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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ABSTRACT

PF-04840082 is a humanized prototype anti-Dickkopf-1 (Dkk-1) immunoglobulin isotype G2 (IgG2) antibody for the treatment of osteoporosis. In vitro, PF-04840082 binds to human, monkey, rat, and mouse Dkk-1 with high affinity. After administration of PF-04840082 to rat and monkey, free Dkk-1 concentrations decreased rapidly and returned to baseline in a dose-dependent manner. In rat and monkey, PF-04840082 exhibited non-linear pharmacokinetics (PK) and a target-mediated drug disposition (TMDD) model was used to characterize PF-04840082 versus Dkk-1 concentration response relationship. PK/pharmacodynamic (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 that were too high to ensure safety of clinical trials. The pharmacological equilibrium approach calculated receptor occupancy (RO) based on equilibrium dissociation constant alone and did not take into account rate of turnover of the target or antibody–target complex kinetics and, as a result, it likely produced a substantial overprediction 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.

Osteoporosis is a bone disease characterized by low bone mineral density that leads to bone fragility and, subsequently, 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/low-density lipoprotein receptor-related protein 5 (LRP5) regulates bone mass and 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 LRP5/6 and frizzled proteins. Wnt signaling is tightly regulated by antagonists that include secreted molecules such as Dickkopf-1 (Dkk-1). Binding of Dkk-1 to the

ABBREVIATIONS: Dkk-1, Dickkopf-1; AUC0-tlast, area under the concentration time curve to time of last observation; AUC0-inf, area under the concentration time curve extrapolated to infinity; Caverage, average concentration; Cmax, maximum concentration; Cmin, minimum concentration; CV, coefficient of variability; FDA, Food and Drug Administration; FIH, first in human; HED, human equivalent dose; IgG2, immunoglobulin isotype G2; kabs, absorption rate constant; KDE, equilibrium dissociation constant; kelim, elimination rate constant; koff, dissociation rate constant; kobs, association rate constant; koff, dissociation rate constant; t1/2, 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; PK/PD, pharmacokinetic/pharmacodynamic; RO, receptor occupancy; t1/2, time of last observation; TMDD, target-mediated drug disposition; MSD, Meso Scale Discovery; PBS, phosphate-buffered saline; BSA, bovine serum albumin; QC, quality control; ADA, antidrug antibodies.
LRP5/6 receptor and Kremen-1/2 coreceptor 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 osteopetrosis 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 caused by 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 by 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 in vitro with high affinities (K_d < 100 pM).

Clinical starting doses of biotherapeutic drugs are traditionally estimated by using no adverse effect level (NOAEL) data in toxicology species as recommended by the Food and Drug Administration (FDA) (Food and Drug Administration, 2005). However, new guidance from the European Medicines Agency (European Medicines Agency, 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 pharmacokinetic/pharmacodynamic (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 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 FeRn 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 nuclear factor-κB, respectively) that exhibit nonlinear 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 after 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 pharmacodynamics of the response. This was coupled with knowledge of target level in healthy versus diseased subjects, target turnover rates, and antibody-target association/dissociation rates to support FIH doses in the clinic. In this article, the NOAEL and MABEL methods of estimating starting doses of the Dkk-1 antibody are compared.

### Materials and Methods

#### Test Materials.
Monoclonal anti-Dkk-1 antibodies were made at Genovac (Freiburg, Germany) by immunization of BALB/c mice with full-length recombinant human Dkk-1 protein (R&D Systems, Minneapolis, MN). A single mouse IgG1κ isotype antibody (JC18) was humanized and affinity-matured by using a library scanning mutagenesis strategy (J. Pons and D. M. Stone, manuscript in preparation). In comparison with 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 PC38377 (Paralkar et al., 2009).

#### Biacore Experiment.
Interactions between PF-04840082 and human, mouse, rat, or cynomolgus monkey Dkk-1 were analyzed by using a Biacore 3000TM system equipped with a CMS 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 BioEvaluation v.4.0 software. Affinities were deduced from the quotient of the kinetic rate constants (K_d = k_on/k_off).

#### Animal Studies.
All animal studies were conducted in accordance with animal care and use protocols approved by the Institutional Animal Care and Use Committee. The program of humane animal care and use at Pfizer Global Research and Development has been evaluated for its compliance with the U.S. Animal Welfare Act and The Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, 1996).

#### PK/PD Study in Rats.
Experiments were conducted in female Sprague-Dawley rats (n = 40, body weight 250–350g; Charles River Laboratories Inc., Wilmington, MA). PF-04840082 was administered once weekly intravenously for 6 consecutive weeks (n = 8/dose) at 0.1, 1, 10, and 100 mg/kg. One group of rats (n = 8) was 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, predose, and at 1, 3, 8, 24, 48, 72, 168, 240, 336, 408, 504, 576, 672, 744, 840, 912, and 1008 h after the 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.

#### PK/PD Study in Cynomolgus Monkeys.
The animal care and experimental procedures of this study were 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 fascieu-
Acquisition of Human Serum Samples for Dkk-1 Analysis. Human serum samples from healthy and diseased subjects were purchased from Bio-reclamation Inc. (Nassau, NY). Bio-reclamation Inc. obtained consent from all donors, permitting use of their blood samples for scientific research. All donor identifications were blinded.

Blood samples were collected by Bio-reclamation Inc. at FDA-inspected paid donor collection facilities in accordance with an established standard operating procedure. Blood donors were screened according to the following inclusion criteria: 1) The donor could not have donated blood in the previous 8 weeks (56 days) or donated a double unit of red cells using an apheresis machine in the past 16 weeks. 2) The donor had to be between 18 and 65 years old with a weight of over 110 pounds and a hematocrit between 38 and 55%. Blood pressure had to be in the range of 90 to 180 mm Hg (systolic) and 50 to 100 mm Hg (diastolic). Pulse had to be between 50 and 100 beats per min. The temperature of the donor had to be between 97 and 99.5°F. 3) The donor had to be generally healthy and free from major diseases, including cancer, heart disease, hepatitis, HIV/AIDS, tuberculosis, etc., and not have taken pre-scription medication in the past 12 months. 4) Female donors could not be pregnant.

For determination of Dkk-1 concentrations blood samples were selected from premenopausal (n = 50), postmenopausal (n = 50), osteopenic (n = 50), and osteoporotic donors (n = 50). Osteopenia and osteoporosis were diagnosed based on bone mineral density of lumbar spine and femur by a physician according to the following World Health Organization diagnosis criteria: normal, T-score at or above −1 S.D.; osteopenia, T-score between −1 and −2.5 S.D.; and osteoporosis, T-score at or below −2.5 S.D. T-Score is a comparison of a subject’s bone mineral density with that of a healthy 30-year-old of the same sex and ethnicity.

Free and Total Dkk-1 Assay. The Assay Designs (Ann Arbor, MI) human Dkk-1 ELISA System was validated to measure total Dkk-1 in human serum according to the manufacturer’s instructions with minor modification. R&D Systems’ recombinant human Dkk-1 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 were used for the remaining steps according to the kit’s instruction with the following modifications. R&D Systems’ recombinant Dkk-1 and human Dkk-1 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-assay and interassay coefficient of variability (CV), dilutional linearity, spike recovery, and freeze-thaw stability. Intra-assay and interassay 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 from serum samples was about 80% for all assays. Serum Dkk-1 measurement was not affected after samples were subjected to five 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 method using a Meso Scale Discovery (MSD) system (Gaithersburg, MD). Samples were diluted in a phosphate-buffered saline (PBS) buffer containing 1% bovine serum albumin (BSA) to a final minimum required dilution (MRD) of 1:2 to 1:150 followed by an additional 10- to 4000-fold dilution to reduce background interference and to fall within the linear range of the assays (~5–130 ng/ml on assay plate). PF-04840082 calibration and quality-control (QC) standards were diluted into the same matrix composition as samples. Ninety-six-well MSD high bind plates 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 h. The plates were then washed with three wash cycles with PBS buffer containing 0.05% Tween 20, followed by incubation with a MSD ruthenylated goat anti-human IgG antibody for 1 h. After washing, MSD read buffer T (4×) with surfactant was added to each well and immediately 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 four-parameter fitting with 1/y^2 weighting in 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 LLOQ was 5 ng/ml on plate (i.e., 10 ng/ml at lowest MRD of 1/2). The performance of the assay was monitored by the inclusion of 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 CV of the QC samples was 2.8% (precision), and the mean relative error was 15.2% (accuracy). The mean CV 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 enzyme-linked immunosorbent assay method. Samples were diluted in a PBS buffer (containing 3% BSA and 0.05% Tween 20) to a final MRD of 1:4 to 1:100 followed by an additional 10- to 500-fold dilution to reduce background interference and fall within the linear range of the assays (~8–100 ng/ml on assay plate). PF-04840082 calibration and QC 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 h. The plates were then washed with three wash cycles, followed by incubation with biotinylated mouse anti-human IgG2 (Invitrogen, Carlsbad, CA) for 1 h. The plates were then washed with three wash cycles with PBS buffer containing 0.05% Tween 20. After washing, plates were developed by color reaction for 10 min with 3,3’,5’,5’-tetramethylbenzidine substrate then stopped with 2 M H2SO4. Blank matrix optical density values were background-subtracted after the absorbance optical density 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 by using four-parameter fitting with uniform weighting in SoftMax Pro 4.8 (Molecular Devices, Sunnyvale, CA). Serum concentrations of anti-Dkk-1 humanized antibody in unknown samples were interpolated from this standard curve.

The LLOQ was 8 ng/ml on plate (i.e., 22 ng/ml at lowest MRD of 1/4). The performance of the assay was monitored by the inclusion of QC samples prepared in control monkey serum. QC samples were prepared (n = 4) at...
Preclinical PK/PD Modeling of a Dkk-1 Antibody

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 CV of the QC samples was 2.6% (precision), and the mean relative error was 8.7% (accuracy). The mean CV of the dilution QC samples was 2.4% (precision), and the mean relative error was 5.8% (accuracy).

Antidrug Antibody Assay. The presence of antidrug antibodies (ADA) against PF-04840082 in rat and monkey was measured with a bridging ligand binding assay using the MSD platform. 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 h 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 h at room temperature. The plate was washed, and after addition of 150 μl of MSD read buffer (2×), the plate was read on a MSD Sector Imager 6000.

Noncompartmental Pharmacokinetic Analysis. Pharmacokinetic analysis was performed by using WinNonLin Enterprise Edition computer software, version 5.2 (Pharsight Corp., Cary, NC). The terminal elimination slope (λz) was determined by linear regression of the log plasma concentration time profile. The terminal elimination half-life was calculated from 0.693/λz.

Behavior. Antibody in the central compartment (volume V1) binds (rate constant, k12) and to ensure accurate estimation of model parameters. In the model and baseline is equal to the concentration of Dkk-1 (Baseline) 0 h. Variation of Dkk-1 concentrations in vehicle-treated monkeys was characterized by using a simple cosine function as described by (Chakraborty et al., 1999):

\[
k_{\text{in}} = (\text{Baseline} + \text{Amplitude} \times \cos(\text{Time}) - \text{PeakTime}) \times (\frac{2\pi}{\text{Period}}) \times k_{\text{el,target}}
\]

Variation of Dkk-1 concentrations in vehicle-treated monkeys was characterized by using a fourth-order polynomial model:

\[
k_{\text{in}} = (A \times \text{Time}^4 - B \times \text{Time}^3 + C \times \text{Time}^2 - D \times \text{Time} + \text{Baseline}) \times k_{\text{el,target}}
\]

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 predose (t = 0 h).

Dkk-1 concentrations below the LLOQ has been described previously (Beal, 2001). The system of differential equations was solved numerically with NONMEM software, version V (Icon Development Solutions, Ellicott City, MD), running in a DOS shell under Windows XP using CompQ Visual Fortran version 6.6 (Intel, Santa Clara, CA). 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, and 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) NOAEL in toxicology species, 2) MABEL using in vitro binding data and equilibrium-based calculations, and 3) MABEL calculated using a TMDD PK/PD model.

NOAEL. Based on FDA guidance (Food and Drug Administration, 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:

\[
\text{HEDmonkey} = \frac{\text{NOAEL}_\text{rat}}{3.1} \quad \text{and} \quad \text{HEDrat} = \frac{\text{NOAEL}_\text{rat}}{3.2}
\]

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

Equilibrium Calculations. A formula to calculate maximal receptor occupancy based on the $k_{\text{el}}$ of the mAb–target interaction was

\[
\frac{dC_{\text{target}}(nM)}{dt} = k_{\text{in}} - k_{\text{el,target}} \cdot C_{\text{target}} - k_{\text{off}} \cdot m\text{Ab}_{\text{serum}} \cdot C_{\text{target}} + k_{\text{off}} \cdot C_{\text{complex}} \quad (3)
\]

\[
\frac{dC_{\text{complex}}(nM)}{dt} = -k_{\text{off}} \cdot C_{\text{complex}} + k_{\text{on}} \cdot m\text{Ab}_{\text{serum}} \cdot C_{\text{target}} - k_{\text{calc,complex}} \cdot C_{\text{complex}} \quad (4)
\]

where $C_{\text{mAb}_{\text{serum}}}$ is equal to free concentrations of PF-04840082, $K_d = k_{\text{off}} \cdot k_{\text{on}} \cdot [\text{mAb}]_{\text{total}} = m\text{Ab}_{\text{serum}} + C_{\text{complex}}$ and $[\text{Target}]_{\text{total}} = C_{\text{target}} + C_{\text{complex}}$.
used to estimate MABEL (Duff, 2006). MABEL was defined as the dose that results in 10% peak RO.

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

(7)

**Human PK/PD Simulations Using the TMDD Model.** Simulations of Dkk-1 and PF-04840082 concentrations in humans were performed by using the TMDD model in Berkeley-Madonna (v8.3.9; University of California, Berkeley, CA) to predict MABEL and efficacious dose for the treatment of osteoporosis. Human model parameters were either taken from the literature (subcutaneous bioavailability, \(k_{\text{mAb}}\)), measured experimentally (\(k_{\text{on}}, k_{\text{off}}\)), or scaled from rat and monkey parameters via the principles of allometry (V1, \(k_\text{all mAb}, k_\text{all targets}, k_\text{all complex}\)).

Allometric extrapolations were achieved by using a simple power model of the form: \(Y = a \cdot \text{BW}^b\), where \(Y\) is the parameter of interest, \(\text{BW}\) 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 \(\delta\) was assumed to equal 1 (Wang et al., 2008).

MABEL was estimated be the dose that resulted in 10% reduction in Dkk-1 levels. Prior experiments in an ovariectomized 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 (\(K_d < 2, <30, \text{and} <100 \text{pM, respectively}\)). In most cases, on rates (\(k_{\text{on}}\)) were too fast and off rates (\(k_{\text{off}}\)) were too slow to be measured precisely by the Biacore instrumentation. PF-04840082 also binds to cynomolgus monkey Dkk-1, although \(K_d\) could not be determined.

**Dkk-1 Expression in Premenopausal, Postmenopausal, Osteopenic, and Osteoporotic Women**

The concentrations of Dkk-1 in serum samples from premenopausal (\(n = 50\)), postmenopausal (\(n = 50\)), osteopenic (\(n = 50\)) and osteoporotic (\(n = 50\)) women are shown in Table 1. There was no significant difference in Dkk-1 concentrations between premenopausal and postmenopausal 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 premenopausal women (mean 2.2 ng/ml) compared with 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 doses of PF-04840082 in osteoporotic women.

**Rat Pharmacokinetics and Pharmacodynamics**

The mean free PF-04840082 concentration versus time profiles after weekly intravenous administration to female Sprague-Dawley rats are shown in Fig. 2. Noncompartmental pharmacokinetic parameters are shown in Table 2. The pharmacokinetics of PF-04840082 were nonlinear across the dose range with supraproportional increases in AUC with dose up to 10 mg/kg. This indicates a higher rate

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Dkk-1 concentrations in premenopausal, postmenopausal, osteopenic, and osteoporotic female subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>(N)</td>
</tr>
<tr>
<td>----------</td>
<td>-----</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>50</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>50</td>
</tr>
<tr>
<td>Osteopenic</td>
<td>50</td>
</tr>
<tr>
<td>Osteoporotic</td>
<td>50</td>
</tr>
</tbody>
</table>

\(a\) A normal T-score is \(\geq -1.0\). Osteopenia is defined as a T-score of \(< -1.0\) and \(> -2.5\), and osteoporosis is defined as a T-score of \(< -2.5\) or lower.

\(b\) Values are reported as mean ± S.D.

[Fig. 2. Observed and model-predicted free PF-04840082 concentrations versus time after weekly intravenous administration of PF-04840082 to Sprague-Dawley rats. Symbols represent the mean observed data (± S.D.). Lines represent the predicted profiles from the model, except for the 0.1 mg/kg dose at time points after 3 h, where model predictions are represented by ○.]
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 by using a qualitative ADA assay, and data from these rats were removed from the analysis and the plots.

Table 2: Noncompartmental pharmacokinetic parameters for free PF-04840082 concentrations in Sprague-Dawley rats after intravenous administration of PF-04840082.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>AUC₀–168 h (µg · hr/ml)</th>
<th>Cₘ₃₃ (µg/ml)</th>
<th>Cₚₚ (µg/ml)</th>
<th>Cₘ₃₃ (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>0.205 ± 0.172</td>
</tr>
<tr>
<td>1</td>
<td>850 ± 88.8</td>
<td>24.6 ± 1.6</td>
<td>5.06 ± 0.528</td>
<td>3.87 ± 0.50</td>
</tr>
<tr>
<td>10</td>
<td>13,300 ± 1000</td>
<td>252 ± 29.7</td>
<td>79.0 ± 5.94</td>
<td>439 ± 116</td>
</tr>
<tr>
<td>100</td>
<td>141,000 ± 18,700</td>
<td>2660 ± 313</td>
<td>841 ± 110</td>
<td>439 ± 116</td>
</tr>
</tbody>
</table>

a Cₚₚ = AUC₀–inf /τₐ₀ of first dosing interval (not for the entire duration of study because of sparse sampling).

b Cₘ₃₃ = first dosing interval (0–168 h).

Fig. 3. Observed and model-predicted free Dkk-1 concentrations versus time after weekly intravenous administration of PF-04840082 to Sprague-Dawley rats. Symbols represent the mean observed data (± S.E.), and lines represent the predicted profiles from the model.
Mean free Dkk-1 concentrations in the same study are plotted in Fig. 3. Free Dkk-1 concentrations decreased rapidly after 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 Fig. 4, and noncompartmental pharmacokinetic parameters of PF-04840082 in cynomolgus monkey are shown in Table 3. The pharmacokinetics of PF-04840082 were nonlinear 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 to 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 after dose is likely to be caused by formation of anti-PF-04840082 antibod-

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**TABLE 3**

Mean noncompartmental pharmacokinetic parameters for free PF-04840082 concentrations in cynomolgus monkeys after single intravenous administration (n = 1/sex/dose group)

<table>
<thead>
<tr>
<th>Dose</th>
<th>AUC₀-tₜₐₙₜ</th>
<th>AUC₀-inf</th>
<th>CL</th>
<th>t₁/₂</th>
<th>C_max</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/kg</td>
<td>µg·hr/ml</td>
<td>µg·hr/ml</td>
<td>ml/day/kg</td>
<td>day</td>
<td>µg/ml</td>
</tr>
<tr>
<td>0.1</td>
<td>36.4</td>
<td>37.2ᵃ</td>
<td>64.6ᵇ</td>
<td>−1ᵇ</td>
<td>2.32</td>
</tr>
<tr>
<td>1</td>
<td>1720</td>
<td>1810</td>
<td>13.8</td>
<td>2.6, 4.4ᵇ</td>
<td>22.3</td>
</tr>
<tr>
<td>10</td>
<td>48,100</td>
<td>55,900</td>
<td>4.56</td>
<td>12.5</td>
<td>242</td>
</tr>
<tr>
<td>100</td>
<td>643,000</td>
<td>845,000</td>
<td>2.85</td>
<td>13.3</td>
<td>3040</td>
</tr>
</tbody>
</table>

ᵃ n = 1 because of AUC₀-inf > 120% AUC₀-tₜₐₙₜ.  
ᵇ Individual t₁/₂ values reported because of calculations from different time intervals.
ries, so these data were removed from the analysis and the plots. However, this could not be confirmed with a qualitative ADA assay.

Individual free Dkk-1 concentrations in the same study are plotted in Fig. 5. Free Dkk-1 concentration decreased rapidly after 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 (Fig. 1) in both the monkey and the rat.

![Fig. 5. Observed and model-predicted free Dkk-1 concentrations versus time after single intravenous administration of PF-04840082 to cynomolgus monkeys. Open symbols represent the observed data from female monkeys, and solid symbols represent the observed data from male monkeys. Solid lines represent the model predicted profiles for male monkeys, and dashed lines represent the model predicted profiles for female monkeys.](image-url)
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 Figs. 2 and 3, and in monkey they are shown in Figs. 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 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 that results in 10% peak RO. The MRSD for PF-04840082 using this method was estimated to be $1 \times 10^{-6}$ mg/kg (Fig. 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 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 Fig. 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.

Nonlinear 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 caused by TMDD and is shown in Fig. 7.

**Discussion**

**PK/PD Understanding of PF-04840082 in Rat and Monkey.** In the rat and monkey studies, PF-04840082 exhibited nonlinear pharmacokinetics with higher rates of clearance and shorter elimination half-life values at lower doses. This is often indicative of 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 because 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 (2001). This model accounts for specific and nonspecific distribution and elimination of the drug molecule and provides flexibility to account for target dynamics. In other cases, where PK and PD have been simul-

### 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; Allometric scaling from rat and monkey</td>
</tr>
<tr>
<td>mAb (nonspecific) elimination rate constant (day −1)</td>
<td>0.03</td>
<td>Tang et al., 2004; Hayashi et al., 2007; Allometric scaling from rat and monkey</td>
</tr>
<tr>
<td>Dkk-1 levels (ng/ml) in postmenopausal womena</td>
<td>2.9 ± 4.3</td>
<td></td>
</tr>
<tr>
<td>Dkk-1 levels (ng/ml) in osteoporosis patientsb</td>
<td>10.6 ± 6.5</td>
<td></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 (k_{on}, nM −1 day −1)</td>
<td>112.3</td>
<td>Human Biacore data</td>
</tr>
<tr>
<td>mAb-Dkk-1 dissociation rate (k_{off}, 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>

a Mean levels of Dkk-1 in postmenopausal women were used for MABEL predictions of starting dose in phase 1 clinical trials.
b Mean levels of Dkk-1 in osteoporosis patients were used for efficacious dose predictions.

Fig. 7. TMDD model-predicted free PF-04840082 concentrations (top) and predicted free Dkk-1 concentrations (as percentage of baseline levels; bottom) in humans after subcutaneous administration of PF-04840082 once a 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. The anticipated LLOQ of the PF-04840082 bioanalytical assay is shown to represent the futility of dosing lower than predicted MABEL (top).
taneously 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 after intravenous 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 (Pep-pard 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 (0.052 l/kg) but higher than plasma volume in rat (0.147 l/kg).

**Target Understanding.** The TMDD model has been used previously to describe the PK/PD relationship of antibodies in vivo and 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 affects the dose-response relationship to a lesser extent within the range relevant to most antibodies (Meno-Tetang and Lowe, 2005; Agoram, 2009). To make meaningful predictions of clinical doses of a Dkk-1 antibody, efforts were made to characterize target levels and turnover rates before 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 that indicate that Dkk-1 is a master regulator of bone remodeling (Diarra et al., 2007) and Dkk-1 baseline levels are approximately five times higher in disease state (ovariectomized) mice compared with healthy mice (M. 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).

**Target Turnover.** 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. Mono-Tetang and Lowe (2005) showed that IgE elimination half-life values range from 5 to 8 h in mouse to 2.7 days in human, 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 FIH doses, on the basis of PK/PD modeling, is essential (European Medicines Agency, 2007). A new parameter, 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 NOAEL in designing FIH dose levels of high-risk therapeutics in recent European regulatory guidance (European Medicines Agency, 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 that 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 known PK of monoclonal antibodies (eq. 7). This method predicts a starting dose using the MABEL principle, which is defined as the dose that 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 overpredict 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 (Fig. 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 FIH 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 that 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 postmenopausal 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 TMDM model was 0.008 mg/kg. This dose is associated with minimal antici-
ployed 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 more than 50% over the dosing interval is 3.74 mg/kg given subcutaneously once a month. Nonlinear PK is predicted in the clinic (Fig. 7), with PF-04840082 exhibiting a higher clearance and shorter half-life at lower doses because of TMDM. A PK/PD model-based approach to MABEL dose calculations was concluded to be more likely to be predictive for Dkk-1 because 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 mecha-
nistic TMDM 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.

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