Pharmacokinetic-Pharmacodynamic Modeling of Biomarker Response and Tumor Growth Inhibition to an Orally Available Heat Shock Protein 90 Inhibitor in a Human Tumor Xenograft Mouse Model

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ABSTRACT

PF04942847 [2-amino-4-{4-chloro-2-[2-(4-fluoro-1H-pyrazol-1-yl)ethoxy]-6-methylphenyl}-N-(2,2-difluoropropyl)-5,7-dihydro-6H-pyrrrolo[3,4-d]pyrimidine-6-carboxamide] was identified as an orally available, ATP-competitive, small-molecule inhibitor of heat shock protein 90 (HSP90). The objectives of the present study were: 1) to characterize the pharmacokinetic-pharmacodynamic relationship of the plasma concentrations of PF04942847 to the inhibition of HSP90-dependent protein kinase, AKT, as a biomarker and 2) to characterize the relationship of AKT degradation to tumor growth inhibition as a pharmacological response (antitumor efficacy). Athymic mice implanted with MDA-MB-231 human breast cancer cells were treated with PF04942847 once daily at doses selected to encompass ED50 values. Plasma concentrations of PF04942847 were adequately described by a two-compartment pharmacokinetic model. A time delay (hysteresis) was observed between the plasma concentrations of PF04942847 and AKT degradation; therefore, a link model was used to account for the hysteresis. The model reasonably fit the time courses of AKT degradation with the estimated EC50 of 18 ng/ml. For tumor growth inhibition, the signal transduction model reasonably fit the inhibition of individual tumor growth curves with the estimated EC50 of 7.3 ng/ml. Thus, the EC50 for AKT degradation approximately corresponded to the EC50 to EC80 for tumor growth inhibition, suggesting that 50% AKT degradation was required for significant antitumor efficacy (50–80%). The consistent relationship between AKT degradation and antitumor efficacy was also demonstrated by applying an integrated signal transduction model for linking AKT degradation to tumor growth inhibition. The present results will be helpful in determining the appropriate dosing regimen and guiding dose escalation to achieve efficacious systemic exposure in the clinic.

Introduction

The complexity of drug action and its therapeutic response can benefit from pharmacokinetic-pharmacodynamic (PKPD) modeling, which is increasingly being applied in drug discovery and development areas such as the selection of drug candidates with the most favorable PKPD properties and the prediction of exposure response in patients with the aim of optimizing the design of early clinical trials. The investigation of timing and interplay of PKPD relationships can be of great value in understanding drug action and finding a drug dosing regimen that results in optimal therapeutic outcome (Derendorf et al., 2000; Lesko et al., 2000; Chien et al., 2005;...
Workman et al., 2006; Cohen, 2008). The use of PKPD modeling in this context relies on the prediction of the time course of drug action in patients using nonclinical information, because nonclinical studies are useful alternatives for investigating PKPD relationships to get insight into the in vivo mechanism of drug action. The integration of PKPD modeling and simulation has provided valuable opportunities for accelerating the evaluation of new chemical entities in the clinic and in principle contribute to shortening the overall period of drug development process.

Heat shock protein 90 (HSP90) is a member of the molecular chaperone family and regulates the conformation, stability, and activity of numerous key signaling proteins, including steroid receptors (e.g., androgen and estrogen receptors), transcription factors (e.g., hypoxia-inducible factor 1α), and protein kinases (e.g., AKT, Cdk4, ErbB family, Raf-1). These client proteins are involved in a large variety of biological processes such as cell proliferation, cell transformation, and tumor progression. Thus, targeting HSP90 offers an opportunity for the inhibition of multiple pathways in human cancers (Whitesell and Lindquist, 2005; Mahalingam et al., 2009; Holzeiberlein et al., 2010; Trepel et al., 2010). A number of mutant oncoproteins such as mutant epidermal growth factor receptor, mutant B-Raf, and v-Src require HSP90 activity to maintain their stability and function, whereas their wild-type counterparts are not or only weakly dependent on HSP90 activity, suggesting that cancer cells greatly depend on HSP90 activity associated with a malignant overstressed condition and oncogene addiction (Whitesell et al., 1994; Shimamura et al., 2005; Grbovic et al., 2006; Mahalingam et al., 2009; Trepel et al., 2010). Accordingly, HSP90 inhibitors may have therapeutic advantages against cancer cells compared with normal cells.

Several natural products, such as geldanamycin and radicicol (Fig. 1), bind the amino-terminal ATP pocket of HSP90 to inhibit ATPase activity, which subsequently leads to client protein degradation mediated through the ubiquitin ligase machinery (Roe et al., 1999; Connell et al., 2001). Progress in the clinical evaluation of targeting HSP90 in cancer has also been evident in the last decade (Li et al., 2009; Trepel et al., 2010). The first HSP90 inhibitor, 17-allylamino-17-demethoxygeldanamycin (17-AAG) (one of the geldanamycin analogs), entered phase I clinical trials in 1999, and a second inhibitor, 17-dimethylamino-ethylamino-17-demethoxygeldanamycin (17-DMAG), another geldanamycin analog, entered clinical trials in 2004. Extensive efforts in drug discovery with rational drug design have successfully advanced more than 10 HSP90 inhibitors into clinical trials for patients with cancer. However, little detailed investigation of PKPD relationships of HSP90 inhibitors to their effects on downstream biomarkers and antitumor efficacy has been published to date. Reported PKPD results were limited largely to simple demonstrations of dose-dependent inhibition of HSP90 client proteins and/or tumor growth inhibition in human tumor xenograft models (Banerji et al., 2005; Massey et al., 2010; Oude Munnink et al., 2010; Sun et al., 2010; Tran et al., 2010). There was one notable study (Xu et al., 2003) showing physiologically based PKPD modeling of 17-AAG and its metabolite for biomarkers in tumor-bearing mice, but a relationship between the inhibitor concentrations or biomarkers versus antitumor efficacy was not addressed.

2-Amino-4-(4-chloro-2-(2-(4-fluoro-1H-pyrazol-1-yl)ethoxy)-6-methylphenyl)-N-(2,2-difluoropropyl)-5,7-dihydro-6H-pyrrolo[3,4-d]pyrimidine-6-carboxamide (PF04942847) (Fig. 1) is an orally available, ATP-competitive HSP90 inhibitor (Ki = 6 nM) that maintains potency toward HSP90 client protein degradation in various cellular assay systems with AKT IC50 values of 20 to 100 nM, which are comparable with 17-DMAG IC50 values (10–90 nM) (Mehta et al., 2011). The objectives of the present study were to characterize 1) the PKPD relationship of plasma concentrations of PF04942847 to AKT degradation in tumors (biomarker response) and 2) the relationship of AKT degradation to antitumor efficacy (pharmacological response) in athymic mice implanted with MDA-MB-231 human breast cancer cells. The extrapolation of the present PKPD relationships to patients by using the combination of in vitro and in vivo data and their model-based analysis can be particularly helpful in determining an appropriate dosing regimen in clinical studies and guiding dose escalation to achieve systemic exposure in patients.

**Materials and Methods**

**Chemicals.** PF04942847 (chemical purity >99%) and a structurally related in-house compound (internal standard for analysis) were synthesized by Pfizer Worldwide Research and Development (San Diego, CA) (Zehnder et al., 2011). All other commercially available reagents and solvents were of either analytical or high-performance liquid chromatography (LC) grade.

**In Vivo PKPD Study.** The experimental designs and methods of the in vitro and in vivo PKPD studies were previously reported in part (Mehta et al., 2011). In brief, 6- to 8-week-old nu/nu athymic female mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained in pressurized ventilated cages. Tumors were established by subcutaneously injecting 2 million MDA-MB-231 human breast cancer cells suspended in a 1:1 (v/v) mixture of Dulbecco’s modified Eagle’s medium and Matrigel Basement Membrane Matrix (BD Bioscience, San Jose, CA) into the flanks of mice (6–8 weeks old). Mice with established tumors of 100 to 200 mm3 for the tumor growth
inhibition study and tumors of 300 to 500 mm³ for the AKT degradation study were selected and randomized to groups approximately 15 days after inoculation. Three repeated oral-dose PKPD studies were conducted with PF04942847 in the MDA-MB-231 xenograft mouse model: study 1, AKT degradation in vivo at the dose of 30 mg/kg once daily for 7 days; study 2, AKT degradation in vivo at the doses of 1, 3, 10, and 50 mg/kg once daily for 4 days; and study 3, tumor growth inhibition at the doses of 1, 3, 10, and 25 mg/kg once daily for 18 days. Blood samples were collected by exsanguination via peri-orbital sinus or cardiac puncture to determine the plasma concentration of PF04942847. Resected tumors were snap-frozen and pulverized using a liquid nitrogen-cooled cryomortar and pestle, and protein lysates were generated (n = 3–4/time point). The level of total AKT protein was determined using LumixBio 100 technology (Luminex, Austin, TX). Tumor volume was also measured during the treatment period by using electronic Vernier calipers (Vernier Software and Technology, Beaverton, OR) and calculated as the product of its length × width² × π/6. Tumor growth inhibition in each treatment group of PF04942847 was calculated as 100 × (1 − ΔT/ΔC), where ΔT and ΔC are the differences in the tumor volumes between the first and last dosing days in the treatment and vehicle control groups, respectively. All of the procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996) and Pfizer Animal Care and Use Committee guidelines. More detailed information about the in vitro and in vivo PKPD studies will be published elsewhere (P. P. Mehta, P. Whalen, P.-P. Kung, S. Yamazaki, and M.-J. Yin, manuscript submitted for publication).

PF04942847 Analysis. Plasma concentrations of PF04942847 were determined by a LC-tandem mass spectrometry (MS/MS) method after protein precipitation of plasma samples. The LC-MS/MS system consisted of Shimadzu LC-10AD VP pumps (Shimadzu, Kyoto, Japan), a CTC PAL autosampler (LEAP Technologies, Carrboro, NC), and a Sciex API 4000 mass spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA) equipped with a turbo ion spray source. Chromatographic separation of the analytes was achieved using a Zorbax XDB-phenyl 5 μm, 2.1 × 50 mm column (Agilent Technologies, Santa Clara, CA) with a simple gradient. The analytes were eluted with mobile phases comprised of water with 0.1% formic acid (solvent A) and acetonitrile with 0.1% formic acid (solvent B) at a flow rate of 0.6 ml/min. The mass spectrometer was operated in the positive ionization mode using multiple reaction monitoring at specific precursor ion product ion transitions of m/z 510 → 415 for PF04942847 and m/z 353 → 249 for internal standard (structure analog of PF04942847), respectively. The total analytical run time was 3 min. Data were processed using Applied Biosystems/ MDS Sciex Analyst software (version 1.4.1). The calibration curve range was 0.25 to 500 ng/ml. The back-calculated calibration standard concentrations were within 15% of their theoretical concentrations with coefficients of variation of less than 15%. The precision and accuracy of the quality-control samples were within 15%.

In Vitro Plasma Protein Binding. The plasma-free fraction of PF04942847 was determined in mouse plasma at 0.05 to 1.5 μg/ml (0.1–3 μM) using the equilibrium dialysis technique described previously (Yamazaki et al., 2008). Pilot experiments revealed that 1) PF04942847 was stable in plasma and 2) protein binding reached equilibrium at 37°C for 6 h. After the incubation, aliquots of plasma and buffer samples were transferred to separate tubes containing either blank buffer or blank plasma, respectively. Samples were extracted with aliquots of acetonitrile/methanol mixture (1:1, v/v) containing the internal standard and analyzed by LC-MS/MSMS as described above. The free fraction in plasma (f₀) was calculated by the following equation:

\[ f₀ = \frac{C_{\text{buffer}}}{C_{\text{plasma}}} \]  

where \( C_{\text{buffer}} \) and \( C_{\text{plasma}} \) denote the concentrations of PF04942847 in buffer and plasma, respectively, after the incubation.

Pharmacokinetic Analysis. A naive-pooled pharmacokinetic analysis was used to determine the pharmacokinetic parameters of PF04942847 in mice. That is, all individual plasma concentrations of PF04942847 (one sample per animal) were pooled together at each dose for the pharmacokinetic analysis as if they came from a single individual (Sheiner, 1984). Pharmacokinetic parameter estimation was performed using a standard two-compartment linear model with a first-order absorption rate as implemented in NONMEM version VI (University of California, San Francisco, CA) (Beal and Sheiner, 1992). The subroutine ADVAN4 with TRANS4 was used for the NONMEM analysis. This PK model was parameterized using absorption rate constant (\( k_a \), 1/h), oral clearance (CL, l/h/kg), intercompartmental clearance (Q, l/h/kg), and oral volumes of distribution in the central compartment \( (V_1, l/kg) \) and peripheral compartment \( (V_p, l/kg) \). Residual variability was characterized by a proportional error model. Pharmacokinetic parameters obtained from the naive-pooled pharmacokinetic model, which provided a reasonable fit to the observed data, were used to simulate plasma concentrations as a function of time after oral dose administration to drive the time-dependent pharmacodynamic model.

PKPD Modeling. The AKT degradation in tumor to plasma concentration of PF04942847 was modeled using a link model (an effect-compartment model) (Sheiner et al., 1979). In brief, the effect site concentration of PF04942847 \( (C_e, \text{ng/ml}) \) was expressed by the following differential equation:

\[ \frac{dC_e}{dt} = k_{eo} \cdot (C_p - C_e) \]  

where \( k_{eo} \) is the rate constant (h⁻¹) for equilibration with the effect site, and \( C_p \) is the plasma concentration of PF04942847 (ng/ml).

In the link model, the following equation was used to determine EC50 (the concentration causing one-half maximum effect, \( E_{\text{max}} \)) for the AKT degradation (\( E = \text{AKT ratio to baseline} \)):

\[ E = E_0 \cdot \left(1 - \frac{E_{\text{max}} \cdot C_e}{EC_0 + C_e^2}\right)^{-1} \]  

where \( E_0 \) is the baseline of AKT response, and \( \gamma \) is the Hill coefficient.

This model parameterization ensures that \( E_{\text{max}} \) is estimated with units of fold change with respect to the baseline, \( E_0 \). This link PKPD model for AKT degradation (henceforth referred to as model I) was evaluated against the indirect response model (Daynaka et al., 1993; Jusko and Ko, 1994) and the transduction model (Sun and Jusko, 1998; Mager and Jusko, 2001) for goodness of fit to AKT data.

PKPD models for tumor growth inhibition require the establishment of a base model for tumor growth dynamics over time. In vivo tumor growth curves in xenograft models are known to follow exponential growth, at least in its early phases. Subsequently, the tumor volume follows a linear growth, eventually reaching a plateau (Gompertz, 1825; Bissey et al., 1996). In our approach, the individual tumor growth curves in the control group were first modeled by using a first-order growth rate with or without a logistic function that constrains the maximum tumor volume (Yang et al., 2010). Simple exponential growth model without a logistic function is defined as:

\[ \frac{dT}{dt} = k_{ng} \cdot T \]  

where \( T \) is tumor volume (mm³) and \( k_{ng} \) is the first-order net growth rate constant (h⁻¹).

In contrast to the simple exponential growth model, the logistic tumor growth model is defined as:

\[ \frac{dT}{dt} = k_{ng} \cdot T \cdot (1 - T/T_s) \]  

where \( T_s \) represents the maximum sustainable tumor volume (carrying capacity), which is assumed to be constant whereas the carrying capacity may change over time.
The ratio of $TT_{as}$ approaches zero when $T$ is relatively small, meaning the net growth rate is approximately first-order (i.e., exponential growth). The tumor growth thereafter approaches zero when the ratio of $TT_{as}$ reach unity. Thus, the logistic model is applicable when tumor growth starts to slow down at later growth stages. In the present study, the logistic model was used as the tumor growth model because the model fit the individual tumor growth curves of the control group better than the simple exponential tumor growth model (data not shown).

The response of tumor volume ($T$) to plasma concentration of PF04942847 ($C_p$) was then modeled using a signal transduction model where the drug first acts on a target receptor, which initiates an effect signal through a cascade of transit compartments (Lobo and Balthasar, 2002). The drug-initiated effect signal finally induces the death of a fraction of the cycling cell population. Model differential equations are as follows:

\[
\frac{dT}{dt} = g(T) - K_4 \cdot T
\]

\[
\frac{dK_1}{dt} = \left( \frac{k_{max} \cdot C}{K_{IC50} + C} - K_1 \right)/\tau
\]

\[
\frac{dK_2}{dt} = (K_1 - K_2)/\tau
\]

\[
\frac{dK_3}{dt} = (K_3 - K_4)/\tau
\]

where $g(T)$ is tumor growth function, $K_1$ to $K_4$ refer to the signal transit compartments, $k_{max}$ is the maximum cell-killing rate constant (h$^{-1}$), $K_{IC50}$ is the plasma concentration of PF04942847 (ng/ml) producing 50% of $k_{max}$, and $\tau$ is the mean transit time between the transit compartments (h). The initial conditions for $T$ and $K_1$-$K_4$ are the initial tumor volume and zero, respectively. This PKPD model (basic signal transduction model for tumor growth inhibition) is henceforth referred to as model II.

Because the AKT degradation was reasonably fitted by model I, in addition to model II, where plasma concentrations drive the effect signal, a signal transduction model with an effect compartment (transduction-link model) was applied to characterize the relationship between the plasma concentration of PF04942847 and antitumor efficacy. In this model, the equilibrium rate constant ($k_{eq}$) between $C_p$ and $C_{eq}$ obtained by model I was used to simulate the concentrations of PF04942847 in the effect compartment as a function of time after the oral administration based on the assumption that the drug distribution rate to the effect compartment (i.e., $k_{eq}$) was comparable between the AKT degradation and tumor growth inhibition. Differential equations of the transduction-link model for tumor growth inhibition (henceforth referred to as model III) are as follows:

\[
\frac{dT}{dt} = g(T) - K_4 \cdot T
\]

\[
\frac{dC_p}{dt} = k_{a0} \cdot (C_{eq} - C_p)
\]

\[
\frac{dK_1}{dt} = \left( \frac{k_{max} \cdot C}{K_{IC50} + C} - K_1 \right)/\tau
\]

\[
\frac{dK_2}{dt} = (K_1 - K_2)/\tau
\]

\[
\frac{dK_3}{dt} = (K_3 - K_4)/\tau
\]

\[
\frac{dK_4}{dt} = (K_4 - K_5)/\tau
\]

where, as before, $E$ is the AKT ratio to baseline and $KE_{IC50}$ corresponds to the AKT degradation index producing 50% of $k_{max}$.

All analyses were performed with NONMEM version VI and S-Plus 6.2 (Insightful Corporation, Seattle, WA). The NONMEM sub-routine ADVAN6 was used for model I, whereas the ADVAN8 was used for the other PKPD models. The initial conditions at time 0 for the absorption compartment, AKT degradation, and tumor volume were dose amount (mg/kg), mean AKT baseline ($E_{0} = 1$), and individual tumor volume (mm$^3$) on day 1, respectively. Residual variability was characterized by a proportional error model. In the exponential tumor growth model with a population analysis, an intramural variability for $k_{eq}$ was estimated using an exponential variance model. Model selection was based on a number of criteria such as the
NONMEM objective function value \( (\text{obj}) \), estimates, standard errors, and scientific plausibility as well as exploratory analysis of the goodness-of-fit plots. The difference in the \( \text{obj} \) value between two nested models was compared with a \( \chi^2 \) distribution in which a difference of 6.63 was considered significant at the 1% level (Wahlby et al., 2001).

### Results

**Pharmacokinetics.** The unbound fractions of PF04942847 in mouse plasma (mean 0.33) were concentration-independent at 0.05 to 1.5 \( \mu \)g/ml (0.1–3 \( \mu \)M). Plasma concentrations of PF04942847 at doses of 1 to 50 mg/kg in all studies were adequately described by a two-compartment model with the first-order absorption rate. The observed and model-fitted plasma concentrations of PF04942847 in athymic mice implanted with the human tumor xenografts after oral administration are shown in Fig. 3. Estimates for \( k_{\text{a}} \), \( CL \), \( V_1 \), \( Q \), and \( V_2 \) were 1.6 h\(^{-1} \), 15 l/h/kg, 29 l/kg, 23 l/h/kg, and 75 l/kg, respectively, at doses of 1 to 50 mg/kg (Table 1). The result suggests that oral exposures of PF04942847 increased dose-proportionally at the doses tested.

**PKPD Relationship.** In study 1, we determined the time course of AKT responses at the dose of 30 mg/kg once daily for 7 days. The AKT degradation on day 2 was 50 to 60% at 1 to 7 h postdose and slightly recovered to 40% at 24 h postdose. The AKT degradation on day 4 was almost constant during the dosing interval, i.e., approximately 60 to 70% inhibition at 1 to 24 h postdose. Thereafter the AKT degradation on days 6 and 7 was similar to that on day 4. Thus, the maximum plasma concentrations were observed at 1 h postdose with the apparent \( t_{1/2} \) of 2 to 7 h, whereas the maximum effect of AKT degradation was observed at 1 to 7 h postdose. Furthermore, AKT degradation seemed to reach the steady-state on day 4, whereas negligible accumulation in plasma concentrations of PF04942847 was observed during once-daily repeated doses. Therefore, it was apparent that there was a time delay (hysteresis) between the plasma concentrations of PF04942847 and the AKT response during repeated oral doses of PF04942847.

In study 2, we determined the AKT responses on day 4 at doses of 1, 3, 10, and 50 mg/kg to investigate the dose dependence of AKT degradation. At doses of 1 and 3 mg/kg, the degrees of AKT degradation were approximately 15 and 30%, respectively, at 4 h postdose and returned to the baseline level at 24 h postdose. The AKT degradation at the dose of 10 mg/kg was approximately 40% at 4 h postdose and 25% at 24 h postdose, whereas those at the dose of 50 mg/kg were 70 to 80% at 4 to 24 h postdose. In study 2, along with study 1, we compared the PKPD modeling results of the AKT degradation between the link model (model I), the indirect response model, and the transduction model. It was clear that the time course of AKT degradation in all groups were best described by the link model (\( \text{obj} = -360 \)). The predicted AKT degradation by both the indirect response and transduction models showed relatively steep up and down time courses, resulting in the poor fitting (\( \text{obj} = -287 \) to -292). The observed and model-fitted AKT degradation-time profiles by PF04942847 in the xenograft model are graphically presented in Fig. 4. Model I reasonably fit the time profiles of AKT degradation at all of the doses tested. The EC50 value was estimated to be 18 ng/ml (Table 2), which was equivalent to the free drug concentration of 12 nM.

Regarding tumor growth inhibition in the MDA-MB-231 xenograft model (study 3), some individual tumor growth curves in the control group seemed to reach the plateau phase on days 14 to 18. Thus, the logistic model fit the individual tumor growth curves better than the simple exponential model (\( \text{obj} = 758 \) versus 798). Accordingly, we applied the logistic model as the tumor growth model to model II. The tumor growth inhibition by PF04942847 was estimated directly from the measured tumor volumes to be 47, 64, 76, and 91% at doses of 1, 3, 10, and 30 mg/kg, respectively, on day 18 (the last dosing day). The observed and model-fitted tumor growth inhibition curves by model II are graphically presented in Fig. 5. Model II reasonably fit the individual tumor growth curves in all groups during the PF04942847 repeated-dose treatment with a correlation coefficient of 0.956 (Fig. 6A). The \( \text{obj} \) value was 3226 (Table 3). The \( k_{\text{max}} \) value was estimated to be 0.0066 h\(^{-1} \), indicating the maximum tumor growth inhibition was induced by the maximum drug plasma concentration of 12 nM.
inhibition by PF04942847 was approximately 0.86 (the ratio of $k_{\text{max}}$ to $k_{\text{hep}}$). The $K_{C50}$ was estimated to be 7.3 ng/ml (4.7 nM free). Thus, the $EC_{50}$ value (18 ng/ml) for the AKT degradation is within the range of the $EC_{50}$ to $EC_{90}$ values ($K_{C50}$ to $K_{C90} = 7$ to 30 ng/ml) for the tumor growth inhibition, suggesting 50% AKT degradation is required for 50 to 80% tumor growth inhibition. When the concentrations of PF04942847 in the effect site (i.e., $C_e$), which was simulated by model I (studies 1 and 2), were used to model tumor growth inhibition in model III (study 3), the model-fitted tumor growth curves were superimposable to those from model II, which used the plasma concentrations of PF04942847 (i.e., $C_p$) (Fig. 6B). The obj value was 3248 (Table 3), thus the use of model III did not result in a substantial improvement in goodness of fit. The $K_{C50}$ estimated by model III was 7.8 ng/ml, which was comparable with that from model II.

Furthermore, integrated modeling of the AKT and tumor growth inhibition was performed to further characterize the PKPD relationships of PF04942847 in the xenograft models (studies 1–3). The integrated model IV also reasonably fit the individual tumor growth curves in all groups. The relationship between the observed and predicted tumor volumes was almost superimposable to that by model II (Fig. 6C). The obj value was 3263 (Table 3). The $KE_{50}$ value was estimated to be 0.36, indicating $E$ of approximately 0.7 corresponded to be 50% of tumor growth inhibition. Relationships between the AKT degradation versus tumor cell-killing rate ($k$) or AKT degradation index ($1/E - 1$) are presented in Fig. 6D. These relationships suggest that approximately 30% of AKT degradation is required for 50% of tumor growth inhibition in the MDA-MB-231 xenograft model.

**Discussion**

The present study provides novel information on the PKPD relationship for PF04942847, an orally available HSP90 inhibitor, in a human tumor xenograft model. The pharmacodynamic biomarker response, measured as AKT degradation in tumors, was delayed relative to the plasma concentrations of PF04942847. Slow distribution of PF04942847 to tumors may be one of the reasons for this delayed response (hysteresis). This observation therefore positioned PF04942847 as an interesting compound for investigating the application of PKPD models that have been proposed to characterize the hysteresis. In the link model (model I) providing the best fit to the observed biomarker data, it is assumed that the rate of onset and offset of biomarker response is governed by the rate of drug distribution to and from a hypothetical “effect site.” However, many drugs show delayed response for other reasons, particularly because of indirect mechanisms of action such as stimulation or inhibition of formation ($k_{\text{in}}$) or loss ($k_{\text{out}}$) of substance controlling the physiological response (Daynkea et al., 1993; Jusko and Ko, 1994). The $k_{\text{in}}$ value (0.012 h$^{-1}$) for PF04942847 estimated by the link model was equivalent to a drug distribution half-life of approximately 60 h. Consistently, PF04942847 showed relatively large oral volumes of distribution (29 and 75 l/kg for $V_1$ and $V_2$, respectively) in the present xenograft mouse model. A large volume of distribution of PF04942847 was also observed in rats and dogs (8–10 l/kg) after a single intravenous administration (in-house data). PF04942847 was also distributed extensively into tumors of the MDA-MB-231 xenograft mouse model with a tumor/plasma area under the curve ratio of 6.8 after repeated oral administration; the observed steady-state half-lives in tumor (100–120 h) were >10-fold longer than those in plasma (5–10 h). Moreover, in the drug discovery process, we observed that the estimated volumes of distribution of potent HSP90 inhibitors in rodents were larger than those of less potent inhibitors when structurally similar compounds were compared. This observation suggests that large volumes of distribution of HSP90 inhibitors may be, in part, related to HSP90 binding because HSP90 is one of the most abundant proteins in unstressed eukaryotic cells, accounting for 1 to 2% of cytosolic protein (Parsell and Lindquist, 1993; Csermely et al., 1998). Collectively, these findings suggest that the main reason for the observed hysteresis is a rate-limiting distribution from plasma to the effect site, i.e., tumors. The factors controlling AKT levels might be of negligible importance to the observed hysteresis.

We further investigated the relationship between the AKT degradation and tumor growth inhibition by using model IV, which simultaneously accounted for AKT and tumor growth.
inhibition. Results suggest that approximately 30% of AKT degradation is required for 50% of tumor growth inhibition in the xenograft model (Table 3). For these quantities to be properly estimated by PKPD modeling, it is required that the model can in principle cover the full dynamic range of PD measurements. An alternative integrated tumor growth inhibition model using the percentage of biomarker inhibition (%I) as the forcing function has been reported by Wong et al. (2009) and Salpatti et al. (2010): $k = k_{\text{max}} \times \%I / (I_{50} \times \%I^*)$, where $k$ is tumor cell-killing rate constant (h^{-1}), $k_{\text{max}}$ is

TABLE 3
Pharmacodynamic parameter estimates of PF04942847 for three different tumor growth inhibition models in athymic mice bearing MDA-MB-231 tumors

Precision of the estimates is expressed as S.E. in parentheses.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Model II</th>
<th>Model III</th>
<th>Model IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{\text{ng,h}}$, h^{-1}</td>
<td>(7.8 \times 10^{-3} (4.0 \times 10^{-4})^a)</td>
<td>(7.8 \times 10^{-3} (4.0 \times 10^{-4})^a)</td>
<td>(7.8 \times 10^{-3} (4.0 \times 10^{-4})^a)</td>
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<td>$V_{\text{ss粉碎}}$, mm$^3$</td>
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<td>(2300 (200)^a)</td>
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<tr>
<td>$h_{\text{max}}$, h^{-1}</td>
<td>(6.6 \times 10^{-3} (4.9 \times 10^{-4}))</td>
<td>(6.6 \times 10^{-3} (4.9 \times 10^{-4})^b)</td>
<td>(6.6 \times 10^{-3} (4.9 \times 10^{-4})^b)</td>
</tr>
<tr>
<td>$K_{C_{\text{10}}}$, ng/ml</td>
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<td>(7.8 (2.5))</td>
<td>N.A.</td>
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<tr>
<td>$h_{\text{e0,b}}$, h^{-1}</td>
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<td>(0.012 (0.056)^c)</td>
<td>(0.012 (0.056)^c)</td>
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<tr>
<td>$K_{E_{50}}$</td>
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<td>(0.36 (0.12))</td>
<td>(0.36 (0.12))</td>
</tr>
<tr>
<td>$\tau$, h</td>
<td>(9.6 \times 10^{-4} (1.8 \times 10^{-5}))</td>
<td>(3.2 \times 10^{-3} (1.1 \times 10^{-5}))</td>
<td>(1.3 \times 10^{-3} (1.0 \times 10^{-5}))</td>
</tr>
<tr>
<td>obj</td>
<td>3226</td>
<td>3248</td>
<td>3263</td>
</tr>
</tbody>
</table>

N.A., not applicable.
a Estimated from individual tumor growth curves of vehicle-treated animals.
b Estimated by model II.
c Estimated by the link model (see Table 2).
the maximum tumor killing rate constant (h⁻¹), and \( K_{I_{50}} \) is defined as \%I where \( k \) is 50% of \( k_{\text{max}} \) in their model (henceforth referred to as model V). In the present study, model IV used an AKT degradation index \((1/E - 1)\) as the forcing function instead of \%I. These functions have different behaviors. The reason we used the inhibition index \((1/E - 1)\) was that model V might not cover the full dynamic range of tumor cell-killing rates. In a typical \( E_{\text{max}} \) model with \( C_p \), i.e., \( E = E_{\text{max}} \times C_p^\gamma / (E_{50}^\gamma + C_p^\gamma) \), \( E \) approaches \( E_{\text{max}} \) when \( C_p \) is much higher than \( E_{50} \) \((C_p \gg E_{50})\). Thus \( E \) ranges from zero to \( E_{\text{max}} \) with an increase in \( C_p \) values from zero to values much higher than \( E_{50} \). In contrast, \( k \) may not reach \( k_{\text{max}} \) in model V because both \%I and \( K_{I_{50}} \) range from 0 to 100%. This implies that \%I cannot be much higher than \( K_{I_{50}} \). Model V thus may not cover the full PD dynamic range. This relationship becomes apparent when the Hill coefficient in model V is fixed to unity (Salphati et al., 2010). The only case where \%I may not reach the full PD dynamic range is when the Hill coefficient is very large, resulting in an extremely steep response curve with the increase in \%I (Wong et al., 2009). However, when our inhibition index \((1/E - 1)\) is used in model IV, the full dynamic range of \( k \) can be covered as shown in Fig. 6D. The functional relationships between the ratio of effect to maximum effect versus inhibition ratio in the \( E_{\text{max}} \) model using the inhibition ratio or the inhibition index are graphically presented in Supplemental Fig. 1.

After all of this has been said, model IV also includes weaknesses that should be addressed. First, the \( K_{E_{50}} \) value is not as simple to interpret as \( E \) or \%I, whose value directly corresponds to 50% of \( k_{\text{max}} \). As a result, a back calculation from the estimated \( K_{E_{50}} \) to \( E \) is required to determine the \( E \) value producing 50% of \( k_{\text{max}} \). Second, because the reciprocal value of \( E \) is introduced as the inhibition index the model cannot handle 100% inhibition, i.e., \( E = 0 \). Furthermore, and perhaps more importantly, because HSP90 is a member of the molecular chaperone family that regulates the conformation, stability, and activity of numerous key signaling proteins, including kinases, the integrated tumor growth models used in the present study may not be exactly reflected by its biological mechanism of action. In other words, tumor growth inhibition by HSP90 inhibitor should be a result not only of the AKT degradation, but also of other biological responses related to HSP90 client proteins, e.g., Her2 inhibition, HSP70 induction, etc.

We analyzed tumor growth inhibition using the three different forcing functions in the models, \( C_p \) (model II), \( C_e \) (model III), or the inhibition index, i.e., \( 1/E - 1 \) (model IV). These three models showed almost superimposable relationships between observed and predicted individual tumor volumes (Fig. 6A–C), which might suggest that any one of these models could sufficiently characterize the existing PKPD relationship among drug concentration, biomarker response, and tumor growth inhibition. The comparison of the \( E_{50} \) values between model I (18 ng/ml) and model II (7.3 ng/ml) suggests that 50% of AKT degradation is required for significant antitumor efficacy (>50%). We have previously reported a similar approach for cMet kinase inhibitor, \((R)-3-[(2,6-dichloro-3-fluoro-phenyl)-ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)-pyridin-2-amine (PF02341066), where \( E_{50} \) for the inhibition of cMet phosphorylation corresponded to the \( E_{50} \) for tumor growth inhibition, suggesting that >90% inhibition of cMet phosphorylation is required for tumor growth inhibition by >50% (Yamazaki et al., 2008). We believe that the comparison of PD parameters estimated from the separate models should be a reasonable

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**Fig. 7.** PKPD modeling summary of AKT degradation and antitumor efficacy by PF04942847 in a human tumor xenograft model. \( C_p \), plasma concentration of PF04942847; \( C_e \), PF04942847 concentration in the effect compartment; \( C_{p,\text{ave}} \), average plasma concentration of PF04942847 during dosing interval; \( C_{e,\text{ave}} \), average concentration of PF04942847 in the effect compartment during dosing interval; \( E \), the ratio of AKT degradation to control value; \( T \), tumor volume (mm²); \( k \), tumor cell-killing rate (h⁻¹); \( 1/E - 1 \), the AKT degradation index.
approach to understanding the PKPD relationship, especially when biomarker response is directly linked to tumor growth estimation, e.g., for tyrosine kinase inhibitors. For PF04942847, however, a HSP90 inhibitor would be expected to cause tumor growth inhibition through a variety of additional HSP90-related biomarker responses. Therefore, more mechanistic PKPD models have the potential to be more useful for understanding the PKPD relationships; however, they would require more complex study design, e.g., multiple biomarker responses and intensive sampling at multiple doses in context to the relationship between biomarkers and the rate of each biosignal turnover. Despite these objective difficulties, the PKPD field is clearly moving toward more mechanistic modeling to have a deeper understanding of drug action and its therapeutic response.

In conclusion, the PKPD relationship among plasma concentrations of PF04942847, AKT degradation, and tumor growth inhibition in a human tumor xenograft model was well characterized in a quantitative manner using PKPD modeling (Fig. 7). The EC50 value (18 ng/ml) for the AKT degradation approximately corresponded to the EC90 to EC30 values (7–30 ng/ml) for the tumor growth inhibition in the MDA-MB-231 xenograft model, suggesting that 50% of AKT degradation was required for significant antitumor efficacy (50–80%). This relationship was further supported by an integrated model indicating that >30% of AKT degradation was required for >50% of tumor growth inhibition in the xenograft model. Thus, the EC50 value for the AKT degradation could be considered for setting minimum target efficacious concentrations in the clinic. The PKPD modeling results also provide insights in the factor that determines the time course of AKT degradation. That is, the distribution process of PF04942847 to target tissues could be a rate-limiting step in the pharmacodynamic process. Based on the present nonclinical PKPD modeling, an efficacious clinical dose of PF04942847 could be projected with accurately predicted or estimated pharmacokinetic parameters in patients by simulating target efficacious concentrations achieving more than 50% of AKT degradation. We believe that these PKPD results will be helpful in determining the appropriate dosing regimen and guiding dose escalation to achieve efficacious systemic exposure in the clinic.

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