. The residual variability (%CV) of the PK model was 26 and 81 for high concentration and low concentration, respectively.

2.2.6 What are the PD characteristics of denosumab?

In clinical studies, treatment with 60 mg of denosumab resulted in rapid reduction in the bone resorption marker, sCTXI within 6 hours of SC administration by approximately 70% (Studies 20030216 [Figure 10] and 20040132), with reductions of approximately

85% occurring by 3 days (Study 20010223). Peaks in sCTX1 levels were each time before the dose was due and valleys were at 3 months after the dose.

Figure 10: sCTX1 levels by visit in Study 20030216



sCTX1 reductions were maintained over the 6-month dosing interval (Figure 11). At the end of each dosing interval, sCTX1 reductions were partially attenuated from maximal reduction of $\geq 87\%$ to $\geq 45\%$ (range 45% to 80%), reflecting the reversibility of the effects of denosumab on bone remodeling once serum levels diminish (Studies 20040135, 20040138, 20040132, and 20030216; Table 8). These effects were sustained with continued treatment. Consistent with the physiological coupling of bone formation and resorption in skeletal remodeling, subsequent reductions in bone formation markers were observed beginning 1 month after the first dose of denosumab.

Figure 11: Mean Serum Denosumab Concentration and Mean Percent Change From Baseline for Serum CTX1 and Lumbar Spine BMD Following Two 60-mg Q6M Doses of Denosumab to Postmenopausal Women with Low BMD (Study 20010223)

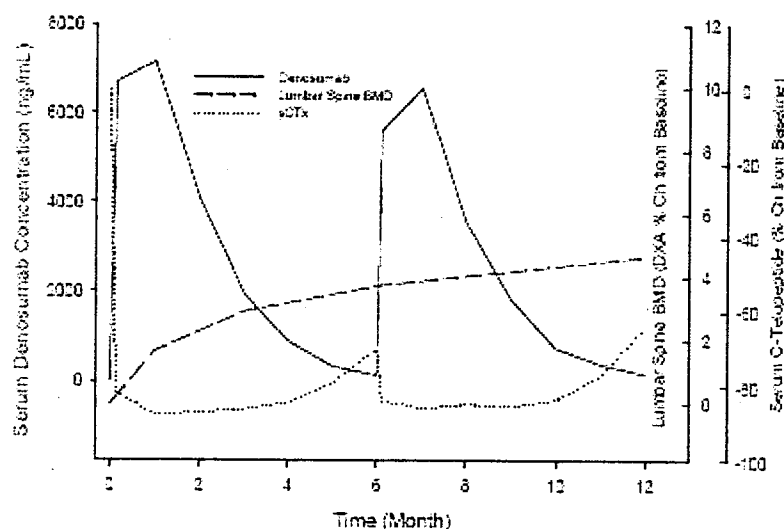


Table 8: Summary Statistics for Percent Reduction in sCTX1 at 1 Month after SC Administration of 60 mg Denosumab

	Healthy Adults	Postmenopausal women			Subjects with cancer and bone loss associated with HALT	
		With low BMD		With osteoporosis	Breast	Prostate
Study No.	20050146	20040132	20050233 ^a	20030216	20040135	20040138 ^b
N	140	160	22	93	109	671
Mean	82	87	90	87	88	87
SD	11	8	6	8	8	13

BMD = bone mineral density, HALT = hormone ablation therapy

^a Change from Study 20010223 baseline in subjects who were switched from placebo in Study 20010223 to 60 mg Q6M denosumab in Study 20050233; this study included women with low BMD and osteoporosis.

^b Excluding subject with outlier sCTX1 value (Subject 138134013 with 1060% change from baseline).

Bone turnover markers generally reached pretreatment levels within 9 months after the last 60 mg SC dose (Studies 20040132 and 20010223). Upon reinitiation, the degree of inhibition of sCTX1 by denosumab was similar to that observed in patients initiating denosumab treatment.

In pivotal Studies 20040135 and 20040138 for cancer indications, baseline sCTX1 were similar between treatment groups. Median percentage changes from baseline in concentrations of sCTX1 at the time points assessed were greatest in the denosumab group at Month 1. Treatment with denosumab resulted in sustained decreases in concentrations of sCTX1 relative to placebo at each post-baseline assessment ($p < 0.0001$ at all time points).

In a clinical study (Study 20050241) of postmenopausal women with low bone mass ($n=20$) who were previously treated with alendronate with a median duration of 3 years, those transitioning to receive alendronate experienced additional reduction in sCTX1, compared with women who remained on alendronate. Fourteen days after dosing, the mean percent change from baseline in sCTX1 concentration was approximately -60% and -73% for subjects in the 15 mg and 60 mg denosumab groups, respectively. These changes were generally maintained from study Day 14 through study Day 84. The mean percent change from baseline for sCTX1 for subjects who continued on alendronate treatment ranged from approximately -40% to +20%, with no change, on average at study Day 107. Please refer to Section 2.4.2 for further discussion.

2.3 Intrinsic Factors

2.3.1 What intrinsic factors (age, gender, race, weight, height, disease, genetic polymorphism, pregnancy, and organ dysfunction) influence exposure (PK usually) and/or response, and what is the impact of any differences in exposure on efficacy or safety responses?

Gender

Mean serum denosumab concentration-time profiles for healthy men ≥ 50 years in Study 20030148 (Figure 12) were also similar to those observed for postmenopausal women administered the same doses in Study 20010124 (Figure 13).

Figure 12: Mean (\pm SD) Serum Concentrations of Denosumab After Single-dose SC Administration to Healthy Men 50 Years and Older (Study 20030148)

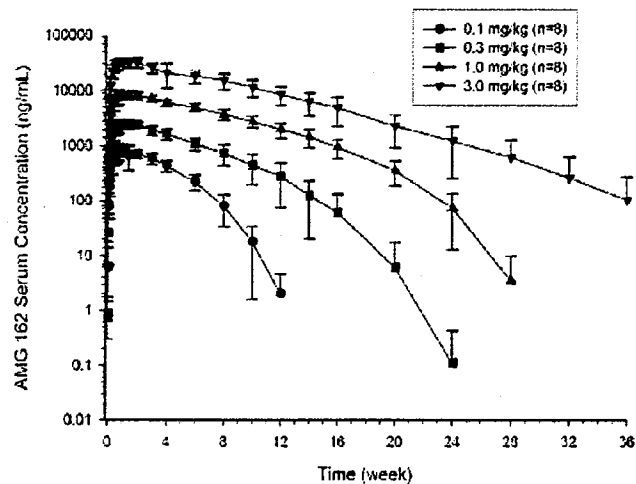
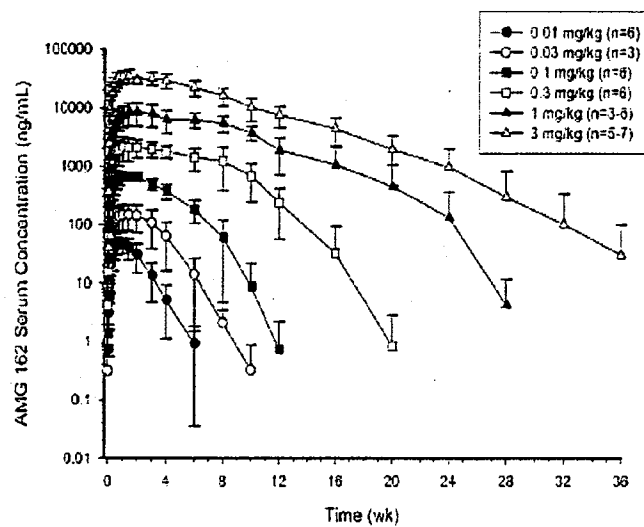


Figure 13: Mean (\pm SD) Serum Concentration-time Profiles of Denosumab in Healthy Postmenopausal Women (Study 20010124, SC Single-dose Cohorts)



Body Weight

Although body weight was identified as a covariate for clearance, body weight did not appear to affect the incidence of new vertebral fracture and change in the BMD levels (Figures 14 and 15).

Figure 14: The Incidence of Any New Vertical Fracture versus Body Weight (From Study 20030216)

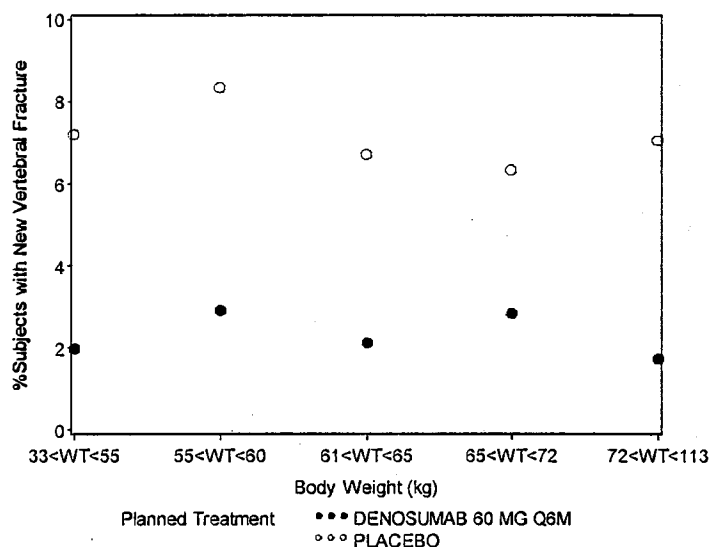
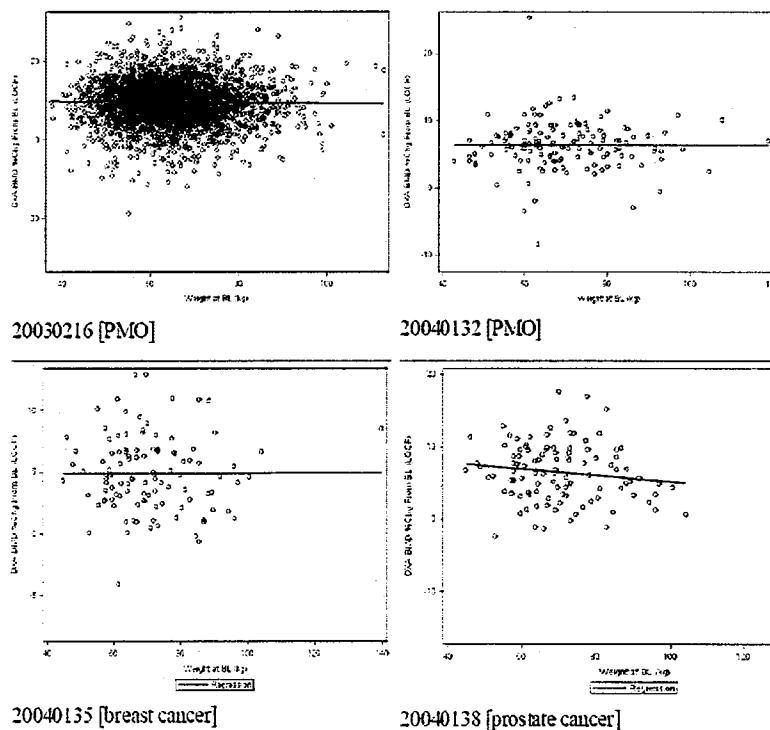


Figure 15: Scattered Plots of Lumbar Spine Bone Mass Density versus Body Weight (From Studies 20030216, 20040132, 20040135, and 20040138)



Denosumab PK parameters are dependent on body weight. However, differences in exposure do not affect response to denosumab. Figure 5 in Section 2.2.5.1 further illustrates that exposures following a weight based dose of 1 mg/kg and a fixed dose of 60 mg to postmenopausal women are comparable. Therefore, fixed dose of 60 mg

appears to be appropriate for all patients recommended for use. Please refer to Sections 1.1.1 and 1.1.2 of Dr. Ping Ji's pharmacometrics review attached separately.

Other Intrinsic Factors

Based on the pharmacometrics review attached separately, Sponsor's population PK analysis is generally adequate and the population PK analysis showed that denosumab's bioavailability is 61%. Age and gender are not significant covariates in the population PK analysis. Subject type as solid tumor and race (as black and Hispanic) were identified as covariates for clearance in the population PK model (Amgen Pharmacometric report 100957) and the significant covariates identified by the sponsor were reproduced (see Section 3 of Dr. Ping Ji's pharmacometrics review attached separately). The PK of denosumab did not appear to be affected by race and solid tumor as shown in the simulation results.

2.3.2 Based upon what is known about exposure-response relationships and their variability and the groups studied, healthy volunteers vs. patients vs. specific populations, what dosage regimen adjustments, if any, are recommended for each of these groups? If dosage regimen adjustments are not based upon exposure-response relationships, describe the alternative basis for the recommendation.

2.3.2.1 Pediatric patients

A pediatric indication is not being sought at this time, and a pediatric waiver has been requested.

2.3.2.2 Renal impairment

Study 20040245 investigating the effect of renal impairment on the PK of denosumab was completed in male and female patients with varying degrees of renal impairment and a normal renal function control group. During screening, subjects were assigned to a renal function group based on creatinine clearance (CrCL) as calculated by the Cockcroft-Gault equation. All subjects were administered a single SC dose of 60 mg denosumab. The renal function groups are defined in Table 9.

Table 9: Definition of Renal Impairment Cohorts.

Group	Creatinine Clearance (CrCL), ml/min
Normal	CrCL > 80
Mild chronic kidney disease (CKD)	CrCL 50 - 80
Moderate CKD	CrCL 30 - 49
Severe CKD	CrCL < 30
End-stage renal disease (ESRD)	hemodialysis

Blood samples for analysis of denosumab were collected up to 113 days after dosing. Specifically, PK samples were collected at pre-dose, and post-dose Days 2, 3, 6, 8, 11, 15, 22, 29, 43, 57, 85, and 113. A total of 55 subjects were enrolled in this study. The

patient demographics between cohorts were comparable and a summary of the demographics can be found in the individual study report.

Overlap was observed in denosumab maximum concentrations and exposures across renal function groups, and no clear trend was apparent between denosumab PK and renal function group (Figure 16). Mean C_{max} and $AUC_{0-16 \text{ weeks}}$ values differed by 4% to 48% when each renal impairment group was compared with the normal renal function group (Table 10). Linear regression analyses did not demonstrate a significant relationship between baseline CrCL and any of the denosumab PK parameters (Table 13). Based on these data, the conclusion can be made that the PK profile of denosumab was not significantly affected by varying degrees of renal impairment.

Figure 16: C_{max} (Panel A) and $AUC_{0-16 \text{ weeks}}$ (Panel B) vs. Renal Function Group After SC Administration of 60 mg Denosumab.

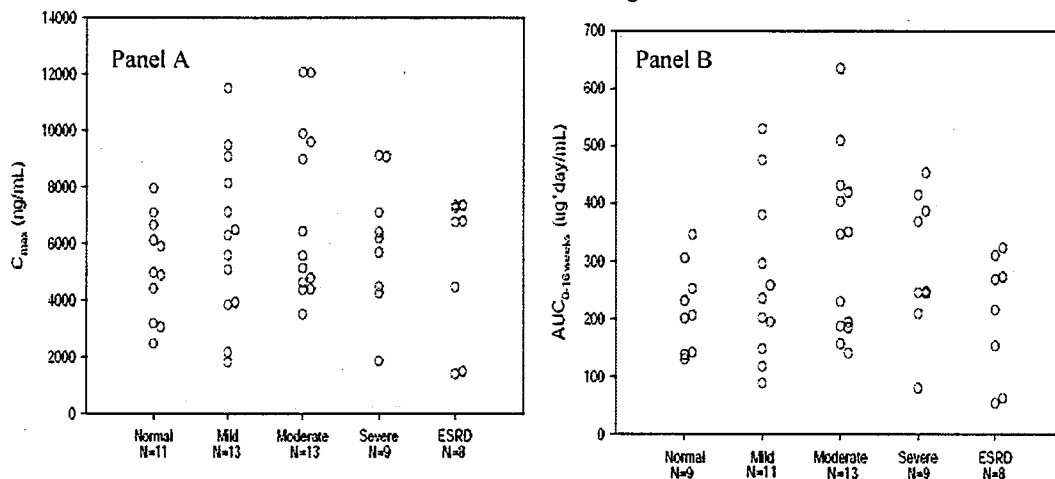


Table 10: Mean (SD) [%CV] Serum Denosumab Pharmacokinetic Parameter Estimates After SC Administration of 60 mg Denosumab.

PK Parameter	Renal Impairment Group					p-Value	
	Normal (n=11)	Mild (n=13)	Moderate (n=13)	Severe (n=9)	ESRD (n=8)	Jonckheere-Terpstra ^d	Linear Regression ^e
AUC0-16weeks ^a ($\mu\text{g}\cdot\text{d}/\text{ml}$)	217 (76) [35]	266 (143) [54]	322 (154) [48]	295 (120) [41]	208 (107) [51]	0.595	0.173 (estimate=-0.0028)
% Change vs. Normal ^b	-	23%	48%	36%	-4%		
Cmax ($\mu\text{g}/\text{ml}$)	5160 (1770) [34]	6200 (2880) [46]	7040 (3060) [43]	6020 (2320) [39]	5370 (2590) [48]	0.511	0.334 (estimate=-0.0018)
% Change vs. Normal ^c	-	20%	36%	17%	4%		
Tmax (day)	10 (3-14)	10 (2-28)	10 (3-28)	10 (7-14)	10 (5-21)	-	-

^a N for AUC0-16weeks: 9, 11, 13, 9, 8 for Normal, Mild, Moderate, Severe, end ESRD, respectively

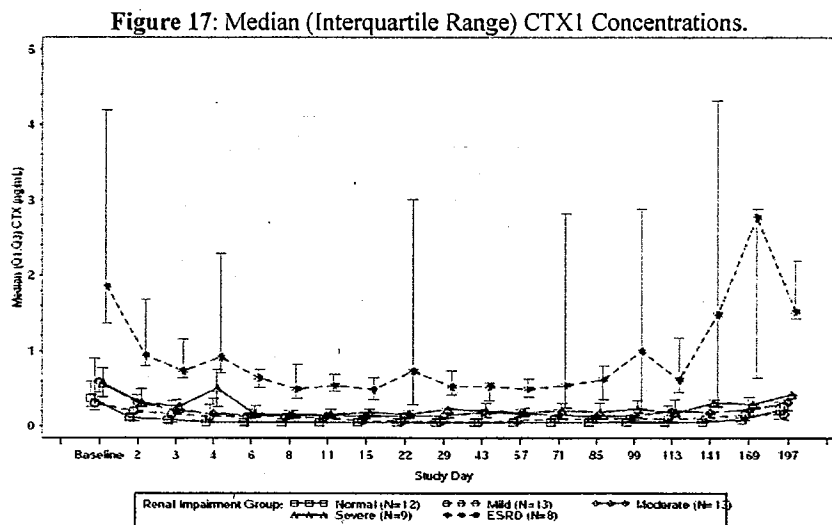
^b % Change vs Normal was calculated as the mean % difference in AUC and Cmax for each renal group compared to the normal group.

^c Tmax is presented as median (range).

^d p-value was obtained by sponsor from nonparametric Jonckheere-Terpstra trend test.

^e Regression analysis of the relationship between CrCL and PK Parameters

Denosumab treatment resulted in decreases from baseline in sCTX1 concentration in all of the renal function groups, which were sustained from the first observation at Day 2 through the end of study (Figure 17). Median percent decreases from baseline in sCTX1 concentrations during the study (65% to 85% once maximal reductions were achieved) were generally similar across the renal function groups (see the individual study review for complete data).



In conclusion, renal impairment does not appear to alter denosumab PK. Denosumab treatment resulted in decreases from baseline in sCTX1 concentration in all of the renal function groups, which were sustained from the first observation at Day 2 through the end of study.

2.3.2.3 Hepatic impairment

No clinical studies have been conducted to evaluate the effect of hepatic impairment on the PK of denosumab.

2.3.2.4 What pregnancy and lactation use information is there in the application?

No data regarding the excretion of denosumab in the milk of humans or animals was provided.

2.3.3 Immunogenicity (NOT applicable to small molecule drugs)

2.3.3.1 What is the incidence (rate) of the formation of the anti-product antibodies (APA), including the rate of pre-existing antibodies, the rate of APA formation during and after the treatment, time profiles and adequacy of the sampling schedule?

Overall, the incidence rate of developing binding antibodies was 0.5% (43 of 8,113) in denosumab-treated subjects and 0.3% (16 of 5,320) placebo-treated subjects in the studies included in this BLA. The rate of positive pre-existing binding antibodies was 0.2% in placebo patients and 0.1% in denosumab patients. In most of these subjects, the antibodies were transiently detected. Samples for immunogenicity were collected at adequate time points to assess for anti-product antibody formation at early onset, during study, and during study follow-up. Table 11 summarizes the sample collection times in the pivotal trials. For information on immunogenicity sampling in other clinical trials conducted, see the individual study reviews. In summary, the denosumab immunogenicity incidence is low and not associated with any clinical consequence.

Table 11: Immunogenicity Sample Collection Times in the Pivotal Trials.

Pivotal Study #	Patient Population	Immunogenicity Sample Collection Times
20030216	PMO	<ul style="list-style-type: none"> Day 1 (baseline) Months 1, 6, 12, 18, 24, 30, and 36/early termination
20040138	Prostate Cancer	
20040132	PMO	<ul style="list-style-type: none"> Day 1 (baseline) Months 1, 6, 12, 18, and 24/early termination
20040135	Breast Cancer	

2.3.3.2 Does the immunogenicity affect the PK and/or PD of the therapeutic protein?

No evidence of altered PK or PD has been observed in subjects who tested positive for binding antibodies. Examples of patients with positive samples for immunogenicity in the 4 pivotal trials are presented in Tables 12 and 13.

Denosumab concentrations were determined in all patients at 1 month post-dose. Patients with positive samples for immunogenicity during the study each had denosumab concentrations at 1 month within the range at that time point observed for all patients in the denosumab treatment arm.

Table 12: Serum Denosumab Concentrations at Month 1 Post-dose for Antibody-positive Patients.

Study #	Subject #	Time of Positive Ab Results (month)	Denosumab Conc at 1 Month post-dose (ng/ml)	Range of Denosumab Conc at 1 Month post-dose (ng/ml)*
20040132	132105014	1	4710	1620 [†] , 11800
	132103012	24	5140	
20030216	6633313	12	4900	842, 17100
20040135	135185004	1, 12	3020	4130, 12200
	135434001	18	8070	
20040138	138646019	1	13800	<0.8, 13200
* Range of denosumab concentrations observed at 1 Month post-dose for antibody negative-subjects.				
[†] Excludes 2 subjects as described in Section 10 of the 24-month study report.				

Percent change from baseline in lumbar spine and total hip BMD at Month 12 (or 24) were determined in all patients. Patients with positive samples for immunogenicity during the study each had percent change BMD values within the range at that time point observed for all denosumab-treated patients in the study.

Table 13: Percent Change from Baseline in Lumbar Spine and Total Hip BMD at Month 12 (or 24) for Antibody-positive Patients.

Study #	Subject #	Time of Positive Ab Results (month)	Lumbar Spine BMD (%)	Lumbar Spine BMD Range (%)	Total Hip BMD (%)	Total Hip BMD Range (%)
20040132	132105014	1	4.59	-4.2, 21.2	0.75	-2, 6.5
	132103012*	24	12.65	-8.4, 25.4	7.89	-3.1, 7.9
20030216	6633313	12	NR	N/A	5.77	-12.5, 15.1
20040135	135185004	1, 12	3.46	-4.1, 12.9	4.21	-12.5, 13.3
	135434001*	18	9.19	-2.4, 17.7	6.51	-12.5, 13.3
20040138	138646019	1	4.36	-6.8, 18.2	5.74	-6.8, 11
NR, not reported; N/A, not applicable						
*Patient BMD and study range values reported at Month 24 due to timing of positive antibody result.						

No evidence of altered PK or PD has been observed in subjects who tested positive for binding antibodies.

2.3.3.3 Do the anti-product antibodies have neutralizing activity?

Neutralizing antibodies have not been detected in any subject who tested positive for binding antibodies.

2.3.3.4 What is the impact of anti-product antibodies on clinical efficacy?

The denosumab immunogenicity incidence was low and was not associated with any clinical consequences.

2.3.3.5 What is the impact of anti-product antibodies on clinical safety? (e.g., infusion-related reactions, hypersensitivity reactions, cross-reactivity to endogenous counterparts, etc.)?

No evidence of altered safety profiles has been observed in subjects who tested positive for binding antibodies.

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence dose-exposure and/or -response and what is the impact of any differences in exposure on response?

The extrinsic factor influence on dose-exposure and/or –response was not explored.

2.4.2 Drug-drug interactions

None of the general drug-drug interaction questions in the QBR is applicable to this biologics product, denosumab. Because denosumab is a monoclonal antibody and is not eliminated via hepatic metabolic mechanisms (e.g., by CYP enzymes), hepatic impairment and drug interaction studies (e.g., with CYP inhibitors or inducers) were not considered appropriate by the Sponsor and have therefore not been conducted.

However, considering that the effect of denosumab, an anti-cytokine antibody, on CYP activities is unknown, a PMC recommendation is being made to the Sponsor to address denosumab's effect on CYP activities and drug interaction potential (see Section 1.2 for details).

In a clinical study (Study 20050241) of postmenopausal women with low bone mass (n=20) who were previously treated with alendronate with a median duration of 3 years, those transitioning to receive denosumab experienced additional reduction in sCTX1, compared with women who remained on alendronate. As shown in Table 12, the PK of denosumab was not altered in subjects who transitioned from bisphosphonates to denosumab.

Table 12: Mean (SD) Denosumab Serum PK Parameters following 60 mg SC Dose (Study 20050234)

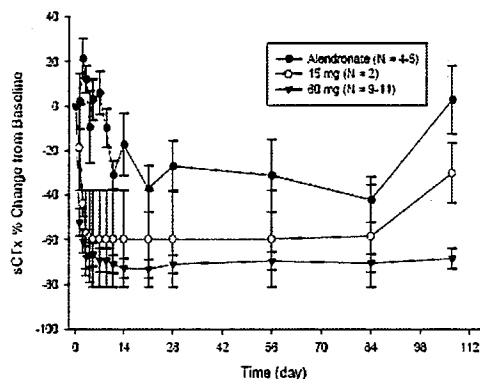
Treatment Groups	T _{max} ^a (day)	C _{max} (ng/ml)	AUC _{0-t} (µg·day/ml)
Alendronate pre-exposed postmenopausal women (n=12)	13 (3, 28)	7570 (4410)	332 (176)
Postmenopausal women (n=73) ^b	10 (3, 21)	6750 (1890)	316 (101)

^a Median (range)

^b Study 20050146 (vial)

Mean percent change from baseline for sCTX1 versus time profiles are presented in Figure 18. Fourteen days after dosing, the mean percent change from baseline in sCTX1 concentration was approximately -60% and -73% for subjects in the 15 mg and 60 mg denosumab groups, respectively. These changes were generally maintained from study Day 14 through study Day 84. The mean percent change from baseline for sCTX1 for subjects who continued on alendronate treatment ranged from approximately -40% to +20%, with no change, on average at study Day 107. In this study, change in serum calcium was similar between the two groups.

Figure 18: Mean (± SE) Serum C-Telopeptide Percent Change From Baseline Profiles for Postmenopausal Women With Low Bone Mass Density Following a Single Subcutaneous Administration of Denosumab 15 mg or 60 mg Dose, or Continuation on Alendronate Therapy (Study 20050241)



2.5 General Biopharmaceutics

Sections 2.5.1 through 2.5.9 are not applicable to therapeutic proteins.

2.5.10 What is the PK and PD comparability of the proposed to-be-marketed formulation to pivotal clinical trial?

The biopharmaceutics program demonstrated the PK and PD comparability between denosumab drug substance and drug product produced for the pivotal Phase 3 clinical trials and those intended for commercial use.

(b) (4)

3 REFERENCES

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4. Greenspan SL, Rosen HN, Parker RA. Early changes in serum N-telopeptide and Ctelopeptide cross-linked collagen type 1 predict long-term response to alendronate therapy in elderly women. *J Clin Endocrinol Metab*. 2000;85:3537-3540.
5. Hayashi N, Tsukamoto Y, Sallas WM, Lowe PJ. A mechanism-based binding model for the population pharmacokinetics and pharmacodynamics of omalizumab. *Br J Clin Pharmacol*. 2006;63(5):548-561.
6. Ng CM, Stefanich E, Anand BS, Fielder PJ, Vaickus L. Pharmacokinetics/pharmacodynamics of nondepleting anti-CD4 monoclonal antibody (TRX1) in healthy human volunteers. *Pharm Res*. 2006;23(1):95-103.
7. Ng CM, Joshi A, Dedrick RL, Garovoy MR, Bauer RJ. Pharmacokineticpharmacodynamic-efficacy analysis of efalizumab in patients with moderate to severe psoriasis. *Pharm Res*. 2005;22(7):1088-1100.

4 LABELING

The following are Clinical Pharmacology relevant parts of the Sponsor's proposed labeling with preliminary labeling recommendations from the Clinical Pharmacology review team. Labeling review will be completed later at the time of label negotiation with the Sponsor.

(b) (4)



5 APPENDIX

5.1 Summary of Clinical Pharmacology and Biopharmaceutics Studies

Table 5.1-1. Clinical Pharmacology and Biopharmaceutics Study Designs and Key Results

Study Number	Study Design & Objectives	Study Population	Key Results
Healthy volunteer PK and initial tolerability studies			
20010124	Phase 1, randomized, double-blind, placebo-controlled, single- and multiple-dose study to assess safety, tolerability, PK, PD, and antibody response	Healthy, postmenopausal women; Age: 40 to 70 yr	Denosumab was generally well tolerated and demonstrated dose-dependent, nonlinear PK. Denosumab doses ≥ 1.0 mg/kg maintained decreases in bone turnover markers for ≥ 6 months post-dose.
20030148	Phase 1, randomized, blinded, placebo-controlled, single-dose study to assess PK, PD, safety, and tolerability	Healthy men; Age: ≥ 50 yr	Denosumab was well tolerated and demonstrated dose-dependent, nonlinear PK. All doses resulted in a rapid suppression of bone turnover markers relative to placebo; suppression was more sustained at higher doses. No differences in PK were apparent between men 50 to 64 years and ≥ 65 years of age.
20030164	Phase 1, randomized, double-blind, placebo-controlled, single-dose study to assess safety, tolerability, PK, and PD	Postmenopausal Japanese women; Age: 40 to 64 yr	Denosumab was well tolerated; demonstrated dose-dependent, nonlinear PK; and rapidly suppressed bone turnover markers relative to placebo. The duration of suppression increased with dose.
20030180	Phase 1, randomized, blinded, placebo-controlled, single-dose study to assess PK, PD, safety, and tolerability	Healthy postmenopausal women; Age: 40 to 64 yr	Denosumab was well tolerated; demonstrated dose-dependent, nonlinear PK; and rapidly suppressed bone turnover markers relative to placebo. The duration of suppression increased with dose.
Patient PK and initial tolerability studies			
20010123	Phase 1, randomized, double-blind, active-controlled, double-dummy, single-dose study to assess the safety, tolerability, PD compared with pamidronate, PK, and antibody response for Denosumab	Men or women with either multiple myeloma or breast cancer, with documented bone lesions/metastases, and estimated survival ≥ 6 months	All doses of denosumab were well tolerated and resulted in rapid suppression of uNTX/Cr. The extent of suppression was dose-dependent for denosumab doses ≤ 1.0 mg/kg. Levels were notably suppressed through 12-weeks postdose for all doses, except 0.1 mg/kg in subjects with BC. Denosumab demonstrated dose-dependent, nonlinear PK.
20040176	Phase 1, open-label, dose-ascending, single- and multiple-dose study	Japanese women with confirmed breast cancer,	Denosumab was well tolerated when administered SC as a single dose of 60 or 180 mg and as

	to assess safety, PK, antibody response, and PD	radiological evidence of ≥ 1 bone metastasis, and ECOG score ≤ 2	multiple doses of 180 mg. Denosumab demonstrated approximately dose-linear PK. As expected, an approximate 2.2-fold accumulation was observed by the third dose relative to the first dose for the 180-mg Q4W cohort. All doses resulted in a rapid and sustained suppression of bone turnover markers.
Intrinsic factor PK study			
20040245	Phase I, open-label, single-dose study to assess PK, safety, and tolerability	Men and women with normal renal function (healthy subjects) and varying degrees of renal impairment	Renal impairment does not impact the PK of denosumab and, therefore, no dose adjustments are required when denosumab is administered to patients with impaired renal function. Denosumab treatment resulted in rapid and sustained suppression in serum CTX1 concentration in all of the renal function groups. In addition, the incidence and types of adverse events reported in this study were generally similar to those reported in other studies with denosumab. As expected with an antiresorptive therapeutic and consistent with other studies, transient decreases in median serum calcium concentration were observed following administration of denosumab. The potential for hypocalcemia in subjects with severe kidney disease and ESRD, however, appeared greater compared with subjects with mild or moderate kidney disease and subjects with normal renal function. This observation is likely due to the fact that subjects with severe kidney disease or ESRD rely more heavily on the bone to provide a source of calcium, due to their impaired ability to reabsorb calcium from the urine and to absorb calcium in the gastrointestinal tract. Therefore, with antiresorptive therapy, these subjects may be more susceptible to reductions in serum calcium. In this study, supplementation with calcium and vitamin D was effective in mitigating the risk of clinically significant hypocalcemia
Extrinsic factor PK study			

20050241	Phase 1, randomized, open-label, single-dose study to assess safety (changes in serum calcium for subjects switched from alendronate to denosumab)	Postmenopausal women who have received alendronate (70 mg QW or equivalent) for ≥ 1 year with low BMD ($-4 \leq T\text{-score} \leq -1$ for spine or total hip); Age: ≤ 80 yr	Transitioning from alendronate to denosumab was generally well tolerated. No clinically significant differences between serum calcium profiles were observed after subjects transitioned from alendronate to denosumab. Denosumab demonstrated dose-dependent, nonlinear PK. All doses tested resulted in a rapid suppression of serum CTX1 relative to alendronate, with a greater extent and duration of suppression observed for the 60-mg dose than the 15-mg dose.
Dose-ranging studies			
20010223	Phase 2, randomized, double-blind, placebo and active-controlled, dose-finding study to assess efficacy (BMD), selection of denosumab dose regimen for future studies, safety, and tolerability	Postmenopausal women with low BMD ($-4.0 \leq T\text{-score} \leq -1.8$ for lumbar spine or $-3.5 \leq T\text{-score} \leq -1.8$ for total hip or femoral neck); Age: ≤ 80 yr	Denosumab was well tolerated and effectively increased trabecular and cortical BMD and decreased bone turnover markers in postmenopausal women with low BMD. The effects of denosumab are reversible, since BMD returned to baseline levels upon discontinuation of treatment. The dose selected for future clinical trials in the bone loss setting was 60 mg Q6M because no additional efficacy for BMD and bone turnover markers was observed at higher doses. Denosumab displayed dose-dependent, nonlinear PK, which did not change with time or upon multiple Q3M or Q6M dosing.
20050172	Phase 2, randomized, double-blind, placebo controlled, dose-response study to assess efficacy (BMD), safety, tolerability, selection of denosumab dose for future studies, PK, and PD	Japanese women with PMO ($-4.0 \leq T\text{-score} \leq -2.5$ for lumbar spine or $-3.5 \leq T\text{-score} \leq -2.5$ for total hip or femoral neck); Age: ≤ 80 yr	Denosumab was well tolerated and effectively increased trabecular and cortical BMD and decreased bone turnover markers in Japanese postmenopausal women with osteoporosis. Denosumab displayed dose-dependent, nonlinear PK, which did not change with time or upon multiple Q6M dosing.
Biopharmaceutics studies			
20050146	Phase 1, randomized, open label, single-dose study to compare PK of denosumab after administration with a PFS vs. a GS (drawn from a vial)	Healthy volunteers; Age: 18 to 65 yr	Denosumab PFS is bioequivalent to denosumab GS. Denosumab administered from a PFS and in a GS was considered well tolerated in this study.
20050227	Phase 1, randomized, openlabel, single-dose	Healthy volunteers;	Denosumab ACO is bioequivalent to denosumab ATO. Denosumab

	study to compare PK of denosumab drug substance produced at ACO vs. ATO	Age: 18 to 65 yr	ACO and ATO were considered well tolerated in this study.
20060286	Phase 1, randomized, open label, single-dose study to compare PK of denosumab drug substance produced at ATO vs. BIP	Healthy volunteers; Age: 18 to 65 yr	Denosumab BIP is bioequivalent to denosumab ATO. Denosumab BIP and ATO were considered well tolerated in this study.

Please refer to the individual study reviews for details of the Clinical Pharmacology and Biopharmaceutics studies.

5.2 Summary of denosumab and sCTX1 bioanalytical assay performances

Table 5.2-1: Denosumab Clinical Studies included in the Summary of Clinical Pharmacology, Summary Statistics for Standard and QC Inter-Assay Performance for Serum Denosumab and CTX1 Concentrations

Study #	Sample Type	Analyte			
		Denosumab		CTX1	
		Accuracy (%Diff)	Precision (%CV)	Accuracy (%Diff)	Precision (%CV)
Healthy Volunteer Pharmacokinetics and Initial Tolerability Studies					
20010124	Standards	-2 to 3	6 to 10	NA	NA
	Quality Controls	-4 to 3	8 to 17	NA	NA
20030148	Standards	-8 to 7	2 to 4	-8 to 7	2 to 8
	Quality Controls	-3 to 7	5 to 7	-8 to 1	4 to 8
20030184	Standards	-8 to 8	2 to 4	-8 to 8	2 to 4
	Quality Controls	-5 to 8	8 to 10	-5 to 4	3 to 6
20030190	Standards	-9 to 7	2 to 3	-5 to 7	2 to 5
	Quality Controls	-1 to 11	5 to 8	-3 to 3	3 to 8
Patient Pharmacokinetics and Initial Tolerability (Advanced Cancer) Studies					
20010123	Standards	-4 to 3	5 to 9	NA	NA
	Quality Controls	-7 to 2	8 to 14	NA	NA
20040178	Standards	-11 to 10	2 to 8	-4 to 4	2 to 7
	Quality Controls	-8 to 7	5 to 8	-2 to 5	3 to 7
Intrinsic Factor Pharmacokinetics Study					
20040245	Standards	-11 to 8	2 to 3	-3 to 8	2 to 7
	Quality Controls	-7 to 4	6 to 9	1 to 7	4 to 7
Extrinsic Factor Pharmacokinetics Study					
20050241	Standards	-9 to 10	2 to 4	-4 to 2	3 to 9
	Quality Controls	8	5 to 8	4 to 15	6 to 12
Other Studies Contributing Pharmacokinetic and Pharmacodynamic Data					
Biopharmaceutics Studies					
20050148	Standards	0 to 2	2 to 4	-5 to 8	2 to 6
	Quality Controls	-4 to -2	4 to 8	1 to 8	4 to 7
20050227	Standards	-1 to 2	1 to 5	-4 to 5	2 to 8
	Quality Controls	-1 to 1	4 to 8	-1 to 4	3 to 9
20060286	Standards	-2 to 3	2 to 4	-4 to 5	2 to 8
	Quality Controls	0 to 2	4 to 7	1 to 9	4 to 8
20060446	Standards	-2 to 2	2 to 4	-6 to 7	2 to 7
	Quality Controls	-4 to -1	4 to 5	2 to 7	4 to 7
Dose-ranging Studies in Postmenopausal Women					
20010223	Standards	-9 to 7	3	-7 to 7	2 to 5
	Quality Controls	-3 to 5	7 to 9	-6 to 3	4 to 6
20050172	Standards	-7 to 8	2 to 4	-4 to 3	4 to 10
	Quality Controls	2 to 8	5 to 9	7 to 25	4 to 10
Safety and Efficacy Studies in the Prevention and Treatment of PMO					
20030218	Standards	-8 to 8	3 to 4	-4 to 3	2 to 12
	Quality Controls	1 to 4	7 to 12	0 to 3	4 to 16
20040132	Standards	-10 to 8	3	-3 to 3	2 to 8
	Quality Controls	-6 to 4	7 to 12	1 to 3	4 to 8
20050233	Standards	-7 to 7	3 to 4	-5 to 2	3 to 9
	Quality Controls	3 to 4	8 to 10	-8 to 20	4 to 13
Safety and Efficacy Studies in the Treatment of Bone Loss Associated with HALT					
20040135	Standards	-11 to 8	2 to 3	-3 to 2	2 to 7
	Quality Controls	-6 to 8	7 to 11	0 to 4	3 to 8
20040138	Standards	-11 to 8	3	-4 to 3	2 to 9
	Quality Controls	-7 to 4	7 to 11	0 to 4	4 to 12
Safety and Efficacy Studies in Other Indications					
20040144	Standards	-11 to 8	3	-4 to 3	2 to 9
	Quality Controls	-7 to 3	8 to 10	-1 to 3	4 to 8
20040113	Standards	-11 to 8	3	-3 to 4	3 to 8
	Quality Controls	-6 to 4	7 to 10	-2 to 5	4 to 16
20040114	Standards	-10 to 7	3	-3 to 3	2 to 8
	Quality Controls	-8 to 5	8 to 13	-1 to 3	4 to 10
20050134	Standards	-9 to 9	3 to 4	-4 to 3	4 to 10
	Quality Controls	4 to 8	6 to 11	-16 to 18	4 to 17

5.2 Clinical Pharmacology Filing Memo

Office of Clinical Pharmacology Biologics License Application Filing and Review Form				
General Information About the Submission				
	Information		Information	
BLA Number	124-329, 129-331	Brand Name	Proia	
OCP Division	BCP3	Generic Name	Denosumab	
Medical Division	DRUP	Drug Class	Monoclonal antibody	
OCP Reviewer	Chongwoo Yu, Ph.D	Indication(s)	Treatment (BLA 124-329) and prevention (BLA 129-331) of osteoporosis in postmenopausal women	
OCP Team Leader	Myoung Jin Kim, Pharm. D.	Dosage Form	Injection	
Secondary Reviewer	Wu Lin, Ph.D.	Dosing Regimen	60 mg/ml every 5 months	
Date of Submission	December 19, 2010	Route of Administration	subcutaneous	
Estimated Due Date of OCP Review	August 13, 2011	Supplier	Amgen	
FEUFA Use Date	October 19, 2010	Priority Classification	Standard	
Division Due Date	September 28, 2010			
CTx, CTy, and CTz Information				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments if any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Biophysical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:				
Isotopic characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase II -				
Healthy Volunteers -				
single dose:	X	3		20030148, 20030149, 20030150
multiple dose:	X	1		20040154
Pediatric -				
single dose:	X	1		20040173
multiple dose:	X	1		20040175
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
in-vivo effects on primary drug:				
in-vivo effects of primary drug:				
in-vitro:				
Subpopulation studies -				
ethnicity:				Pop-PK
gender:				Pop-PK
pediatric:	NA			Pediatric value request
geriatric:				Pop-PK
renal impairment:		1		20040245
hepatic impairment:	NA			

QC:				
Phase 1:		1		20050241
Phase 2:		5		20050175, 20040144, 20040153, 20040114, 20050134
Phase 3:		11		20030245, 20040132, 20050144, 20050179, 20050234, 20050233, 20060232, 20050229, 20040133, 20040138, 20050229
PK/PD:				
Phase 1 and/or 2, proof of concept:		1		20010223
Phase 3 clinical trial:				
Population Analysis -				
PK:	x	1		100657
PD:	x	1		110134
II. Biocompatibility				
Absolute biocompatibility:				
Relative biocompatibility -				
cellular as reference:				
in vitro formation as reference:				
Biocompatibility studies -				
traditional design; days / rats dose:		4		20050145, 20050227, 20060139, 20050445
injection design; days / rats dose:				
Food-drug interaction studies:	NA			
Discussion:				
IRATC:				
Re-evaluation request based on OQ2				
IC3 class				
III. Other CPB Studies				
Genetic/pharmacokinetic studies:				
Chromopharmacokinetics				
Pediatric development plan				
Immunogenicity profile		1		20060237
Literature References				
Total Number of Studies		32		
Other comments:				
	Comments			
GBR questions (any issues to be considered)	1. Acceptability of comparability studies. 2. Sufficient drug interaction information? 3. Population PK analyses. 4. Acceptability of immunogenicity. 5. Sufficient biorelevant assay validation information?			
Other comments or information not included above	1. Need to request a consult to the OCP Pharmacokinetics (PM) group. 2. Need to request a consult to the Office of Biotechnology Products (OBP) for review of antibody assays 3. Need to request a consult to OGI for inspection of sites where the comparability studies were conducted.			

5.3 Pharmacometric Review

Attached Separately

OFFICE OF CLINICAL PHARMACOLOGY:
PHARMACOMETRIC REVIEW

Application Number	BLA125320 BLA125331 BLA125332 BLA125333
Submission Number (Date)	Dec 19, 2008
Clinical Division	Division of Reproductive and Urology Products (DRUP) Division of Biologics and Oncology Products (DBOP)
Primary PM Reviewer	Ping Ji, Ph.D.
Secondary PM Reviewer	Pravin Jadhav, Ph.D.

1	Summary of Findings	2
1.1	Key Review Questions	2
1.1.1	Is there evidence to support fixed dosing regimen (60 mg Q6M) for all patients?	2
1.1.2	Are the proposed labeling claims based on PopPK analysis appropriate?..	4
1.1.3	Does exposure-response analysis provide evidence of effectiveness and are there any exposure related safety events?	4
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1 SUMMARY OF FINDINGS

1.1 Key Review Questions

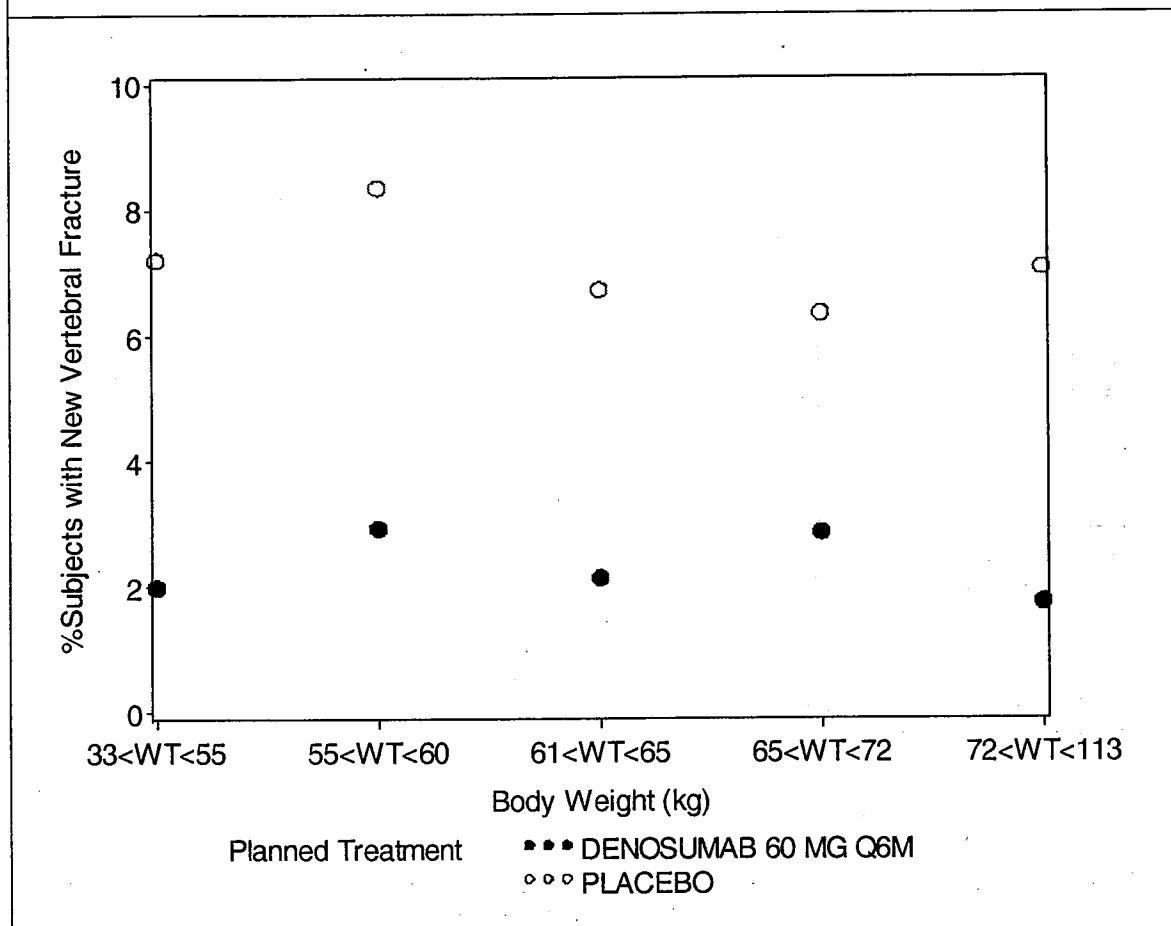
The purpose of this review is to address the following key questions.

1.1.1 Is there evidence to support fixed dosing regimen (60 mg Q6M) for all patients?

Yes, fixed dosing regimen (60 mg Q6M) is appropriate for all the patients.

The effect of body weight on the incidence of new vertebral fracture over the 36 months period, the primary efficacy point, was investigated in study 200302016. Body weight did not appear to affect the incidence of new vertebral fracture over the 36 months period.

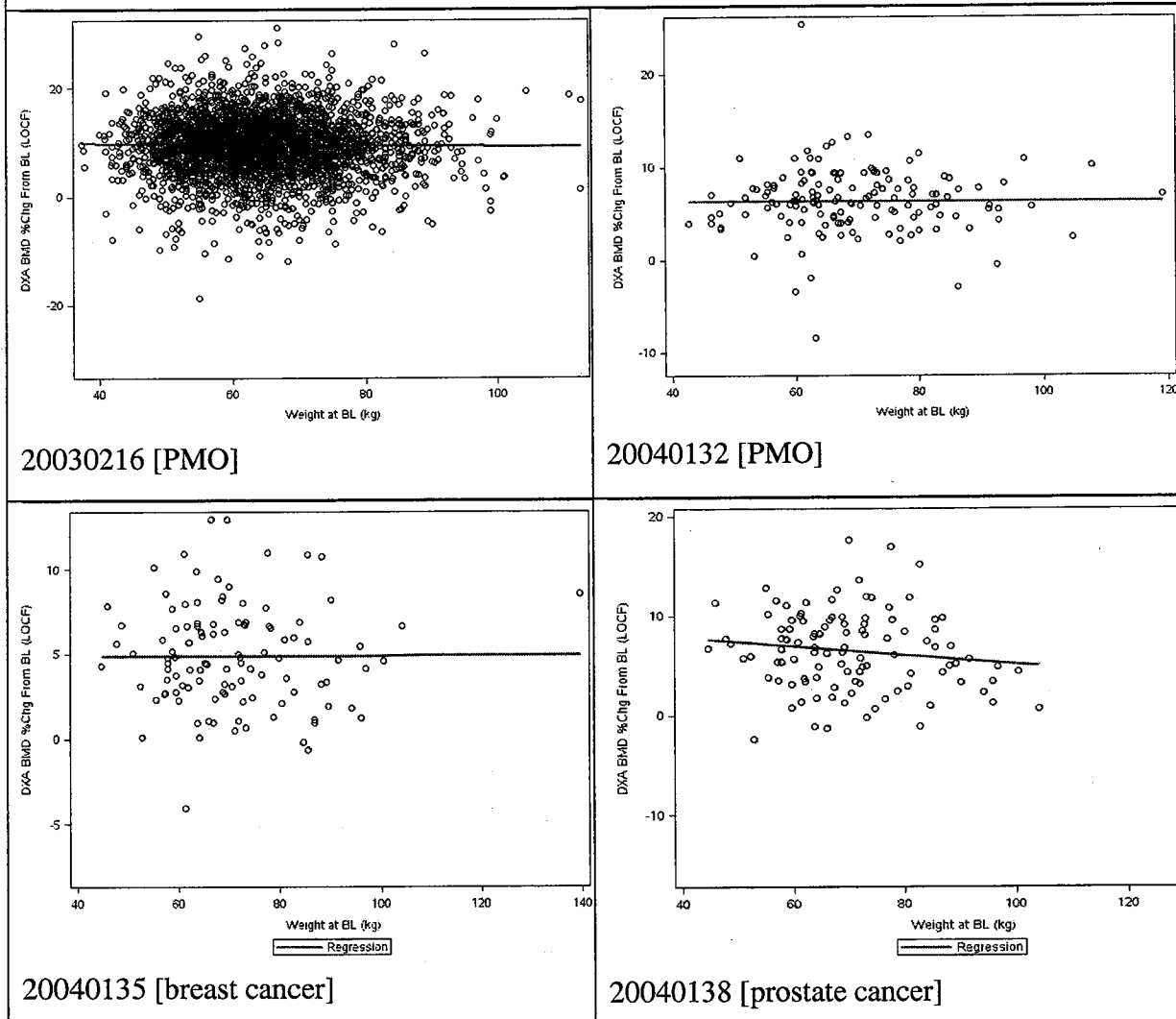
Figure 1. The incidence of any new vertebral fracture versus body weight in study 200302016.



Data used in creating this plot are summarized in Appendix 1

The effect of body weight on the bone mineral density (BMD) in lumbar spine was also explored in the phase III pivotal efficacy studies (200302016 [PMO], 20040132 [PMO 24 month data], 20040135 [breast cancer patients], and 20040138 [prostate cancer patients]). The increase of body weight was not associated with any change in the BMD level.

Figure 2. Scatter plots of lumbar spine bone mineral density versus body weight in studies 200302016, 20040132, 20040135, and 20040138.



Data Source:

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[\\Cbsap58\m\CTD_Submissions\STN12532\0000\m5\datasets\20040132-36-Month\analysis\aslbase.xpt abmdxa.xpt](#)

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[\\Cbsap58\m\CTD_Submissions\STN12532\0000\m5\datasets\20040138\analysis\aslbase.xpt abmdxa.xpt](#)

Denosumab pharmacokinetic parameters are dependent on body weight. However, differences in exposure do not affect response to denosumab, therefore, fixed dose for all patients can be supported.

1.1.2 Are the proposed labeling claims based on PopPK analysis appropriate?

Yes, the proposed labeling appeared to be appropriate.

Age and gender are not significant covariates in the population PK analysis (See Appendix 4 for distribution of subject demographics). Subject type as solid tumor and race as black and Hispanic were identified as covariates for clearance in the population PK model (*Amgen Pharmacometric report 100957*). However, the pharmacokinetics of denosumab did not appear to be affected by race and solid tumor as shown in the simulation results.

Table 1. Mean and SD of the AUC, C_{max}, and the duration the serum concentration above 200 ng/mL after 60 mg single dose for black, Hispanic and solid tumor subjects with respect to the Caucasian postmenopausal women (reference).

Parameter	Reference		Black		Ratio	
	Mean	SD	Mean	SD	Mean	Quartiles
AUC (mg·h/L)	10200	4820	9300	4470	0.908	0.928, 0.884
C _{Max} (ng/mL)	11300	4630	10300	4120	0.915	0.938, 0.912
T > 200 ng/mL (days)	130	28.7	127	29.1	0.979	0.952, 1.00
	Reference		Hispanic		Ratio	
	Mean	SD	Mean	SD	Mean	Quartiles
AUC (mg·h/L)	10200	4820	9770	4630	0.954	0.948, 0.942
C _{Max} (ng/mL)	11300	4630	10700	4300	0.951	0.966, 0.952
T > 200 ng/mL (days)	130	28.7	129	29.4	0.990	0.952, 1.00
	Reference		Solid Tumor		Ratio	
	Mean	SD	Mean	SD	Mean	Quartiles
AUC (mg·h/L)	10200	4820	9920	4580	0.969	0.980, 0.959
C _{Max} (ng/mL)	11300	4630	11100	4530	0.979	0.974, 0.998
T > 200 ng/mL (days)	130	28.7	129	28.6	0.995	0.952, 1.00

Mean (SD) bodyweight in kg for each population: Reference 66.0 (10.2); Black 72.2 (13.9); Hispanic 68.5 (12.4); Solid Tumor 69.6 (15.7)

Source: page 63 of Amgen pharmacometrics report: 109957.

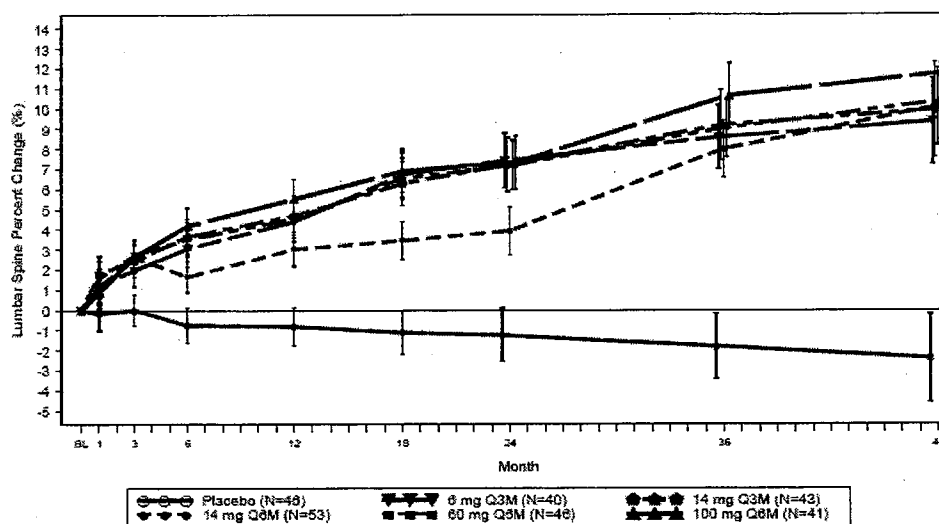
1.1.3 Does exposure-response analysis provide evidence of effectiveness and are there any exposure related safety events?

Effectiveness

Denosumab treatment was associated with increase in BMD of the lumbar spine in dose dependent manner. The effect was significant compared to placebo. Denosumab treatment was associated with significant decrease in the bone-turnover-marker (BALP, CTX, or NTX_UCR) of the lumbar spine than placebo treatment.

The effect of denosumab dose on BMD of the lumbar spine in postmenopausal women with low BMD was investigated in study 20010223. All drug treated cohorts had significantly greater increase in BMD of the lumbar spine than placebo cohort. Across denosumab dose cohorts, the magnitude of the increase in BMD was similar with the exception of the 14-mg-Q6M dose cohort.

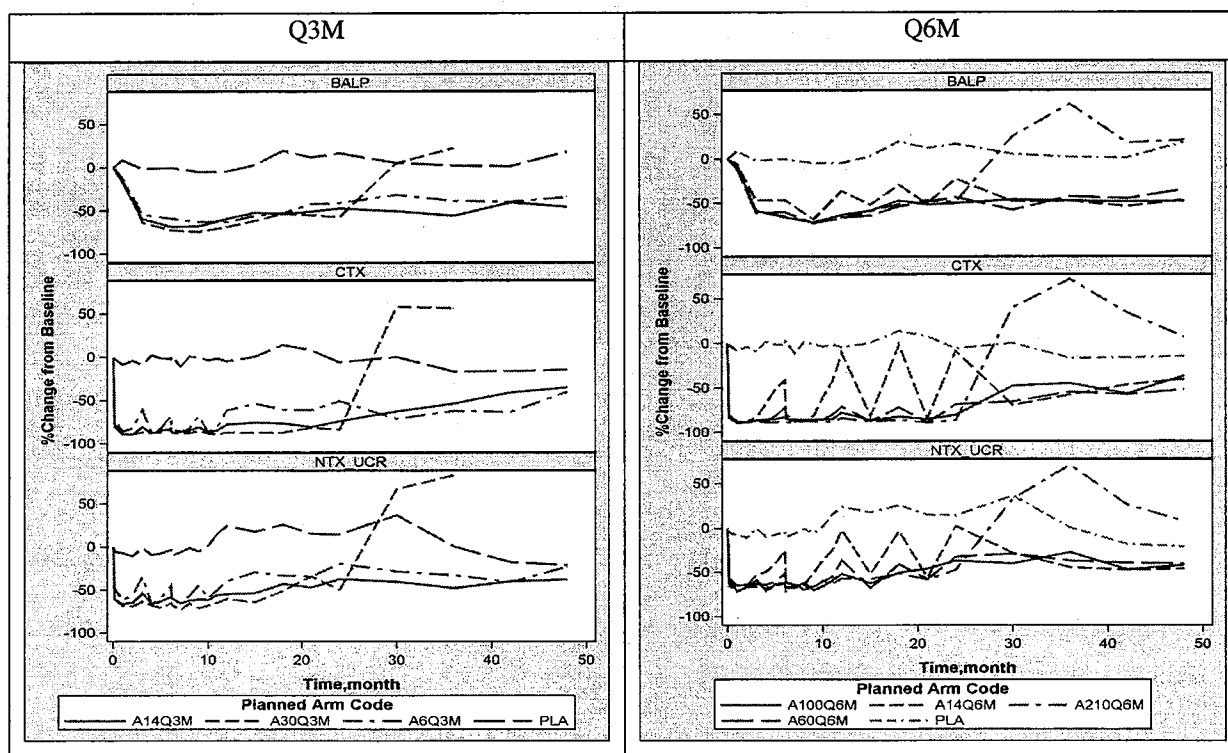
Figure 3. %Change (95% CI) from baseline in lumbar spine BMD for subjects in the ~~comparator~~ treatment cohorts (Study 20010223)



Source: Page 66 in Module 2.72. Summary of Clinical Pharmacology Studies

Further, Denosumab treatment resulted in a rapid and sustained decrease in median serum C-TX of type I collagen (CTX), bone-specific alkaline phosphatase (BALP), and N-telopeptide (NTX) concentrations (Figure 4). Across the denosumab dose cohorts, the maximal suppression of serum turnover markers was similar.

Figure 4. Median percent change from baseline in the bone-turnover-marker (CTX, BALP, and NTX_UCR) versus time (study 20010223).



Data Source:

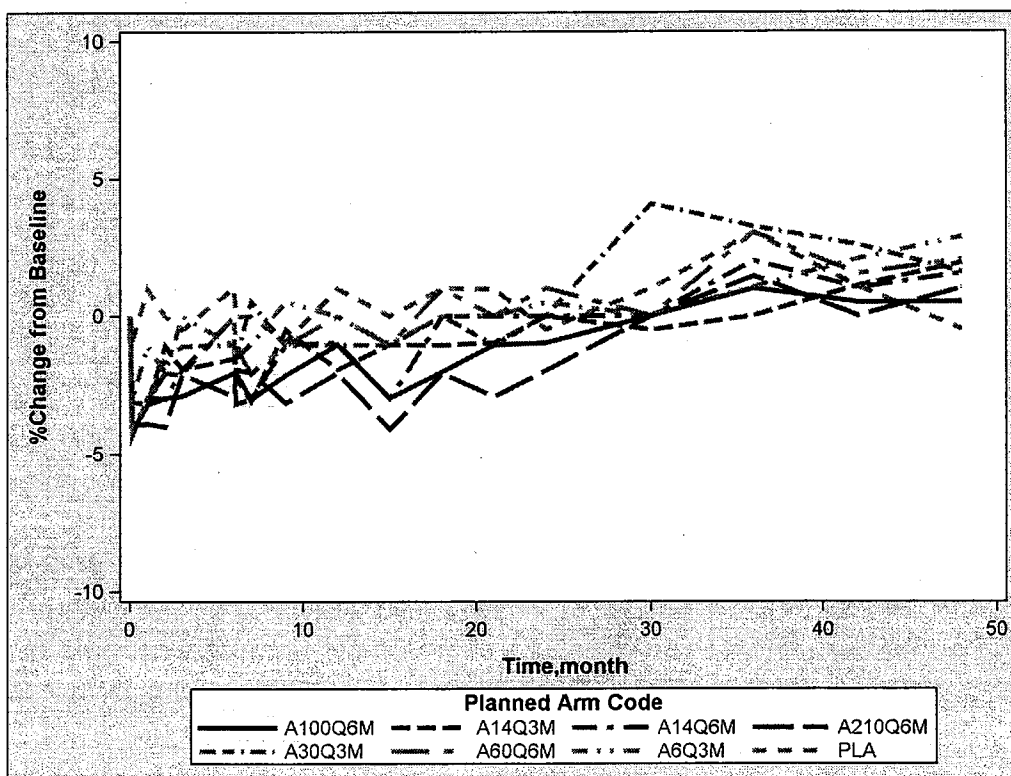
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Safety

The overall safety profile was comparable between denosumab group and placebo group. Denosumab administration was associated with mild transient decreases in the concentration of serum calcium, an event of interest.

The overall incidence of adverse events, serious adverse events, and adverse events leading to treatment withdrawal were generally similar between denosumab and placebo groups. In this application, hypocalcemia was considered an event of interest due to the potential for denosumab to lower serum calcium levels. The effects of denosumab dose on the percent change of calcium levels were investigated in study 20010223 as shown in Figure 5. Denosumab administration was associated with mild transient decrease in serum calcium level. However, no subjects had albumin-adjusted serum calcium below the normal range at the scheduled visits. For details, please refer to the medical review.

Figure 5. Median % change from baseline in plasma calcium.



Data Source:

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1.1.4 Does immunogenicity affect the PK and effectiveness?

No. The incidence of immunogenicity of denosumab in humans appears to be low and the presence of immunogenicity did not appear to affect the PK and effectiveness.

Overall, 43 out of 8113 (0.5%) denosumab-treated subjects were positive for development of binding antibodies. In most of these subjects, the antibodies were transiently detected. In addition, neutralizing antibodies were not been detected in any subject. Table 2 summarizes the serum denosumab concentrations at 1 month postdose and the changes in lumbar spine and total hip

BMD at month 12 or 24 for subjects who developed transient binding antibodies to denosumab during the phase 3 studies in subjects with low BMD (*Study 20040132*) or PMO (*Study 20030216*) or bone loss due to HALT (Studies 20040135 and 20040138). The serum denosumab concentrations and changes in BMD for these subjects were all within the ranges observed for the other subjects in the studies, except for the month-1 serum denosumab concentration for subject 138646019 in Study 20040138. This subject had the highest serum concentration at that time point; however, this value was < 5% greater than that of the next highest value in an antibody-negative subject. Thus, there is no evidence that the transient occurrence of binding antibodies to denosumab alters its pharmacokinetic or pharmacodynamic profiles.

Table 2. Serum denosumab concentrations at Month 1 and percent change from baseline in lumbar spine and total hip BMD at Month 12 or 24 for antibody-positive subjects.

(Studies 20040132, 20040135, 20040138, and 20030216)

Study	Subject	Time of Positive Ab Result (month)	C1month (ng/mL)	Range C1month ^a (ng/mL)	Lumbar Spine BMD (%)	Range: Lumbar Spine BMD (%)	Total Hip BMD (%)	Range: Total Hip BMD (%)
20040132	132103012 ^b	24	5140	1620 ^c , 11800	12.85	-8.4, 25.4	7.89	-3.1, 7.9
20040132	132105014	1	4710	1620 ^c , 11800	4.59	-4.2, 21.2	0.75	-2.0, 8.5
20040135	135135004	1, 12	3020	1430, 12200	3.48	-4.1, 12.9	4.21	-12.5, 13.3
20040135	135434001 ^b	18	9070	1430, 12200	9.19	-2.4, 17.7	8.51	-12.5, 13.3
20040138	138646019	1	13800	<0.9, 13200	4.38	-8.8, 18.2	5.74	-8.8, 11.0
20030216	0633313	12	4900	842, 17100	NR	-5.1, 15.9	5.77	-12.5, 15.1

Ab = antibody; BMD = bone mineral density (percent change from baseline at month 12); C1month = serum denosumab concentration at 1 month postdose; NR = not reported

^a For antibody-negative subjects

^b Lumbar spine and total hip BMD individual values and ranges at month 24 (due to timing of positive antibody result).

^c Excludes 2 subjects as described in Section 10 of the Study 20040132 24-month clinical study report


Source: Page 131 in Module 2.7.2 Summary of clinical pharmacology studies

1.2 Recommendations

None.


Signature Page

Pharmacometrics Reviewer:



Ping Ji, Ph.D., 8/21/2009

Pharmacometrics Team Leader:



Pravin Jadhav, Ph.D., 8/21/2009

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS

FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Office of Clinical Pharmacology Biologics License Application Filing and Review Form				
General Information About the Submission				
	Information		Information	
BLA Number	125-320, 125-331	Brand Name	Prolia	
OCP Division	DCP3	Generic Name	Denosumab	
Medical Division	DRUP	Drug Class	Monoclonal antibody	
OCP Reviewer	Chongwoo Yu, Ph.D	Indication(s)	Treatment (BLA 125-320) and prevention (BLA 125-331) of osteoporosis in postmenopausal women	
OCP Team Leader	Myong Jin Kim, Pharm. D.	Dosage Form	Injection	
Secondary Reviewer	Ike Lee, Ph.D.	Dosing Regimen	60 mg/ml every 6 months	
Date of Submission	December 19, 2009	Route of Administration	subcutaneous	
Estimated Due Date of OCP Review	August 19, 2009	Sponsor	Amgen	
PDUFA Due Date	October 19, 2009	Priority Classification	Standard	
Division Due Date	September 26, 2009			
Clin. Pharm. and Biopharm. Information				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments if any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	x			
Tabular Listing of All Human Studies	x			
HPK Summary	x			
Labeling	x			
Reference Bioanalytical and Analytical Methods	x			
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:				
Plasma protein binding:				
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	x	3		20030148, 20030164, 20030180
multiple dose:	x	1		20010124
Patients-				
single dose:	x	1		20010123
multiple dose:	x	1		20040176
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				Pop-PK
gender:				Pop-PK
pediatrics:	NA			Pediatric waiver request
geriatrics:				Pop-PK
renal impairment:		1		20040245
hepatic impairment:	NA			

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

PD:				
Phase 1:		1		20050241
Phase 2:		5		20050172, 20040144, 20040113, 20040114, 20050134
Phase 3:		11		20030216, 20040132, 20050141, 20050179, 20050234, 20050233, 20060232, 20060289, 20040135, 20040138, 20050209
PK/PD:				
Phase 1 and/or 2, proof of concept:		1		20010223
Phase 3 clinical trial:				
Population Analyses -				
PK:	x	1		109957
PD:	X	1		110184
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				
Bioequivalence studies -				
traditional design; single / multi dose:		4		20050146, 20050227, 20060286, 20060446
replicate design; single / multi dose:				
Food-drug interaction studies:	NA			
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Immunogenicity profile		1		20060237
Literature References				
Total Number of Studies		32		
Other comments				
	Comments			
QBR questions (key issues to be considered)	1. Acceptability of comparability studies. 2. Sufficient drug interaction information? 3. Population PK analyses. 4. Acceptability of Immunogenicity. 5. Sufficient bioanalytical assay validation information?			
Other comments or information not included above	1. Need to request a consult to the OCP Pharmacometrics (PM) group. 2. Need to request a consult to the Office of Biotechnology Products (OBP) for review of antibody assays 3. Need to request a consult to DSI for inspection of sites where the comparability studies were conducted.			

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

On initial review of the NDA/BLA application for filing:

	Content Parameter	Yes	No	N/A	Comment
Criteria for Refusal to File (RTF)					
1	Has the applicant submitted bioequivalence data comparing to-be-marketed product(s) and those used in the pivotal clinical trials?	x			
2	Has the applicant provided metabolism and drug-drug interaction information?		x		See filing memo below
3	Has the sponsor submitted bioavailability data satisfying the CFR requirements?		x		See filing memo below
4	Did the sponsor submit data to allow the evaluation of the validity of the analytical assay?	x			
5	Has a rationale for dose selection been submitted?	x			
6	Is the clinical pharmacology and biopharmaceutics section of the NDA organized, indexed and paginated in a manner to allow substantive review to begin?	x			
7	Is the clinical pharmacology and biopharmaceutics section of the NDA legible so that a substantive review can begin?	x			
8	Is the electronic submission searchable, does it have appropriate hyperlinks and do the hyperlinks work?	x			
Criteria for Assessing Quality of an NDA (Preliminary Assessment of Quality)					
Data					
9	Are the data sets, as requested during pre-submission discussions, submitted in the appropriate format (e.g., CDISC)?			x	
10	If applicable, are the pharmacogenomic data sets submitted in the appropriate format?			x	
Studies and Analyses					
11	Is the appropriate pharmacokinetic information submitted?	x			
12	Has the applicant made an appropriate attempt to determine reasonable dose individualization strategies for this product (i.e., appropriately designed and analyzed dose-ranging or pivotal studies)?	x			
13	Are the appropriate exposure-response (for desired and undesired effects) analyses conducted and submitted as described in the Exposure-Response guidance?	x			
14	Is there an adequate attempt by the applicant to use exposure-response relationships in order to assess the need for dose adjustments for intrinsic/extrinsic factors that might affect the pharmacokinetic or pharmacodynamics?	x			
15	Are the pediatric exclusivity studies adequately designed to demonstrate effectiveness, if the drug is indeed effective?			x	Pediatric waiver submitted
16	Did the applicant submit all the pediatric exclusivity data, as described in the WR?			x	Pediatric waiver submitted
17	Is there adequate information on the pharmacokinetics and exposure-response in the clinical pharmacology section of the label?	x			

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

General				
1 8	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x		
1 9	Was the translation (of study reports or other study information) from another language needed and provided in this submission?		x	

IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

- N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

- *We acknowledge the submission of the comparability studies in your submission. We notice the following and these will be review issues:*
 - *There is no direct bridging between the ATO vial and the ACO PFS formulation.*
 - *Pharmacodynamics comparability in target population needs to be assessed.*
- *We remind you that bioanalytical method validation reports for all analytes (i.e., biomarkers) assessed to support this application need to be submitted.*
- *We recommend that you assess denosumab's effect on CYP activities in vitro.*

Chongwoo Yu

1/28/2009

Reviewing Clinical Pharmacologist

Date

Hae Young Ahn

1/28/2009

Team Leader/Supervisor

Date

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Filing Memo

Clinical Pharmacology Review

BLA: 125-320 and 125-331
Compound: Prolia (60 mg/ml Denosumab)
Sponsor: Amgen

Date: 1/26/2009
Reviewer: Chongwoo Yu, Ph.D.

Introduction:

Denosumab is a fully human IgG2 monoclonal antibody that inhibits receptor activator of nuclear factor kappa B (RANK) ligand, for the treatment (BLA 125-320) and prevention (BLA 125-331) of osteoporosis in postmenopausal women (PMO) and for the treatment and prevention of bone loss in patients undergoing hormone ablation therapy (HALT) for breast (BLA 125-332) or prostate (BLA 125-333) cancer. The Division of Reproductive and Urologic Products (DRUP) will be responsible for reviewing BLAs 125-320 and 125-331. Denosumab is considered to be a new molecular entity (NME). The proposed proprietary name for denosumab in these indications is Prolia™.

Denosumab drug product is supplied as a single-use, sterile, preservative-free solution intended for delivery by subcutaneous injection, supplied in either a 60 mg/ml prefilled syringe (PFS) or 60 mg/ml vial presentation with a 1.0 ml deliverable volume to support dosing of 60 mg every 6 months (Q6M).

This marketing application includes 30 clinical studies in normal volunteers and patients with osteoporosis (approximately 10,500 subjects), bone loss associated with hormone ablation therapy (approximately 1700 subjects), rheumatoid arthritis, and advanced cancer performed from June 2001 to September 2008. Twenty-three of the 30 clinical studies supporting this marketing application contributed data on the safety, tolerability, and pharmacokinetic (PK) profiles for denosumab. Eight of these 23 studies were primarily designed as clinical pharmacology studies to assess healthy volunteer pharmacokinetics and initial tolerability (Studies 20010124, 20030148, 20030164, and 20030180), patient PK and initial tolerability (Studies 20010123 and 20040176), intrinsic factor PK (Study 20040245), or extrinsic factor PK (Study 20050241). The other 15 studies were primarily designed to address other objectives (i.e., "Biopharmaceutic" and "Efficacy and Safety" studies), but provide supportive PK and pharmacodynamic (PD) data. Of these 15 studies

(b) (4)

(b) (4)

The PMO clinical development program is supported by 2 pivotal phase 3 studies (Studies 20030216 and 20040132). Study 20030216 was a 3-year randomized, double-blind, placebo controlled study in postmenopausal women with osteoporosis to determine whether denosumab treatment can reduce the incidence of new vertebral (primary endpoint), and nonvertebral and hip fractures (secondary endpoints) as compared with control. Study 20040132 is a randomized, double-blind, placebo-controlled study in postmenopausal women with low bone mass to determine whether denosumab treatment can prevent lumbar spine bone loss.

The Sponsor believes that the criteria for priority review are met for denosumab for the indications listed above and justifications were submitted in this marketing application.

Bioavailability and ADME (Absorption, Distribution, Metabolism, and Excretion):

Safety, tolerability, PK, PD, and exposure-response properties of denosumab were characterized in healthy volunteers and in patients with low BMD or osteoporosis or bone loss associated with HALT. The dose-exposure-response relationship for denosumab has also been studied. Data obtained in these characterizations were instrumental in the selection of the dose regimens for phase 2 and 3 studies, the evaluation of the effect of covariates (sex, body weight/body mass index (BMI), race, and age) on the PK and PD of denosumab, and exploring the relationship between serum concentrations of denosumab and calcium. Oral absorption studies have not been conducted because denosumab is administered subcutaneously. Bioavailability, plasma protein binding, and other human biomaterials studies were not considered appropriate by the Sponsor and have therefore not been conducted.

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS

FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

Drug-drug interactions:

Because denosumab is a monoclonal antibody and is not eliminated via hepatic metabolic mechanisms (e.g., by cytochrome P450 (CYP) enzymes), drug-drug interaction studies (e.g., with CYP inhibitors) were not considered appropriate by the Sponsor and have therefore not been conducted. However, studies including transition from a bisphosphonate to denosumab or studies including concomitant hormone ablation therapies allowed an indirect evaluation of drug interactions when compared to results from other studies.

Special population:

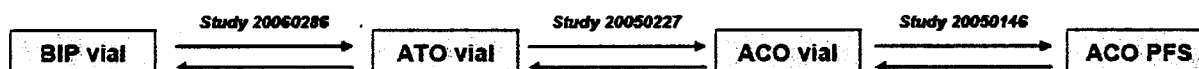
A study evaluating the effect of renal impairment on the PK of denosumab have been conducted and data is available for review. Because denosumab is a monoclonal antibody and is not eliminated via hepatic metabolic mechanisms (e.g., by CYP enzymes), hepatic impairment were not considered appropriate by the Sponsor and have therefore not been conducted.

Population PK/PD Analyses:

Population PK and PD analyses in healthy subjects, postmenopausal women with low BMD or osteoporosis, and subjects with cancer were performed using NONMEM. Population PK and PD reports are submitted for review.

Comparability studies for drug substance and drug product presentation

Data from biopharmaceutics studies that were conducted to demonstrate clinical comparability between denosumab drug substance produced for the pivotal phase 3 studies and that intended for commercial use and clinical comparability between the drug product presentations proposed for commercial use are submitted in this marketing application. Drug substance for the pivotal phase 3 studies was manufactured at Amgen Thousand Oaks (denosumab ATO). In preparation for commercialization of denosumab, manufacturing process was subsequently scaled-up and transferred from ATO to both Amgen Colorado (ACO) and Boehringer Ingelheim Pharma GmbH & Co Kg (BI Pharma), with minor changes to improve process robustness and ensure facility fit. Drug product preparations proposed for commercial use in this marketing application are the 60 mg/ml vial and 60 mg/ml PFS. It is noted that PK and PD profiles were only assessed in healthy volunteers.



Bioanalytical Method validation:

Serum denosumab and serum type 1 C-telopeptide (CTX1) concentrations in study samples were determined with an enzyme-linked immunosorbent assay (ELISA) following validated analytical procedures. For immunogenicity testing, sensitive and specific assays were developed and validated for detecting antibodies in nonclinical and clinical studies. Samples were screened for binding antibodies using an electrochemiluminescent (ECL) bridging immunoassay. If confirmed positive or reactive samples were then characterized for neutralizing antibodies using a cell-based chemiluminescent mRNA expression assay. Bioanalytical method validation reports are submitted for review.

Food Effect:

The effect of food on denosumab PK has not been studied.

Genomics Information:

This submission contains limited genomics information. The potency of denosumab's drug substance has been assessed using 3 different assays: homogenous time resolved fluorescence (HTRF), Reporter Gene, and tartrate-resistant acid phosphatase (TRAP) assays

Immunogenicity:

Immunogenicity testing (using validated assays) has been performed in all denosumab clinical studies. More than 13,000 subjects have been tested for antidenosumab antibodies in studies described in this marketing application, including > 8000 subjects who have received at least 1 dose of denosumab.

Recommendation:

**CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS
FILING FORM/CHECKLIST FOR NDA/BLA or Supplement**

The Office of Clinical Pharmacology/Division of Clinical Pharmacology 3 finds that the Clinical Pharmacology section for BLAs 125-320 AND 125-331 is fileable with potential review issues.

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS FILING FORM/CHECKLIST FOR NDA/BLA or Supplement

General				
18	Are the clinical pharmacology and biopharmaceutics studies of appropriate design and breadth of investigation to meet basic requirements for approvability of this product?	x		
19	Was the translation (of study reports or other study information) from another language needed and provided in this submission?		x	

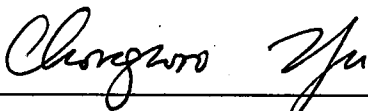
IS THE CLINICAL PHARMACOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the clinical pharmacology perspective, state the reasons and provide comments to be sent to the Applicant.

- N/A

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.


- *We acknowledge the submission of the comparability studies in your submission. We notice the following and these will be review issues:*
 - *There is no direct bridging between the ATO vial and the ACO PFS formulation.*
 - *Pharmacodynamics comparability in target population needs to be assessed.*
- *We remind you that bioanalytical method validation reports for all analytes (i.e., biomarkers) assessed to support this application need to be submitted.*
- *We recommend that you assess denosumab's effect on CYP activities in vitro.*



Reviewing Clinical Pharmacologist

1/28/2009

Date



Team Leader/Supervisor

1/28/2009

Date