The Application of Target Information and Preclinical Pharmacokinetic/Pharmacodynamic Modeling in Predicting Clinical Doses of a Dickkopf-1 Antibody for Osteoporosis

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Running Title Page: Preclinical PK/PD modeling of a Dkk-1 antibody

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List of non-standard abbreviations:
AUC_{0-\text{last}}, Area under the concentration time curve, to time of last observation; AUC_{0-\text{inf}}, Area under the concentration time curve, extrapolated to infinity; C_{\text{average}}, Average concentration; C_{\text{max}}, Maximum concentration; C_{\text{min}}, Minimum concentration; CV, Coefficient of variability; Dkk-1, Dickkopf-1; EMEA, European medicines agency; FDA, Food and drug administration; FIH, First in human; HED, Human equivalent dose;
IgG₂, Immunoglobulin isotype G₂;  \( k_a \), Absorption rate constant;  \( K_d \), Dissociation constant;  \( k_{el} \), Elimination rate constant;  \( k_{on} \), Association rate constant;  \( k_{off} \), Dissociation rate constant;  \( \lambda_z \), Slope of terminal phase;  LLOQ, Lower limit of quantification;  LRP5, Low density lipoprotein receptor related protein;  MABEL, Minimum anticipated biological effect level;  MRD, Minimum required dilution;  MRSD, Maximum recommended starting dose;  NOAEL, No adverse effect level;  PD, Pharmacodynamics;  PK, Pharmacokinetics;  PK/PD, Pharmacokinetic/Pharmacodynamic;  RO, Receptor occupancy;  SC, subcutaneous;  \( t_{1/2} \), Half-life;  \( t_{last} \), Time of last observation;  TMDD, Target mediated drug disposition

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PF-04840082 is a humanized prototype anti Dickkopf-1 (Dkk-1) IgG₂ antibody for the treatment of osteoporosis. In vitro, PF-04840082 binds to human, monkey, rat and mouse Dkk-1 with high affinity. Following administration of PF-04840082 to rat and monkey, free Dkk-1 concentrations decreased rapidly and returned to baseline in a dose dependant manner. In rat and monkey, PF-04840082 exhibited non-linear pharmacokinetics (PK) and a target mediated drug disposition (TMDD) PK/PD model was used to characterize PF-04840082 versus Dkk-1 concentration response relationship. The PK/PD modeling enabled estimation of antibody non-target mediated elimination, Dkk-1 turnover, complex formation and complex elimination. The TMDD model was translated to human to predict efficacious dose and minimum anticipated biological effect level (MABEL) by incorporating information on typical IgG₂ human PK, antibody-target association/dissociation rates, Dkk-1 expression and turnover rates. The PK/PD approach to MABEL was compared with the standard ‘no adverse effect level’ (NOAEL) approach to calculating clinical starting doses and a pharmacological equilibrium method. The NOAEL method gave estimates of dose which were too high to ensure safety of clinical trials. The pharmacological equilibrium approach calculated receptor occupancy (RO) based on $K_d$ alone and did not take into account rate of turnover of the target or antibody-target complex kinetics and as a result likely produced a substantial over prediction of RO at a given dose. It was concluded that the calculation of MABEL according to the TMDD model was the most appropriate means for ensuring safety and efficacy in clinical studies.
INTRODUCTION

Osteoporosis is a bone disease characterized by low bone mineral density which leads to bone fragility, and subsequently, to bone fractures. The majority of pharmacological osteoporosis therapies, including bisphosphonates, calcitonin, hormone replacement therapy and selective estrogen receptor modulators, prevent bone loss by reducing bone resorption. Restoration of bone mass in patients suffering from osteoporosis is an area of unmet medical need.

Recently it has been shown that Wnt/ LRP5 regulates bone mass and that activation of Wnt signaling leads to an accrual of bone mass (Gong et al., 2001; Boyden et al., 2002; Little et al., 2002). Wnts are secreted glycoproteins that bind to and activate a receptor complex which includes low-density lipoprotein receptor related protein (LRP5/6) and frizzled proteins. Wnt signaling is tightly regulated by antagonists which include secreted molecules such as Dickkopf-1 (Dkk-1). Binding of Dkk-1 to the LRP5/6 receptor and Kremen-1/2 co-receptor promotes internalization of the receptor complex resulting in dampening of the Wnt signal (Diarra et al., 2007).

Genetic evidence for a central role of the Wnt pathway in maintaining bone mass has come from the identification of both activating and inactivating mutations in the Wnt receptor LRP5. Inactivation of LRP5 results in a decrease in bone mass and causes the autosomal recessive disorder: osteoporosis pseudoglioma syndrome in humans (Gong et al., 2001) and a similar phenotype in LRP5 knockout mice (Holmen et al., 2004). Conversely, the high bone mass phenotype observed in humans was found to be due to a
single point mutation in LRP5 (G171V) that inhibits the ability of Dkk-1 to bind (Boyden et al., 2002; Little et al., 2002). Individuals with high bone mass have markedly reduced risks of skeletal fracture.

A neutralizing Dkk-1 antibody is expected to increase bone mass due to increased bone formation by osteoblasts and thus prevent osteoporotic fractures. PF-04840082 is a humanized prototype anti-Dkk-1 monoclonal antibody for the treatment of osteoporosis. It binds human, mouse, rat and cynomolgus monkey Dkk-1 \textit{in vitro} with high affinities (Kd<100pM).

Clinical starting doses of biotherapeutic drugs are traditionally estimated using no adverse effect level (NOAEL) data in toxicology species as recommended by the FDA (FDA, 2005). However, new EMEA guidance (EMEA, 2007) in response to severe adverse events seen in a first in human (FIH) clinical trial of a CD28 agonist antibody (Duff, 2006), suggests use of a more holistic dose selection approach. The guidance suggests integration of all pharmacology, safety and efficacy testing data gathered during preclinical evaluation of the candidate in a PK/PD modeling framework so that a starting dose can be chosen that would result in a minimum anticipated biological effect level (MABEL).

Despite the large number of antibodies in development, only a handful of reports using preclinical data to predict the clinical pharmacokinetics (PK) or dose of antibodies have been published (Lobo et al., 2004; Agoram, 2009). Allometric power models are
commonly used for interspecies scaling of antibody PK when linear PK is anticipated (Wang et al., 2008). However, unlike small molecules, interaction of an antibody with its target often affects the PK of the antibody. This is known as target mediated drug disposition (TMDD) and is generally characterized by a higher antibody clearance at lower antibody doses (Tabrizi et al., 2006). Upon saturation of the target mediated pathway, typical IgG FcRn catabolic clearance mechanisms predominate, which gives the antibody its characteristic long half-life. TMDD is more common for monoclonal antibodies directed against proteins expressed on cell membranes where receptor mediated endocytosis results in drug elimination. However, TMDD has also been observed with soluble targets: omalizumab and denosumab are antibodies for soluble targets (IgE and receptor activator of NFkB respectively) which exhibit non-linear elimination kinetics (Hayashi et al., 2007; Marathe et al., 2008).

As a result, PK/PD understanding requires knowledge of the antibody, target and antibody-target interactions. The highest value comes from linking PK with PD response to predict drug exposure and effect following a given dose (Agoram et al., 2007). Mechanistic PK/PD modeling thus offers a rational and effective means of predicting both human PK and clinically efficacious dose of antibodies.

In this study, simultaneous characterization of antibody (PF-04840082) and target (Dkk-1) in rat and monkey facilitated understanding of the pharmacokinetics and the pharmacodynamics of the response. This was coupled with knowledge of target level in healthy vs. diseased subjects, target turnover rates and antibody-target association/
dissociation rates to support FIH doses in the clinic. In this manuscript, the NOAEL and MABEL methods of estimating starting doses of the Dkk-1 antibody are compared.
METHODS

Test materials

Monoclonal anti-Dkk-1 antibodies were made at Genovac (Germany) by immunization of Balb/C mice with full-length recombinant human Dkk-1 protein (R&D Systems, Minneapolis, MN). A single mouse IgG1/kappa isotype antibody (JC18) was humanized and affinity matured using a library scanning mutagenesis strategy (Pons et al., 2009; manuscript in preparation). In comparison to the parent mouse antibody, the humanized antibody (PF-04840082) exhibited a >100-fold increase in affinity for both human and mouse Dkk-1. The sequence of PF-04840082 is detailed in patent application PC33877.

Biacore experiment

Interactions between PF-04840082 and human, mouse, rat, or Cynomolgus monkey Dkk-1 were analyzed using a Biacore 3000TM system equipped with a CM5 sensor chip (Biacore AB, Uppsala, Sweden). The association and dissociation phases were monitored during the interaction analysis. The binding responses were double-referenced and fit globally to a simple model using BiaEvaluation v.4.0 software. Affinities were deduced from the quotient of the kinetic rate constants ($K_d = k_{off}/k_{on}$).

Animal studies

All animal studies were conducted in accordance with animal care and use protocols approved by the Institutional Animal Care and Use Committee (IACUC). The program of humane animal care and use at Pfizer Global Research and Development has been evaluated for its compliance with Title 9 CFR, Chapter 1, Subchapter A of the Federal
Animal Welfare Act (AWA) and the *Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Research, 1996).

*Pharmacokinetic/pharmacodynamic study in rats*

Experiments were conducted in female Sprague Dawley rats (n=40, body weight 250-350g, Charles River Laboratories, Wilmington, MA, U.S.A). PF-04840082 was administered once weekly by the intravenous route for six consecutive weeks (n=8/dose) at 0.1, 1, 10 and 100 mg/kg. One group of rats (n=8) were administered vehicle control (20 mM histidine pH 6.5 with 140 mM NaCl). Doses were administered by intravenous bolus administration into the femoral vein via previously implanted indwelling femoral vein cannulas.

Serial blood samples (400-800 µl) were collected initially via the femoral vein cannula and then via the jugular vein under anesthesia once femoral catheter patency was lost, pre-dose and at 1, 3, 8, 24, 48, 72, 168, 240, 336, 408, 504, 576, 672, 744, 840, 912 and 1008 hours post first dose from each treatment group. Serum was obtained by centrifugation and stored at -20°C until analysis for Dkk-1 and PF-04840082 concentrations.

*Pharmacokinetic/pharmacodynamic study in cynomolgus monkeys*

The animal care and experimental procedures of this study was conducted in compliance with the U.S. Animal Welfare Act and the conditions specified in *The Guide for Care and Use of Laboratory Animals* (Institute of Laboratory Animal Research, 1996).
Five male and five female cynomolgus monkeys (Macaca fascicularis), 2 to 5 years of age weighing between 3.2 and 5.2 kg (Charles River Primates, BioResearch Facility, Houston, Texas, U.S.A) were used in this study. Animals (n=1/sex/group) were assigned to 5 groups. PF-04840082 was administered by slow intravenous injection via the saphenous vein to 4 groups of 1 male and 1 female monkey at single doses of 0.1, 1, 10 or 100 mg/kg. The remaining group was administered vehicle control in the same manner. Whole blood samples (~2 ml) were collected pre-treatment and at 0.1, 0.5, 1, 3, 8, 24, 48, 72, 168, 240, 336, 408, 504, 576 and 672 hours post-dose from each treatment group via femoral venepuncture. Serum was separated from whole blood by centrifugation after which samples were stored at -80°C until analysis. Samples were later analyzed for Dkk-1 and PF-04840082 concentrations.

Acquisition of human serum samples for Dkk-1 analysis

Human serum samples from healthy and diseased subjects were purchased from Bioreclamation Inc. (Nassau, NY). Bioreclamation Inc. obtained consent from each donor permitting use of their blood samples for scientific research. All donor identifications were blinded.

Blood samples were collected by Bioreclamation Inc. at FDA inspected paid donor collection facilities in accordance with an established SOP. Blood donors were pre-screened according to the following inclusion criteria: 1. The donor may not have donated blood in the previous 8 weeks (56 days), or donated a double unit of red cells using an aphaeresis machine in the past 16 weeks. 2. The donor must be between 18 and 65 years old, must have a weight of over 110 pounds (lb), and must have a hematocrit
between the range of 38 and 55%. Blood pressure must be in the range of 90 – 180 mmHg (systolic) and 50-100 mmHg (diastolic). Pulse must be between 50 – 100 bpm. The temperature of the donor must be between 97 and 99.5 degrees. 3. The donor must be generally healthy and free from major diseases including cancer, heart disease, hepatitis, HIV/AIDS, TB, etc. and must not take prescription medication in the past 12 month. 4. Female donors must not be pregnant.

For determination of Dkk-1 concentrations blood samples were selected from pre-menopausal (n=50), post-menopausal (n=50), osteopenic (n=50) and osteoporotic donors (n=50). Osteopenia and osteoporosis are diagnosed based on bone mineral density (BMD) of lumbar spine and femur by a physician according to the following World Health Organization diagnosis criteria: Normal: T-Score at or above -1 Standard Deviation (SD); Osteopenia: T-Score between -1 and -2.5 SD; Osteoporosis: T-Score at or below -2.5 SD. T-Score is a comparison of patient's BMD to that of a healthy thirty-year-old of the same sex and ethnicity.

Free and total Dkk-1 assay

Assay Designs (Ann Arbor, Michigan) human Dkk-1 ELISA System (Cat# 900-151) was validated to measure total Dkk-1 in human serum according to the manufacturer’s instructions with minor modification: R&D Systems (Minneapolis, MN) recombinant human Dkk-1 (Cat# 1096-dk-10/cf) was used as assay standards.

To assay free Dkk-1 (unbound to therapeutic antibodies) in rat and monkey serum, anti-Dkk-1 antibodies were used for capturing unbound serum Dkk-1, and reagents from the
Assay Designs human Dkk-1 ELISA System (Catalog # 900-151) were used for the remaining steps according to the kit’s instruction with the following modifications: R&D Systems (Minneapolis, MN) recombinant rat Dkk-1 (Cat# 4010-dk-10/cf ) and human Dkk-1 (Cat# 1096-dk-10/cf) were used as assay standards for rat and monkey assays, respectively.

The lower limit of quantification (LLOQ) of the assays was 0.02 ng/ml for human Dkk-1, 0.1 ng/ml for monkey free Dkk-1, and 0.05 ng/ml for rat free Dkk-1. These assays were evaluated for intra- and inter-assay coefficient of variability (CV), dilutional linearity, spike recovery and freeze-thaw stability. Intra-assay and inter-assay CVs were <10 % and <20 %, respectively. Linear dilution ranges were found to be 1 to 16 fold for rat serum, 1 to 40 fold for monkey serum, and 16 to 512 fold for human serum. Recovery of recombinant Dkk-1 spiked in serum samples was above 80% for all assays. Serum Dkk-1 measurement was not affected after samples were subjected to 5 rounds of freezing and thawing cycles.

Free PF-04840082 antibody assay for rat PK/PD samples

Serum concentrations of free PF-04840082 were determined by an electrochemiluminescence (ECL) method using a Meso Scale Discovery (MSD) system (Gaithersburg, Maryland). Samples were diluted in a PBS buffer containing 1% BSA to a final minimum required dilution (MRD) of 1/2 to 1/150 followed by an additional 10-4000 fold dilution in order to reduce background interference and to fall within the linear range of the assays (~5-1300 ng/ml on assay plate). PF-04840082 calibration and quality control standards were diluted into the same matrix composition as samples. 96-well
MSD high bind plates (Cat# L11XB-3) were coated overnight at 4 °C with hDkk-1-V5-6His (generated in-house) at 5 µg/ml. After inverting the plate to remove the coat, plates were blocked with 1% BSA in PBS. The diluted samples and standards were added to plates (25 µl/ well) and incubated with shaking at room temperature for 2 hours. The plates were then washed with three wash cycles with PBS buffer containing 0.05% Tween-20, followed by incubation with a MSD ruthinylated goat anti-human IgG antibody (Cat# R32AJ-1) for 1 hour. After washing, MSD read buffer T (4x) with surfactant (Cat# R92TC-1) was added to each well and immediate read on a MSD Sector Imager 6000. Blank matrix values were background subtracted from the sample values. The anti-Dkk-1 humanized antibody calibration standards were used to construct a standard curve using 4-parameter fitting with 1/y² weighting in the MSD Discovery Workbench v 3.0 software. Serum concentrations of anti-DKK-1 humanized antibody in unknown samples were interpolated from this standard curve.

The lower limit of quantification (LLOQ) was 5ng/ml on plate (i.e. 10ng/ml at lowest MRD of ½). The performance of the assay was monitored by the inclusion of quality control (QC) samples prepared in control rat serum. QC samples were prepared (n=4) at concentrations representing the top, middle and bottom of the dynamic range of the assay for each MRD level. Dilution QC samples were also prepared (n=4) representing the highest dilution in each sample set. The mean co-efficient of variability of the QC samples was 2.8% (precision) and the mean relative error was 15.2% (accuracy). The mean co-efficient of variability of the dilution QC samples was 2.0% (precision) and the mean relative error was 19.1% (accuracy).
Free PF-04840082 antibody assay for monkey samples

Serum concentrations of free PF-04840082 were determined by an ELISA method. Samples were diluted in a PBS buffer (containing 3% BSA and 0.05% Tween-20) to a final minimum required dilution (MRD) of 1/4 to 1/100 followed by an additional 10-500 fold dilution in order to reduce background interference and to fall within the linear range of the assays (~8-100 ng/ml on assay plate). PF-04840082 calibration and quality control standards were diluted into the same matrix composition as samples. The 96-well immunosorbent assay plates were coated overnight at 4 °C with hDkk-1-V5-6His (generated in-house) at 1.5 µg/ml and then blocked with PBS (containing 3% BSA and 0.05% Tween-20) after washing with PBS buffer containing 0.05% Tween-20. The diluted samples and standards were added to plates (100 µl/well) and incubated with shaking at room temperature for 1 hour. The plates were then washed with three wash cycles, followed by incubation with biotinylated mouse anti-human IgG2 (Invitrogen, Carlsbad, CA) for 1 hour after which horseradish peroxidase–conjugated streptavidin (Jackson ImmunoResearch, West Grove, PA) was added and the plate was further incubated for 30 minutes. After washing, the plates were developed by color reaction for ~10 min with 3,3',5,5'-tetramethylbenzidine (TMB) substrate then stopped with 2M H₂SO₄. Blank matrix OD values were background subtracted after the absorbance OD reading was determined at a wavelength of 450 nm (with subtraction of 650 nm). The anti-Dkk-1 humanized antibody calibration standards were used to construct a standard curve using 4-parameter fitting with uniform weighting in SoftMax Pro 4.8. Serum concentrations of anti-Dkk-1 humanized antibody in unknown samples were interpolated from this standard curve.
The lower limit of quantification (LLOQ) was 8ng/ml on plate (i.e. 32ng/ml at lowest MRD of ¼). The performance of the assay was monitored by the inclusion of quality control (QC) samples prepared in control monkey serum. QC samples were prepared (n=4) at concentrations representing the top, middle and bottom of the dynamic range of the assay for each MRD level. Dilution QC samples were also prepared (n=4) representing the highest dilution in each sample set. The mean co-efficient of variability of the QC samples was 2.6% (precision) and the mean relative error was 8.7% (accuracy). The mean co-efficient of variability of the dilution QC samples was 2.4% (precision) and the mean relative error was 5.8% (accuracy).

**Anti-drug antibody (ADA) assay**

Presence of anti-drug antibodies (ADA) against PF-04840082 in rat and monkey was measured with a bridging ligand binding assay using the Meso Scale Discovery (MSD) platform (Gaithersburg, Maryland). Serum samples (25 µl) diluted 1:10 with assay diluent (3% BSA, 0.05% Tween 20, PBS) were added to a 96-well MSD high bind plate that was coated with PF-04840082 at 1 µg/ml in pH 9.6 carbonate buffer. After incubating for 1 hour at room temperature, the plate was washed and 25 µl of ruthenium labeled PF-04840082 was added to each well at 1 µg/ml and incubated again for 1 hour at room temperature. The plate was washed and following addition of 150 µl of MSD read buffer (2x), the plate was read on a MSD Sector Imager 6000.
Non-compartmental pharmacokinetic analysis

Pharmacokinetic analysis was performed using the WinNonLin Enterprise Edition computer software, Version 5.2 (Pharsight Corp., Cary, NC). The terminal elimination slope ($\lambda_z$) was determined by linear regression of the log plasma concentration time profile. The terminal elimination half-life ($t_{1/2}$) was calculated from $0.693/\lambda_z$. Maximum drug concentration ($C_{\text{max}}$) and minimum drug concentration ($C_{\text{min}}$) values were obtained directly from recorded data. Area under the serum-concentration time curve to the time of the last observation (AUC$_{0-t_{\text{last}}}$) was calculated using the linear trapezoidal rule and extrapolated to infinity (AUC$_{0-\text{inf}}$) using $\lambda_z$. Clearance (CL) was calculated using the relationship dose/AUC$_{0-\text{inf}}$. Average drug concentration ($C_{\text{average}}$) was calculated as AUC$_{0-\text{inf}}$/time of last observation of 1st dosing interval.

Pharmacokinetic/pharmacodynamic analysis

A mechanistic target–mediated drug disposition model (Mager and Jusko, 2001) was used to describe the PK/PD profile of PF-04840082 in rats and monkeys (Figure 1). In brief, the TMDD model assumes that saturable high affinity binding of the antibody (PF-04840082) to the target (Dkk-1) is responsible for the observable nonlinear pharmacokinetic behavior. Antibody in the central compartment (volume V1) binds (rate constant, $k_{on}$) to free Dkk-1 to form an antibody-Dkk-1 receptor complex. Once formed the complex may dissociate (rate constant, $k_{off}$) or the antibody-Dkk-1 complex may be eliminated (rate constant, $k_{el,complex}$). Unbound antibody can also be directly eliminated from the central compartment at a first order rate ($k_{el}$). The model was extended to
account for antibody distribution to non-specific tissue sites which are described by the rate constants $k_{12}$ and $k_{21}$.

The model was implemented as the following set of differential equations:

\[
(1) \quad \frac{dC_{\text{mAbserum}}}{dt} (\text{nM}\ ) = -k_{el} \cdot C_{\text{mAbserum}} - k_{12} \cdot C_{\text{mAbserum}} + k_{21} \cdot C_{\text{mAbtissue}} \\
- k_{on} \cdot C_{\text{mAbserum}} \cdot C_{\text{targ}} + k_{off} \cdot C_{\text{complex}}
\]

\[
(2) \quad \frac{dC_{\text{mAbtissue}}}{dt} (\text{nM}\ ) = -k_{21} \cdot C_{\text{mAbtissue}} + k_{12} \cdot C_{\text{mAbserum}}
\]

\[
(3) \quad \frac{dC_{\text{targ}}}{dt} (\text{nM}\ ) = k_{in} - k_{el} \cdot C_{\text{targ}} - k_{on} \cdot C_{\text{mAbserum}} \cdot C_{\text{targ}} + k_{off} \cdot C_{\text{complex}} \\
+ k_{off} \cdot C_{\text{complex}}
\]

\[
(4) \quad \frac{dC_{\text{complex}}}{dt} (\text{nM}\ ) = -k_{off} \cdot C_{\text{complex}} + k_{on} \cdot C_{\text{mAbserum}} \cdot C_{\text{targ}} - k_{el} \cdot C_{\text{complex}}
\]

Where $C_{\text{mAbserum}}$ is equal to free concentrations of PF-04840082, $K_d = k_{off}/k_{on}$, 

\[
[m\text{Ab}]_\text{total} = C_{\text{mAbserum}} + C_{\text{complex}} \quad \text{and} \quad [T\text{arg}et]_\text{total} = C_{\text{targ}et} + C_{\text{complex}}
\]

Baseline target concentrations varied as a function of time in both rat and monkey. This was incorporated into the target production rate, $k_{\text{targ}}$, using empirical functions to modulate the time course of Dkk-1 concentrations under vehicle treatment as described in equations (5) and (6).

Variation of Dkk-1 concentrations in vehicle treated rats was characterized using a simple cosine function as described by (Chakraborty et al., 1999):

\[
(5) \quad k_{\text{targ}} = (\text{Baseline} + \text{Amplitude}\cdot\cos((\text{Time} - \text{PeakTime})\cdot(\frac{2\pi}{24}))) \cdot k_{\text{targ}et}
\]

Variation of Dkk-1 concentrations in vehicle treated monkeys was characterized using a fourth order polynomial model:
(6) \( k_{in} = (A.Time^4 - B.Time^3 + C.Time^2 - D.Time + Baseline).k_{el_{target}} \)

where A, B, C and D are constants fixed according to the vehicle data in the model and baseline is equal to the concentration of Dkk-1 pre-dose (\( t = 0 \) hr.)

Dkk-1 concentrations below the lower limit of quantification (LLOQ) in rat and monkey were included in the analysis in order to avoid creating a bias in the model fit and to ensure accurate estimation of model parameters. In the model, concentrations below the LLOQ were set to \( \frac{1}{2} \) LLOQ (0.025ng/ml in rat and 0.05ng/ml in monkey). This method of handling concentrations below LLOQ has been described previously (Beal, 2001).

The system of differential equations was solved numerically in the NONMEM software, version V, running in a DOS shell under Windows XP utilizing Compaq Visual Fortran version 6.6. The goodness-of-fit was assessed from the precision of the parameter estimates and correlation matrix of the parameters provided, visual inspection for a random spread of weighted residual against time and predicted concentrations, together with visual inspection of the individual subject predicted versus actual concentration-time plots for a lack of systematic bias at any points in time.

Calculation of clinical doses

The FIH starting dose of PF-04840082 was selected based on three different methodologies: (1) No adverse effect level (NOAEL) in toxicology species (2) Minimum anticipated biological effect level (MABEL) using \textit{in vitro} binding data and equilibrium based calculations (3) MABEL calculated using a TMDD PK/PD model.
Based on Food and Drug administration (FDA) guidance (FDA, 2005), the NOAEL of PF-04840082 was determined in rat and monkey and converted to a ‘Human equivalent dose’ (HED) by normalizing for body surface area as follows:

\[
HED_{\text{monkey}} = \frac{\text{NOAEL}_{\text{monkey}}}{3.1} \quad \text{and} \quad HED_{\text{rat}} = \frac{\text{NOAEL}_{\text{rat}}}{6.2}
\]

A safety factor of 100 fold was then applied to obtain the maximum recommended starting dose (MRSD).

**Equilibrium calculations**

A formula to calculate maximal receptor occupancy based on equilibrium dissociation constant (\(K_d\)) of the mAb-target interaction was used to estimate MABEL (Duff, 2006). MABEL was defined as the dose which results in 10% peak RO.

\[
RO(\%) = \frac{\left[\frac{\text{Dose}}{mAbV1}\right]}{K_d + \left[\frac{\text{Dose}}{mAbV1}\right]} \times 100
\]

**Human PK/PD simulations using the TMDD model**

Simulations of Dkk-1 and PF-04840082 concentrations in humans were performed using the TMDD model in Berkeley-Madonna (v8.3.9, University of California, Berkeley, CA) in order to predict MABEL and efficacious dose for the treatment of osteoporosis. Human model parameters were either taken from the literature (subcutaneous bioavailability, \(k_a\) mAb), measured experimentally (\(k_{on}, k_{off}\), Dkk-1 baseline levels), or
scaled from rat and monkey parameters via the principles of allometry (V1, \(k_{el\, mAb}\), \(k_{el\, target}\), \(k_{el\, complex}\)).

Allometric extrapolations were achieved using a simple power model of the form: \(Y = a \cdot BW^b\), where \(Y\) is the parameter of interest, \(BW\) is the body weight, \(a\) is the allometric coefficient and \(b\) is the allometric exponent. For scaling of elimination rate constants \(b\) was assumed to equal -0.25 and for volume of distribution \(b\) was assumed to equal 1 (Wang et al., 2008).

MABEL was estimated be the dose which resulted in 10% reduction in Dkk-1 levels. Prior experiments in an OVX mouse disease model indicated that 50% reduction in Dkk-1 was required for a statistically significant increase in bone mineral density and this was the target set for predicting efficacious dose.
RESULTS

Biacore data

PF-04840082 binds human, mouse and rat Dkk-1 with high affinity ($K_d < 2pM, <30pM$ and $<100pM$, respectively). In most cases, on rates ($k_{on}$) were too fast and off rates ($k_{off}$) were too slow to be measured precisely by the Biacore instrumentation. PF-04840082 also binds to cynomolgus monkey Dkk-1, although a $K_d$ could not be determined.

Dkk-1 expression in pre-menopausal, post-menopausal, osteopenic and osteoporotic women

The concentrations of Dkk-1 in serum samples from pre-menopausal ($n = 50$), post-menopausal ($n = 50$), osteopenic ($n = 50$) and osteoporotic ($n = 50$) women are shown in Table 1. There was no significant difference in Dkk-1 concentrations between pre- and post-menopausal women in the sample set tested, suggesting that age does not affect Dkk-1 concentrations. There was a significant difference ($p<0.01$) between Dkk-1 levels in pre-menopausal women (mean 2.2 ng/ml) compared to samples from osteopenic women (T score -2.2, mean Dkk-1 9.0 ng/ml) and osteoporotic women (T-score -3.0, mean Dkk-1 10.5 ng/ml). These values were included in the PK/PD model to predict efficacious dose of PF-04840082 in osteoporotic women.

Rat pharmacokinetics and pharmacodynamics

The mean free PF-04840082 concentration versus time profiles following weekly intravenous administration to female Sprague-Dawley rats are shown in Figure 2. Non-compartmental pharmacokinetic parameters are shown in Table 2.
The pharmacokinetics of PF-04840082 were non-linear across the dose range with supra-proportional increases in AUC with dose up to 10 mg/kg. This indicates a higher rate of clearance at the lower doses (0.1 and 1 mg/kg) compared with the higher doses (10 and 100 mg/kg). At later time points, serum PF-04840082 concentrations in some of the rats were lower than expected or below the LLOQ of the assay. Rat anti-PF-04840082 antibodies were confirmed in these samples using a qualitative anti-drug antibody (ADA) assay and data from these rats were removed from the analysis and the plots.

Mean free Dkk-1 concentrations in the same study are plotted in Figure 3. Free Dkk-1 concentrations decreased rapidly following administration of PF-04840082 to rat. At all but the lowest dose, free Dkk-1 concentrations remained suppressed for the duration of the study.

Cynomolgus monkey pharmacokinetics and pharmacodynamics

Individual free serum PF-04840082 concentration versus time profiles in cynomolgus monkeys are shown in Figure 4, and non-compartmental pharmacokinetic parameters of PF-04840082 in cynomolgus monkey are shown in Table 3. The pharmacokinetics of PF-04840082 were non-linear over the dose range tested with higher rates of clearance and shorter half-life values observed at the lower doses. The half-life of PF-04840082 in cynomolgus monkeys ranged from 1-13 days across the dose range.

Loss of exposure in the 1 mg/kg group around 14 days and in one monkey in the 10 mg/kg group around 21 days post dose is likely to be due to formation of anti-PF-04840082 antibodies and these data were removed from the analysis and the plots. However, this could not be confirmed using a qualitative ADA assay.
Individual free Dkk-1 concentrations in the same study are plotted in Figure 5. Free Dkk-1 concentration decreased rapidly following dosing of PF-04840082. Duration of Dkk-1 suppression was dose dependent and returned to baseline at the lower doses. At the highest doses, Dkk-1 remained suppressed for the entire dosing interval.

**PK/PD modeling**

A TMDD model (Mager and Jusko, 2001) was used to simultaneously fit free PF-04840082 and free Dkk-1 concentrations over time (Figure 1) in both the monkey and the rat. This model was chosen as it accounts for non-target specific elimination of antibody, target synthesis and turnover, and complex formation and loss from the serum. Observed versus model predicted concentrations of PF-04840082 and Dkk-1 in rat are shown in Figures 2 and 3 and in monkey are shown in Figures 4 and 5. Parameter estimates from the TMDD model for rat and monkey are shown in Table 4.

**Calculation of clinical doses**

**NOAEL method**

In both rat and monkey, PF-04840082 was well tolerated with no treatment related changes up to the highest dose administered (100 mg/kg). At this dose level 100% RO was achieved and Dkk-1 was suppressed for the entire dosing interval. NOAEL was therefore estimated to be 100 mg/kg. HED was estimated as 16 mg/kg from rat and 32 mg/kg from monkey. After application of a 100 fold safety margin the maximum recommended starting dose (MRSD) was estimated to be 0.16 mg/kg from rat data or 0.32 mg/kg from monkey data.
Equilibrium based approach to MABEL

Using the equilibrium calculation approach (Duff, 2006), MABEL was defined to be the dose which results in 10% peak RO. The MRSD for PF-04840082 using this method was estimated to be $1 \times 10^{-6}$ mg/kg (Figure 6).

PK/PD modeling approach to MABEL

The parameters used in the human simulations are shown in Table 5. From human simulations using the TMDD model the dose predicted to give minimum anticipated biological effect (MABEL) was 0.008 mg/kg. This dose is predicted to transiently reduce Dkk-1 levels by approximately 10% followed by return to baseline. A dose of 0.0008 mg/kg (1/10 of MABEL) was predicted to have no effect. These human simulations of Dkk-1 concentration are shown in Figure 7. The predicted efficacious dose of PF-04840082 to reduce Dkk-1 by >50% over the dosing interval is 3.74 mg/kg given once monthly.

Non-linear pharmacokinetics of PF-04840082 are predicted across the dose range encompassing MABEL (0.008 mg/kg) and the predicted efficacious dose (3.74 mg/kg). This is due to TMDD and is shown in Figure 7.
DISCUSSION

PK/PD understanding of PF-04840082 in rat and monkey

In the rat and monkey studies, PF-04840082 exhibited non-linear pharmacokinetics with higher rates of clearance and shorter elimination half-life values at lower doses. This is often indicative of target mediated drug disposition (TMDD) where interaction of an antibody with its pharmacological target influences disposition at lower doses (Tabrizi et al., 2006; Wang et al., 2008). Empirical PK/PD models consisting of a PK model to describe systemic drug concentrations, which is used as a forcing function to describe PD, are often not appropriate for characterizing TMDD as they do not account for the interdependency of PK and PD. A single model describing drug PK, target dynamics and their interaction was proposed by Mager and Jusko (Mager and Jusko, 2001). This model accounts for specific and non-specific distribution and elimination of the drug molecule as well as providing flexibility to account for target dynamics. In other cases, where PK and PD have been simultaneously analyzed, this model has been used to provide a direct link between dose, exposure and response (Meno-Tetang and Lowe, 2005; Ng et al., 2006; Wu et al., 2006). In this case, the TMDD model was used to simultaneously fit antibody, PF-04840082, and target, Dkk-1, concentrations in the rat and monkey following IV administration of PF-04840082 at several dose levels. The model gave an estimation of non-target mediated pharmacokinetics of PF-04840082 that was fairly consistent with typical IgG2 pharmacokinetics in each species (Peppard and Orlans, 1980; Hinton et al., 2004). Thus, the elimination half-life was 2.5 days in rat and 13.6 days in monkey. Volume of distribution was approximately equivalent to plasma volume in the monkey (0.052L/kg) but higher than plasma volume in rat (0.147L/kg).
Target understanding

The TMDD model has been used previously to describe the PK/PD relationship of antibodies *in vivo* and to predict the human dose-RO response. In these cases, it has been shown that the *in vivo* response is very sensitive to baseline levels and turnover rate of the target. In contrast, antibody affinity impacts the dose response relationship to a lesser extent within the range relevant to most antibodies (Meno-Tetang and Lowe, 2005; Agoram, 2009). In order to make meaningful predictions of clinical doses of a Dkk-1 antibody, efforts were made to characterize target levels and turnover rates prior to drug development.

Baseline levels

The baseline levels of Dkk-1 in patients with osteoporosis were not known and this prompted establishment of reference ranges for human Dkk-1 levels in both healthy subjects and the patient population (osteopenic and osteoporotic patients) that could be used for more informative dose predictions at different stages of clinical development. The analysis showed that Dkk-1 levels were higher in osteopenic and osteoporotic women (T-score < -1) compared with healthy women.

This is consistent with data in mice which indicates that Dkk-1 is a master regulator of bone remodeling (Diarra et al., 2007) and Dkk-1 baseline levels are approximately 5 times higher in disease state (ovariectomised, OVX) mice compared with healthy mice (Li et al, manuscript in preparation). Dkk-1 levels have also been shown to be elevated in bone marrow plasma and peripheral blood of multiple myeloma patients with bone lesions (Tian et al., 2003).
The rate of turnover of target ligands can vary from minutes to days which can have a significant impact on efficacious dose and even potential of the target to be perturbed for clinical benefit. Some ligands have similar kinetics across species, but for others the turnover is not predictable a priori. Meno-Tetang et al (Meno-Tetang and Lowe, 2005) showed that IgE elimination half-life values range from 5-8 hr in mouse to 2.7 days in man which markedly affected the predictions of human effect of an anti-IgE antibody. Interestingly, IgE turnover does scale according to allometric principles.

The elimination half-life of the target Dkk-1 was estimated to be 11 min in rat and 26 min in monkey from the PK/PD modeling indicating that Dkk-1 has a rapid turnover rate. The half-life of the PF-04840082-Dkk-1 complex was estimated to be intermediate between PF-04840082 half-life and Dkk-1 half-life which may reflect the target mediated clearance mechanism. However, depending on the nature of the downstream events resulting from antibody-target binding, the process described could be clearance through antigen processing, or distribution if antibody dissociates from the target after loss of the complex from the serum. It is also possible that binding of the target to the antibody interferes with antibody binding to FcRn and studies are being completed in-house to test this hypothesis.

Prediction of FIH starting doses

For monoclonal antibodies, it is becoming widely recognized that rational selection of safe first-in-human doses, on the basis of PK/PD modeling is essential (EMEA, 2007). A new parameter, minimum anticipated biological effect level (MABEL) involves
extrapolation of observed preclinical PK/PD data to clinical prediction on the basis of a
PK/PD modeling approach. MABEL has been suggested for consideration in addition to
the no adverse effect level (NOAEL) in designing first-in-human dose levels of high risk
therapeutics in recent European regulatory guidance (EMEA, 2007).

Use of the NOAEL method to calculate the FIH starting dose of PF-04840082 yielded
doses predicted to give high receptor occupancy, even when scaled to account for body
surface area and a 100 fold safety factor applied. This approach should ensure adequate
safety margins for PF-04840082 in the clinic. However, the high RO is likely to result in
a highly pharmacologically active dose which was not considered appropriate for a
clinical starting dose.

An alternative approach to FIH starting dose selection was suggested in the Expert Study
Group Report (Duff, 2006) and uses a simple formula based on equilibrium drug-receptor
interaction theory and on known PK of monoclonal antibodies (Equation 7). This method
predicts starting dose using the MABEL principle which is defined as the dose which
results in 10% peak RO. Equation 7 does not consider time course of antibody
concentration, rather it calculates concentration using the relationship dose/volume of
distribution and therefore estimates a fixed (peak) RO. It also assumes excess of drug
compared with target and rapid association-dissociation rates, however, these
assumptions may not always apply to biologics. In particular, this method relies on $K_d$
alone and does not take into account target or target-mAb complex kinetics. Dkk-1 has
been shown to have a high turnover rate and, binding of Dkk-1 by PF-04840082 changes
the kinetics of the target (TMDD). Under these conditions, RO is often not predictable by pharmacological equilibrium approaches and simple $K_d$ based RO calculations have been shown to substantially over predict RO at a particular dose (Agoram, 2009). For Dkk-1, RO calculated using the equilibrium formula estimates a clinical starting dose of $1 \times 10^{-6}$ mg/kg (Figure 6). Use of this approach could result in the selection of doses that are too low in the clinic and delay the progression of a first-in-human study.

In contrast, a PK/PD model based approach to MABEL provides a more mechanistic rationale for starting dose selection. The TMDD model accurately characterized the preclinical data which indicated the role of target turnover in determining percentage of binding of target. This model was adapted for human PK/PD simulations by incorporating literature reported values of IgG2 antibody PK and estimates of both Dkk-1 target kinetics and PF-04840082-Dkk-1 complex kinetics obtained from preclinical modeling. *In vitro* Biacore values for human Dkk-1 were used to determine association and dissociation rates of the complex ($k_{on}$ and $k_{off}$) in the model. Mean Dkk-1 baseline levels in post-menopausal or osteoporotic patients were used to simulate FIH starting doses and clinical efficacious doses for osteoporosis respectively. The estimate of FIH starting dose from the TMDD model was 0.008 mg/kg. This dose is associated with minimal anticipated biological effect and is well below the dose predicted using the NOAEL approach. The predicted efficacious dose for the treatment of osteoporosis to reduce Dkk-1 levels by greater than 50% over the dosing interval is 3.74 mg/kg given subcutaneously 1/month. Non-linear PK is predicted in the clinic (Figure 7), with PF-04840082 exhibiting a higher clearance and shorter half-life at lower doses due
to TMDD. A PK/PD model based approach to MABEL dose calculations was concluded to be more likely to be predictive for Dkk-1 as it integrates information on pharmacology, efficacy and safety in a quantitative manner.

Conclusion

In conclusion, PF-04840082 is a humanized prototype anti-Dkk-1 antibody for the treatment of osteoporosis. A mechanistic TMDD model was used to characterize PF-04840082 versus Dkk-1 concentration response relationship in rat and monkey. This model was translated to human to predict efficacious dose and MABEL by incorporating information on target expression and turnover rates. The proposed approach should provide the most likely drug exposure to ensure the safety and efficiency of clinical studies.
ACKNOWLEDGEMENTS

We would like to thank Carolyn Mallozzi and Vincent Bernardo for their work on PF-04840082 and Dkk-1 assay development respectively. Thanks also to Hugh Barton and Tristan Maurer for critical review of this manuscript.
REFERENCES


(2001) LDL receptor-related protein 5 (LRP5) affects bone accrual and eye development. 


LEGENDS FOR FIGURES

1. Target mediated drug disposition (TMDD) model.

2. Observed and model predicted free PF-04840082 concentrations versus time following weekly intravenous administration of PF-04840082 to Sprague-Dawley rats. Symbols represent the mean observed data (± SD). Lines represent the predicted profiles from the model, except for the 0.1mg/kg dose at time points post 3hrs, where model predictions are represented by open circles.

3. Observed and model predicted free Dkk-1 concentrations versus time following weekly intravenous administration of PF-04840082 to Sprague-Dawley rats. Symbols represent the mean observed data (± SE) and lines represent the predicted profiles from the model.

4. Observed and model predicted free PF-04840082 concentrations versus time following single intravenous administration of PF-04840082 to Cynomolgus monkeys. Symbols represent the observed individual monkey data and lines represent the predicted profiles from the model.

5. Observed and model predicted free Dkk-1 concentrations versus time following single intravenous administration of PF-04840082 to Cynomolgus monkeys. Open symbols represent the observed data from the female monkeys and solid symbols represent the observed data from the male monkeys. Solid lines represent the model predicted profiles.
for male monkeys and dashed lines represent the model predicted profiles for female monkeys.

6. Receptor occupancy calculations for PF-04840082 vs. predicted human dose based on (1) steady state equilibrium method (Duff, 2006) and (2) TMDD PK/PD model

7. TMDD model predicted free PF-04840082 concentrations (top panel) and predicted free Dkk-1 concentrations (as percentage of baseline levels, bottom panel) in humans following subcutaneous administration of PF-04840082 1/month for 6 months. Three different doses were simulated: 0.0008 mg/kg which represents 1/10 of MABEL or a no effect dose, 0.008 mg/kg which represents MABEL and 3.74 mg/kg which represents predicted efficacious dose for osteoporosis. In the top panel, the anticipated LLOQ of the PF-04840082 bioanalytical assay is shown to represent the futility of dosing lower than predicted MABEL.
TABLE 1

Dkk-1 concentrations in pre-menopausal, post-menopausal, osteopenic and osteoporotic female subjects

<table>
<thead>
<tr>
<th>Subject</th>
<th>N</th>
<th>Age</th>
<th>T-Score&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Serum Dkk-1&lt;sup&gt;b&lt;/sup&gt; (ng/ml)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-menopausal</td>
<td>50</td>
<td>33.3 ± 13</td>
<td>&gt;-1.0</td>
<td>2.2 ± 2.2</td>
<td>-</td>
</tr>
<tr>
<td>Post-menopausal</td>
<td>50</td>
<td>54.7 ± 4.4</td>
<td>&gt;-1.0</td>
<td>2.9 ± 4.3</td>
<td>0.35</td>
</tr>
<tr>
<td>Osteopenic</td>
<td>50</td>
<td>61.3 ± 10.6</td>
<td>-2.2 ± 0.5</td>
<td>9.0 ± 4.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Osteoporotic</td>
<td>50</td>
<td>67.4 ± 7.7</td>
<td>-3.0 ± 0.5</td>
<td>10.6 ± 6.5</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

<sup>a</sup> A normal T-score is ≥-1.0, osteopenia is defined as a T-score of <-1.0 and >-2.5, osteoporosis is defined as a T-score of -2.5 or lower.

<sup>b</sup> Values reported as mean ± standard deviation.
TABLE 2
Non-compartmental pharmacokinetic parameters for free PF-04840082 concentrations in Sprague Dawley rats following intravenous administration of PF-04840082

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>AUC$_{0-168}$ hr (µg.hr/mL)</th>
<th>$C_{\text{max}}$ (µg/ml)</th>
<th>$C_{\text{average}}^b$ (µg/ml)</th>
<th>$C_{\text{min}}^c$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>21.2 ± 2.2</td>
<td>1.81 ± 0.16</td>
<td>0.127 ± 0.013</td>
<td>&lt;LLOQ</td>
</tr>
<tr>
<td>1</td>
<td>850 ± 88.8</td>
<td>24.6 ± 1.6</td>
<td>5.06 ± 0.528</td>
<td>0.205 ± 0.172</td>
</tr>
<tr>
<td>10</td>
<td>13300 ± 1000</td>
<td>252 ± 29.7</td>
<td>79.0 ± 5.94</td>
<td>38.7 ± 8.50</td>
</tr>
<tr>
<td>100</td>
<td>141000 ± 18700</td>
<td>2660 ± 313</td>
<td>841 ± 110</td>
<td>439 ± 116</td>
</tr>
</tbody>
</table>

$^a$ Values reported as mean ± standard deviation

$^b$ $C_{\text{average}} = \text{AUC}_{0-\text{inf}} / \text{t}_{\text{last of 1st dosing interval (not for entire duration of study due to sparse sampling)}}.$

$^c$ $C_{\text{min}} = 1^{\text{st}}$ dosing interval (0-168hrs).
TABLE 3
Mean non-compartmental pharmacokinetic parameters for free PF-04840082 concentrations in Cynomolgus monkeys following single intravenous administration (n=1/sex/dose group)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>AUC₀-tlast (µg.hr/mL)</th>
<th>AUC₀-inf (µg.hr/mL)</th>
<th>CL (mL/day/kg)</th>
<th>t½ (day)</th>
<th>Cmax (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>36.4</td>
<td>37.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>~1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.32</td>
</tr>
<tr>
<td>1</td>
<td>1720</td>
<td>1810</td>
<td>13.8</td>
<td>2.6, 4.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.3</td>
</tr>
<tr>
<td>10</td>
<td>48100</td>
<td>55900</td>
<td>4.56</td>
<td>12.5</td>
<td>242</td>
</tr>
<tr>
<td>100</td>
<td>643000</td>
<td>845000</td>
<td>2.85</td>
<td>13.3</td>
<td>3040</td>
</tr>
</tbody>
</table>

<sup>a</sup> n=1 due to AUC₀-inf >120% AUC₀-tlast.

<sup>b</sup> Individual t½ values reported due to calculations from different time intervals.
TABLE 4

Estimated PK/PD Parameters in Monkey and Rat

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate from Rat Model</th>
<th>CV (%)</th>
<th>Estimate from Monkey Model</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V1_{mAb}$ (L/kg)</td>
<td>0.147</td>
<td>1.74</td>
<td>0.052</td>
<td>2.02</td>
</tr>
<tr>
<td>$k_{el mAb}$ (day$^{-1}$)</td>
<td>0.278</td>
<td>3.52</td>
<td>0.051</td>
<td>1.54</td>
</tr>
<tr>
<td>$t_{1/2_mAb}$ (day)$^{a}$</td>
<td>2.5 Derived</td>
<td></td>
<td>13.6 Derived</td>
<td></td>
</tr>
<tr>
<td>$k_{12}$ (day$^{-1}$)</td>
<td>1.03</td>
<td>5.82</td>
<td>0.285</td>
<td>2.70</td>
</tr>
<tr>
<td>$k_{21}$ (day$^{-1}$)</td>
<td>0.842</td>
<td>9.73</td>
<td>0.277</td>
<td>1.25</td>
</tr>
<tr>
<td>$k_{on}$ (nM$^{-1}$ day$^{-1}$)$^{b}$</td>
<td>49.4 Fixed</td>
<td>316</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>$k_{off}$ (day$^{-1}$)$^{b}$</td>
<td>1.72 Fixed</td>
<td>16.2</td>
<td>Fixed</td>
<td></td>
</tr>
<tr>
<td>$K_d$ (pM)$^{c}$</td>
<td>34.8 Derived</td>
<td>51.3</td>
<td>Derived</td>
<td></td>
</tr>
<tr>
<td>$k_{el target}$ (day$^{-1}$)</td>
<td>93.1 1.05</td>
<td>39</td>
<td>2.18</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2 target}$ (min)$^{a}$</td>
<td>11 Derived</td>
<td>26</td>
<td>Derived</td>
<td></td>
</tr>
<tr>
<td>$k_{el complex}$ (day$^{-1}$)</td>
<td>9.54 1.75</td>
<td>0.613</td>
<td>1.57</td>
<td></td>
</tr>
<tr>
<td>$t_{1/2 complex}$ (hr)$^{a}$</td>
<td>1.74 Derived</td>
<td>26.4</td>
<td>Derived</td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Secondary parameter calculated as $t_{1/2} = 0.693/k_{el}$

$^{b}$ $k_{on}$ and $k_{off}$ were determined in a previous run where standard errors could not be estimated in NONMEM. In the final run, $k_{on}$ and $k_{off}$ estimates were fixed to these values and standard errors were achieved.

$^{c}$ Secondary parameter calculated as $K_d = k_{off}/k_{on}$
TABLE 5

Parameter estimates used in human PK/PD simulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Reference/ Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>mAb SC bioavailability (%)</td>
<td>75</td>
<td>(Tang et al., 2004)</td>
</tr>
<tr>
<td>mAb SC absorption rate constant (day-1)</td>
<td>0.5</td>
<td>(Tang et al., 2004; Agoram et al., 2007)</td>
</tr>
<tr>
<td>mAb volume of distribution (mL/kg)</td>
<td>50</td>
<td>(Tang et al., 2004; Agoram et al., 2007)</td>
</tr>
<tr>
<td>mAb (non-specific) elimination rate constant (day-1)</td>
<td>0.03</td>
<td>(Tang et al., 2004; Hayashi et al., 2007)</td>
</tr>
<tr>
<td>Dkk-1 levels (ng/ml) in post-menopausal women&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.9 ± 4.3</td>
<td>Allometric scaling from rat and monkey</td>
</tr>
<tr>
<td>Dkk-1 levels (ng/ml) in osteoporosis patients&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.6 ± 6.5</td>
<td>Allometric scaling from rat and monkey</td>
</tr>
<tr>
<td>Dkk-1 half-life (min)</td>
<td>49</td>
<td>Allometric scaling from rat and monkey</td>
</tr>
<tr>
<td>mAb-Dkk-1 association rate (kon, nM-1day-1)</td>
<td>112.3</td>
<td>Human Biacore data</td>
</tr>
<tr>
<td>mAb-Dkk-1 dissociation rate (koff, day-1)</td>
<td>0.1728</td>
<td>Human Biacore data</td>
</tr>
<tr>
<td>mAb-Dkk-1 elimination rate constant (day-1)</td>
<td>0.3</td>
<td>Allometric scaling from monkey</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean levels of Dkk-1 in post menopausal women were used for MABEL predictions of starting dose in Phase 1 clinical trials.

<sup>b</sup> Mean levels of Dkk-1 in osteoporosis patients were used for efficacious dose predictions.
Figure 1

\[ k_{in} = C_{target} \times k_{el \ target} \]
\[ K_d = \frac{k_{off}}{k_{on}} \]
Figure 3
Figure 5
Figure 6

- Receptor Occupancy (%) vs. PF-04840082 Dose (mg/kg)

- **Line 1**: Receptor occupancy calculated using steady state equilibrium calculation (%)

- **Line 2**: Receptor occupancy calculated from mechanistic PK/PD modeling (%)

- X-axis: PF-04840082 Dose (mg/kg)

- Y-axis: Receptor Occupancy (%)
Figure 7

- 3.74mg/kg (Predicted Efficacious Dose)
- 0.008mg/kg (MABEL)
- 0.0008mg/kg (1/10 of MABEL)
- Anticipated Assay LLOQ

Free PF-04840082 (nM)

Time (day)

DKk-1 concentrations as % of baseline

Time (day)