TITLE: Mechanistic Understanding of Translational Pharmacokinetic-Pharmacodynamic Relationships in Nonclinical Tumor Models: A Case Study of Orally Available Novel Inhibitors of Anaplastic Lymphoma Kinase

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ABBREVIATIONS:

ALK, anaplastic lymphoma kinase; CL/F, oral clearance; Cp, plasma concentration; EC50, drug concentration causing 50% of maximum effect; E0, ALK phosphorylation baseline; Emax, maximum effect; EML4, echinoderm microtubule-associated protein-like 4; ka, absorption rate constant; KC50, the plasma concentration causing 50% of maximum effect; kin, zero-order formation rate constant, Kmax, maximal tumor killing rate constant; kmd, first-order rate degradation rate constant for a modulator; kng, net tumor growth rate constant; kout, first-order degradation rate constant; MET, hepatocyte growth factor receptor; NSCLC, non–small-cell lung cancer; OFV, objective function value; PF06463922, (10R)-7-amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(metheno)pyrazolo[4,3-h][2,5,11]benzoxazacyclotetradecine-3-carbonitrile; PF06471402, ((10R)-7-amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(azeno)pyrazolo[4,3-h][2,5,11]benzoxadiacyclo-
etradecine-3-carbonitrile); PKPD, pharmacokinetic-pharmacodynamic; Tss, maximum sustainable tumor volume; Tsc, tumor stasis concentration; V/F, oral volume of distribution.
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ABSTRACT

The orally available novel small molecules, PF06463922 and PF06471402, are second-generation anaplastic lymphoma kinase (ALK) inhibitors targeted to both naïve- and resistant-patients with non-small cell lung cancer (NSCLC) to the first-generation ALK inhibitor, crizotinib. The objectives of the present study were to characterize and compare pharmacokinetic-pharmacodynamic (PKPD) relationships of PF06463922 and PF06471402 for target modulation in tumor and antitumor efficacy in athymic mice implanted with H3122 NSCLC cells expressing a crizotinib-resistant EML4-ALK mutation (EML4-ALK<sup>L1196M</sup>). Furthermore, the PKPD relationships for these ALK inhibitors were evaluated and compared between oral administration and subcutaneous constant infusion, i.e., between different pharmacokinetic profiles. Oral and subcutaneous pharmacokinetic profiles of these ALK inhibitors were adequately described by a one-compartment pharmacokinetic model. An indirect response model extended with a modulator fit the time-courses of PF06463922- and PF06471402-mediated target modulation (i.e., ALK phosphorylation) with estimated unbound EC<sub>50,in vivo</sub> of 36 and 20 nM, respectively, for oral administration, and 100 and 69 nM, respectively, for subcutaneous infusion. A drug-disease model based on the turnover concept fit tumor growth curves inhibited by PF06463922 and PF06471402 with estimated unbound tumor stasis concentrations of 51 and 27 nM, respectively, for oral administration, and 116 and 70 nM, respectively, for subcutaneous infusion. Thus, the EC<sub>50,in vivo</sub> to EC<sub>60,in vivo</sub> estimates for ALK inhibition corresponded to the concentrations required tumor stasis in all cases, suggesting that the pharmacodynamic relationships of target modulation to antitumor efficacy were consistent among the ALK inhibitors, even
when the pharmacokinetic profiles with different administration routes were considerably different.
INTRODUCTION

Pharmacokinetic-pharmacodynamic (PKPD) modeling is a compelling mathematical approach to quantitatively characterize relationships between drug exposures and biological and/or pharmacological responses as a function of time. Dynamic PKPD modeling can therefore provide valuable insights into understanding in vivo drug action to accelerate evaluation of new molecular entities (Derendorf et al., 2000; Lesko et al., 2000; Chien et al., 2005; Cohen, 2008). Consequently, PKPD modeling is increasingly applied to decision-making processes in drug discovery and development. Recent rapid advances in a number of molecular profiling technologies have enhanced the development of personalized, molecularly targeted therapies based on individual genetic or protein profiles (Meric-Bernstam et al., 2013; Arnedos et al., 2014). Quantitative PKPD modeling is emerging as a valuable approach to translate in vivo drug potency from nonclinical models to patients, and ultimately develop successful molecularly targeted therapies (Meric-Bernstam et al., 2013; Yamazaki, 2013; Stroh et al., 2014).

As an example of personalized medicine, crizotinib (Xalkori®, PF02341066), an orally available potent inhibitor of multiple tyrosine kinases, such as anaplastic lymphoma kinase (ALK) and mesenchymal-epithelial transition factor (MET), was approved by the Food and Drug Administration (FDA) for the treatment of locally advanced or metastatic non–small cell lung cancer (NSCLC) patients who are positive for ALK rearrangement, e.g., echinoderm microtubule-associated protein-like 4 (EML4)-ALK, as detected by an FDA-approved test (FDA, 2014). We previously reported on the PKPD relationships of crizotinib exposure to inhibition of ALK- and MET-
phosphorylation and tumor growth inhibition in nonclinical xenograft models (Yamazaki et al., 2008; Yamazaki et al., 2012; Yamazaki, 2013). The quantitative PKPD modeling results suggested that 50% ALK inhibition corresponded to 50% tumor growth inhibition in an H3122 NSCLC model whereas 90% MET inhibition was required to achieve 50% tumor growth inhibition in a GTL16 gastric cancer model. Unfortunately, clinical responses to crizotinib have not been durable in some of NSCLC patients as tumor cells readily acquire drug resistance through multiple mechanisms, leading to the rapid development of second-generation ALK inhibitors for crizotinib-resistant cancer patients (Choi et al., 2010; Casaluce et al., 2013; Gridelli et al., 2014). A macrocyclic small molecule, PF06463922 (Fig. 1), has recently been identified as a second-generation ALK inhibitor against tumors expressing ALK rearrangements from both crizotinib-naïve and -resistant patients (Johnson et al., 2014). PF06463922 is highly potent against ALK phosphorylation in the cell-based assay (H3122 NSCLC cells expressing EML4-ALK fusion protein) with an $EC_{50,\text{in vitro}}$ of ~2 nM against wild-type EML4-ALK (without ALK mutations) and ~20 nM against one of the most frequently detected crizotinib-resistant EML4-ALK mutations, (i.e., EML4-ALK$^{L1196M}$). Quantitative PKPD modeling results of PF06463922 to inhibition of ALK phosphorylation and antitumor efficacy in an H3122 NSCLC tumor model with EML4-ALK$^{L1196M}$ have recently been reported (Yamazaki et al., 2014). The results demonstrated that $EC_{60,\text{in vivo}}$ for ALK inhibition corresponded to concentration required for tumor stasis ($T_{sc}$), suggesting that ~60% ALK inhibition would be required for tumor stasis (i.e., 100% tumor growth inhibition) in this nonclinical tumor model.
Other potent small molecule ALK inhibitors have also been identified along with PF06463922 in a drug discovery program (Johnson et al., 2014). Among these ALK inhibitors, PF06471402 is a structurally similar analog of PF06463922, differing in a core group between pyrazine and pyridine (Fig 1), while having different pharmacokinetics and pharmacodynamic characteristics. The \( E_{C50, \text{in vitro}} \) for PF06471402 against the wild-type EML4-ALK (~1 nM) and mutant EML4-ALK\(^{L1196M} \) (~6 nM) were ~3-fold lower than those for PF06463922 (Johnson et al., 2014). In vivo estimates for clearance and volume of distribution were 2 to 3-fold higher for PF06471402 relative to PF06463922 in nonclinical species. These novel ALK inhibitors can be positioned as interesting tool compounds to investigate mechanistic aspects of quantitative PKPD relationships. Given that crizotinib and PF06463922 were orally administered to nonclinical models (Yamazaki et al., 2008; Yamazaki et al., 2012; Yamazaki, 2013; Yamazaki et al., 2014), the impact of different pharmacokinetic profiles on the PKPD relationships, e.g., oral administration (clinical administration route) versus constant infusion, has not been investigated yet. The comparison of PKPD relationship arising from different pharmacokinetic profiles can be valuable for translational pharmacology, because pharmacokinetic profiles are generally different (in some cases, substantially different) between nonclinical models and patients. The objectives of the present study were to quantitatively characterize and compare how the systemic exposures of PF06463922 and PF06471402 related to inhibition of ALK phosphorylation in tumor and tumor growth inhibition in athymic mice implanted with H3122 NSCLC cells expressing crizotinib-resistant EML4-ALK\(^{L1196M} \). Furthermore, these mechanistic PKPD relationships were
compared when the pharmacokinetic profiles were different, i.e., oral administration versus subcutaneous constant infusion.
MATERIALS AND METHODS

Chemicals

PF06463922 ((10R)-7-amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(metheno)pyrazolo[4,3-h][2,5,11]benzoxadiazacyclotetradecine-3-carbonitrile), PF06471402 ((10R)-7-amino-12-fluoro-2,10,16-trimethyl-15-oxo-10,15,16,17-tetrahydro-2H-8,4-(azeno)pyrazolo[4,3-h][2,5,11]benzoxadiazacyclotetradecine-3-carbonitrile) and a structurally-related in house compound (internal standard for analysis) were synthesized by Pfizer Worldwide Research and Development (San Diego, CA) (Johnson et al., 2014). All other reagents and solvents were commercially available and were of either analytical or high performance liquid chromatography grade.

In Vivo PKPD Study

The experimental designs and methods of the in vitro and in vivo PKPD studies were previously reported in part (Zou et al., 2013; Yamazaki et al., 2014; Zou et al., 2014). Briefly, four separate PKPD studies were conducted with PF06463922 and PF06471402 in female athymic nu/nu mice implanted with subcutaneous xenografts of H3122 NSCLC cells expressing EML4-ALKL1196M (Table 1). For simplicity, the NSCLC xenograft model with EML4-ALKL1196M is henceforth referred to as an ALK-xenograft model. PF06463922 and PF06471402 were administered to animals orally twice daily, 7-hour apart (studies 1 and 3, respectively) or subcutaneously via ALZET osmotic pumps (Durect Co., Cupertino, CA) at the constant infusion rate of 0.5 μL/h (studies 2 and 4, respectively). Oral doses of PF06463922 and PF06471402 in acidified water were 0.6, 2, 6, 20 and 40 mg/kg/day twice daily up to 13 days (study 1) and 1.2, 4, 12, 40 and
60 mg/kg/day twice daily for 11 days (study 3), respectively. Subcutaneous daily infusion doses of PF06463922 and PF06471402 in the solution of DMSO, PEG400 and Cremophor ELP (45:40:15, v/v) were 0.5, 1.5, 5, 15 and 40 mg/kg/day for 13 days (study 2) and 0.5, 1.5, 5, 15 and 50 mg/kg/day for 12 days (study 4), respectively. The results from the repeated oral-dose studies of PF06463922 have been reported recently (Yamazaki et al., 2014): two separate repeated oral-dose PKPD studies in the previous report (i.e., 4- and 13-day repeated oral-dose studies) were combined in study 1 here. The intent of the previously reported results was to provide a head-to-head comparison between PF06463922 versus PF06471402. Animals were randomized into 6 groups on the 1st dosing day of each study. On the last dosing day, a subset of mice (n= 3/time point) was humanely euthanized at 1, 3, 7, 8 and 24 hours after the first daily dose in studies 1 and 3 or at approximate same time as the ALZET pump implantation in studies 2 and 4 to collect blood and tumor samples. Blood samples were collected by exsanguinations via cardiac puncture to determine plasma concentrations of PF06463922 and PF06471402. Blood samples were also collected from tail vein on days 1, 3 and 7 during the ALZET pump infusion (studies 2 and 4). In addition, a subset of mice (n= 3/time point) was humanely euthanized on day 7 in study 4 to collect blood and tumor samples. Resected tumors were snap-frozen and pulverized using liquid nitrogen-cooled cryomortar. Protein lysates were generated, and the level of total phosphorylated ALK protein (ALK phosphorylation) was determined using a capture ELISA method by using the PathScan® Phospho-ALK (Tyr1604) Chemiluminescent Sandwich ELISA Kit#7020 (Cell Signaling Technology, Danvers, MA) and PathScan® Total ALK Chemiluminescent Sandwich ELISA Kit#7084 (Cell Signaling Technology) according to
the manufacturer’s protocol (studies 1 and 2). The back-calculated calibration standard concentrations were within 15% of their theoretical concentrations with a few exceptions. Tumor volume was measured during the treatment period by electronic Vernier calipers and was calculated as the product of its length $\times$ width$^2 \times 0.4$. Tumor growth inhibition (%) in each PF06463922 and PF06471402 treatment group was calculated as $100 \times (1 - \Delta T/\Delta C)$, where $\Delta T$ and $\Delta C$ are the differences in the median tumor volumes between the first and last dosing days in the treatment and vehicle control groups, respectively. When calculated percent tumor growth inhibition was greater than 100%, tumor regression (%) was also calculated as $100 \times (\Delta T/T_{initial})$, where $T_{initial}$ is the median tumor volume on the first dosing day. All of the procedures were conducted in accordance with the Institute for Laboratory Animal Research Guide for the Care and Use of Laboratory Animals and with Pfizer Animal Care and Use Committee guidelines.

**Analyses of PF06463922 and PF06471402 in Plasma**

Plasma concentrations of PF06463922 and PF06471402 were quantitatively determined by a liquid-chromatography tandem mass spectrometry (LC-MS/MS) method after protein precipitation of plasma samples. The LC-MS/MS system consisted of Waters Acquity UPLC system (Waters, Milford, MA) and an API 5500 triple-stage quadrupole mass spectrometer (Applied Biosystems). Both instruments were controlled by Analyst 1.5.2 software (Applied Biosystems). Chromatographic separation of the analytes was achieved using a reverse phase column (Phenomenex Kinetex phenyl-hexyl, 50×2 mm 1.7 µm) at a flow rate of 0.5 mL/min. A binary mobile phase consisted of water with 0.1% formic acid (A) and acetonitrile with 0.1% formic acid (B). The gradient started at 5% B for 0.2 minutes, increased to 95% B over 1.3 minutes, and then
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held at 95% B for 0.5 minutes. The gradient was returned to the initial condition of 5% B in 0.1 minutes and equilibrated at 5% B for 0.5 minutes before the next injection. The mass spectrometer was operated in the positive ionization mode using multiple reaction monitoring (MRM) at specific precursor ion → product ion transition, \( m/z \) 407.3→228.0 for PF06463922, \( m/z \) 408.2→229.0 for PF06471402 and \( m/z \) 472.3→432.6 for the internal standard. The standard calibration curve was constructed using weighted \((1/x^2)\) linear regression. The calibration curve range was 0.5 to 5000 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%.

**Pharmacokinetic Data Analysis**

A naïve-pooled pharmacokinetic analysis was used to estimate pharmacokinetic parameters of PF06463922 and PF06471402 in ALK-xenograft models. Since full plasma concentration-time profiles in each animal were not available, all individual data at each dose were pooled together for pharmacokinetic analysis as if they came from a single animal (Beal and Sheiner, 1992). Pharmacokinetic analysis was performed with a standard one-compartment model as implemented in NONMEM® version 7.1.2 (University of California at San Francisco, San Francisco, CA) (Sheiner et al., 1979). The one-compartment pharmacokinetic model (subroutine ADVAN2 with TRANS2) was parameterized using absorption rate constant \( (k_{in}, \text{h}^{-1}) \), oral clearance \( (CL/F, \text{L/h/kg}) \) and oral volume of distribution \( (V/F, \text{L/kg}) \). The pharmacokinetic parameters were estimated together at all doses of each study since plasma concentrations of PF06463922 and
PKPD Modeling

**Target Modulation:** The ALK responses in the treatment group were expressed as the ratios to vehicle control animal data, meaning that the ratios of 1 and 0 represent 0 and 100% inhibition, respectively. In oral-dose studies 1 and 3, the ALK rebounds were observed at 24 hour post-dose, i.e., ALK phosphorylation ratios greater than unity (above baseline) in the treatment groups relative to the control group. As previously reported (Yamazaki et al., 2014), the inhibition of ALK phosphorylation in tumor to the plasma concentrations of ALK inhibitors was therefore modeled by an indirect response model with a hypothetical modulator (i.e., precursor model) to take account of the observed ALK rebounds (Jusko and Ko, 1994; Sharma et al., 1998).

The precursor model assumed that a modulator \( M \) was synthesized at a zero-order rate \( k_{in} \) and degraded at a first-order rate \( k_{md} \) while ALK phosphorylation was maintained by a balance of the first-order formation rate provided by the modulator degradation rate \( k_{md} \) and the ALK degradation rate \( k_{out} \). Accordingly, the following differential equations were used to estimate the \( EC_{50} \) required for ALK inhibition:

\[
\frac{dM}{dt} = k_{in} - k_{md} \cdot \left( 1 - \frac{E_{max} \times C_p}{EC_{50} + C_p} \right) \cdot M
\]

\[
\frac{dR}{dt} = k_{md} \cdot \left( 1 - \frac{E_{max} \times C_p}{EC_{50} + C_p} \right) \cdot M - k_{out} \cdot R
\]

where \( C_p \) is the plasma concentration of ALK inhibitors (ng/mL), \( E_{max} \) is maximal effect, \( EC_{50} \) is the plasma concentration of ALK inhibitors (ng/mL) causing one-half \( E_{max}, k_{in} \) is
the zero-order formation rate constant (h\(^{-1}\)), \(k_{mod}\) is the first-order formation rate for ALK phosphorylation provided by the modulator degradation rate (h\(^{-1}\)), \(k_{out}\) is the first-order degradation rate constant (h\(^{-1}\)) for ALK phosphorylation and \(\gamma\) is the Hill coefficient.

**Antitumor Efficacy:** To perform drug-disease modeling, antitumor efficacy to plasma concentration of ALK inhibitors was modeled by a modified indirect response model as reported previously (Yamazaki et al., 2014). Briefly, the individual tumor growth curves in the vehicle control group were first modeled by using a first-order net growth rate without (exponential growth) and with saturation at the maximal sustainable tumor volume (logistic growth). Maximal sustainable tumor volume was assumed to be constant, whereas tumor volume changed over time. The exponential and logistic tumor growth models are as follows:

\[
\frac{dT}{dt} = k_{ng} \cdot T
\]  \hspace{1cm} (3)

\[
\frac{dT}{dt} = k_{ng} \cdot T \cdot (1 - T/T_{ss})
\]  \hspace{1cm} (4)

where \(k_{ng}\), \(T\) and \(T_{ss}\) represent the first-order net growth rate constant (h\(^{-1}\)), tumor volume (mm\(^3\)) and the maximum sustainable tumor volume (mm\(^3\)), respectively.

The logistic tumor growth model is applicable if growth rate starts to slow down in the later stage of tumor growth. The model implies that the net tumor growth rate is roughly first-order (i.e., exponential growth) when \(T\) is relatively small, e.g., during the early stage of tumor growth, since the ratio of \(T/T_{ss}\) approximates near zero. Thereafter, the net tumor growth rate eventually becomes zero when the \(T/T_{ss}\) ratio approaches unity. The logistic model was used in all studies since the model provided better fits to the
individual tumor growth curves of the vehicle control groups compared to the exponential model.

Subsequently, the response of tumor volume ($T$) to plasma concentration of ALK inhibitors ($C_p$) was modeled based on the assumption that ALK inhibitors stimulated the tumor killing rate, thus inhibiting the tumor growth rate:

$$\frac{dT}{dt} = g(T) - \left( \frac{K_{\text{max}} \times C_p^\gamma}{KC_{50}^\gamma + C_p^\gamma} \right) \cdot T$$

where $g(T)$ is the tumor growth function characterized in the vehicle control group, $C_p$ is the plasma concentration of ALK inhibitors (ng/mL), $K_{\text{max}}$ is the maximal tumor killing rate constant (h$^{-1}$) caused by the ALK inhibitors, $KC_{50}$ is the plasma concentration of ALK inhibitors (ng/mL) corresponding to one-half $K_{\text{max}}$ and $\gamma$ is the Hill coefficient.

Tumor stasis concentration ($T_{sc}$), defined as the plasma concentration of ALK inhibitors required to maintain 100% tumor growth inhibition (i.e., zero net tumor growth rate), was calculated using equation 5 with the obtained pharmacodynamic parameter estimates, assuming zero tumor growth rate (i.e., $dT/dt = 0$ as $C_p = T_{sc}$).

**Data Analysis:** All PKPD modeling analyses were performed with NONMEM version 7.1.2 with the subroutine ADVAN8. The initial conditions at time zero for the GI tract compartment, ALK phosphorylation ratio and tumor volume were the dose amount (mg/kg), the ALK baseline ratio (i.e., unity) and the measured initial individual tumor volume (mm$^3$), respectively. Residual variability was characterized by a proportional error model. In the drug-disease model, an inter-animal variability on $k_{ng}$ was estimated by mixed-effect modeling using an exponential variance model. As is customarily done, model selection was based on a number of criteria such as the NONMEM objective.
function values (OFVs), estimates, their standard errors, overall biological and scientific plausibility, and exploratory analysis of standard goodness-of-fit plots. The difference in the OFVs 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 of PF06463922 and PF06471402

The observed and one-compartment model-fitted plasma concentrations of PF06463922 (studies 1 and 2) and PF06471402 (studies 3 and 4) are presented in Fig. 2. Overall, the plasma concentration-time profiles of PF06463922 and PF06471402 in each study were adequately described by the one-compartment model. Pharmacokinetic parameter estimates in each study are summarized in Table 2. The apparent $CL/F$ estimates for PF06463922 (0.84 to 1.1 L/h/kg) were lower than those for PF06471402 (1.7 to 3.3 L/h/kg) while the apparent $V/F$ estimates for PF06463922 (7.0 to 13 L/kg) were smaller than those for PF06471402 (14 to 32 L/kg). The $k_a$ estimates for PF06463922 and PF06471402 were larger for oral administration (1.3 and 7.6 h$^{-1}$, respectively) than subcutaneous infusion (0.062 and 0.036 h$^{-1}$, respectively). The standard errors of the pharmacokinetic parameter estimates in all studies were small (CV<30%). The typical pharmacokinetic parameters thus obtained were used to simulate plasma concentrations as a function of time following oral administration and subcutaneous infusion to drive the pharmacodynamic models for the PKPD analysis.

PKPD Modeling for Target Modulation

ALK phosphorylation was dose-dependently inhibited by PF06463922 and PF06471402 in oral-dose studies 1 and 3, respectively, while the ALK responses in all groups returned to near or above baseline at 24 hour post-dose. ALK phosphorylation was also dose-dependently inhibited by PF06463922 and PF06471402 during subcutaneous infusion in studies 2 and 4, respectively. The observed and precursor model-fitted ALK phosphorylation-time courses in studies 1 to 4 are shown in Fig. 3.
The precursor model reasonably fit both PF06463922- and PF06471402-mediated ALK inhibition including the recovery phase of ALK responses following oral administration in studies 1 and 3, respectively. The precursor model was also able to fit PF06463922- and PF06471402-mediated ALK inhibition in subcutaneous infusion studies 2 and 4, respectively. The \( EC_{50,\text{in vivo}} \) values of PF06463922 and PF06471402 in studies 1, 2, 3 and 4 were estimated to be 58, 162, 40 and 140 ng/mL, respectively (Table 3).

**Drug-Disease Modeling for Antitumor Efficacy**

Dose-dependent tumor growth inhibition by PF06463922 and PF06471402 was observed in all studies with tumor regression seen at the highest two to three doses. The observed percent tumor growth inhibition on the last dosing day was as follows: 57, 87, 101 (3% regression), 121 (63%) and 120% (66%) at the doses of 0.6, 2, 6, 20 and 40 mg/kg/day, respectively, in study 1; 32, 58, 98, 116 (35% regression) and 132% (50%) at the doses of 0.5, 1.5, 5, 15 and 40 mg/kg/day, respectively, in study 2; 60, 75, 109 (20% regression), 119 (37%) and 122% (48%) at the doses of 1.2, 4, 12, 40 and 60 mg/kg/day, respectively, in study 3; and 46, 77, 82, 107 (36% regression) and 109% (40%) at the doses of 0.5, 1.5, 5, 15 and 50 mg/kg/day, respectively, in study 4. The observed tumor volumes and model-fitted typical tumor growth inhibition curves by PF06463922 and PF06471402 in studies 1 to 4 are presented in Fig. 4. The drug-disease model reasonably fit the observed individual tumor volumes in all groups with \( KC_{50} \) estimates of 33, 151, 24 and 117 ng/mL in studies 1, 2, 3 and 4, respectively (Table 4). The estimated \( K_{\text{max}} \) (0.0091 to 0.023 h\(^{-1}\)) was 1.1 to 1.5-fold higher than the estimated \( k_{ng} \) (0.0073 to 0.0162 h\(^{-1}\)). The calculated tumor stasis concentrations (\( T_{sc} \)) from the
pharmacodynamic parameters obtained in studies 1, 2, 3 and 4 were 83, 188, 56 and 143 ng/mL, respectively.

**Quantitative Comparison of PKPD Relationships**

Systemic exposure-response curves of PF06463922 and PF06471402 for ALK and tumor growth inhibition based on the pharmacodynamic parameters (e.g., $EC_{50,\text{in vivo}}$ and $E_{\text{max}}$) obtained from ALK-xenograft models are graphically presented in Fig. 5. Since the model-predicted maximal antitumor efficacy by both PF06463922 and PF06471402 was tumor regression (i.e., >100% tumor growth inhibition), the tumor growth inhibition on the y-axis of Fig. 5 ranges from 0 to 120% while the range of ALK inhibition is 0 to 100%. In all studies, the plasma concentration-ALK response curves for PF06463922 and PF06471402 are shifted to the right compared to the tumor growth inhibition curves. The efficacious concentrations of PF06463922 and PF06471402, summarized as $EC_{50,\text{in vitro}}, EC_{50,\text{in vivo}}$ and $EC_{60,\text{in vivo}}$ for target modulation and $Tsc$ for antitumor efficacy, are shown in Table 5. These concentrations in vivo were converted from total (bound plus unbound) to unbound concentrations using the unbound fraction in mouse plasma (0.25 and 0.20 for PF06463922 and PF06471402, respectively). In oral-dose studies 1 and 3, the $EC_{50,\text{in vivo}}$ to $EC_{60,\text{in vivo}}$ ($EC_{50-60,\text{in vivo}}$) estimates for ALK inhibition (i.e., 36 to 52 nM free and 20 to 32 nM free, respectively) were comparable to the $Tsc$ (51 and 27 nM free, respectively). Similarly, the $EC_{50-60,\text{in vivo}}$ estimates for ALK inhibition in subcutaneous infusion studies 2 and 4 (i.e., 100 to 119 nM free and 69 to 96 nM free, respectively) were comparable to the $Tsc$ (116 and 70 nM free, respectively). Thus, the $EC_{50-60,\text{in vivo}}$ estimates for ALK inhibition consistently corresponded to the $Tsc$ estimates in all studies, despite the differences in the inhibitors, i.e., PF06463922 and
PF06471402, and their pharmacokinetic profiles, i.e., oral administration and subcutaneous infusion.
DISCUSSION

Using a quantitative mathematical modeling approach, the present study with the ALK inhibitors, PF06463922 and PF06471402, demonstrated a consistent in vivo pharmacodynamic relationship of target modulation (inhibition of ALK phosphorylation) to antitumor efficacy (tumor growth inhibition) as summarized in Table 5 and Fig. 5. That is, the $EC_{50-60,\text{in vivo}}$ estimates for both PF06463922- and PF06471402-mediated ALK inhibition consistently corresponded to the concentrations required for 100% tumor growth inhibition (estimated as $Tsc$). Furthermore, the pharmacodynamic relationships of these ALK inhibitors were also consistent between the different pharmacokinetic profiles, i.e., oral administration and subcutaneous constant infusion. Collectively, these findings suggested that ~60% ALK inhibition would be required for tumor stasis in ALK-xenograft models for any ALK inhibitor and any pharmacokinetic profile.

To characterize the inhibition of ALK phosphorylation, a precursor indirect response model was applied here based on a previous report (Yamazaki et al., 2014). The precursor model is based on the concept of turnover required to maintain ALK phosphorylation constant by a balance of formation and degradation rates. In addition, a hypothetical modulator was incorporated as a precursor to take account of the observed ALK rebounds (Sharma et al., 1998). The OFVs provided by the precursor models were smaller than those by the indirect response models (without a modulator) in all studies (Supplemental Table 1), indicating improved model performance. As shown in Supplemental Figure 1, the indirect response model was not able to account for the ALK rebound, particularly at 24 hours post-dose, in oral-dose studies 1 and 2. The indirect response model also tended to under-predict ALK inhibition on day 7 in subcutaneous
infusion study 4. Since the biological mechanism for ALK rebounds in an ALK-tumor model still remains unclear, several other feedback and pool models were applied to the present results (Gabrielsson and Weiner, 2000). None of these PKPD models except for the precursor model could acceptably fit the time-course of ALK responses in the present oral-dose studies.

To characterize tumor growth inhibition by ALK inhibitors, the drug-disease (tumor growth inhibition) model used in the present study was also based on the turnover concept. The model assumed that ALK inhibitors stimulated a tumor killing rate in turn inhibiting a net tumor growth rate ($k_{ng}$), supposedly maintained by a balance of formation and degradation rates. Thus, when an estimated maximal tumor killing rate ($K_{max}$) was larger than $k_{ng}$, a model-predicted maximal antitumor efficacy was greater than 100% tumor growth inhibition, i.e., tumor regression ensued. In all studies, the $K_{max}$ estimates were 1.1 to 1.5-fold larger than the $k_{ng}$ estimates (Table 4), suggesting that the model-predicted maximal antitumor efficacy of both PF06463922 and PF06471402 was tumor regression, as suggested by the observed results. In a previous report (Yamazaki et al., 2012), the observed maximal antitumor efficacy by the first-generation ALK inhibitor, crizotinib, was near tumor stasis in an H3122 wild-type xenograft model (without mutations) at the highest dose of 200 mg/kg tested, resulting in tumor stasis being the model-predicted maximal efficacy. Moreover, crizotinib failed to exhibit significant antitumor efficacy in an ALK-xenograft model with crizotinib-resistant mutant EML4-ALK$^{L1196M}$ at twice-daily oral doses of 150 mg/kg/day, which yielded unbound plasma concentrations in mice higher than in patients at the clinically recommended twice-daily oral doses of 250 mg (Zou et al., 2013). The second-generation ALK inhibitors,
PF06463922 and PF06471402, attained tumor regressions in the ALK-xenograft model with EML4-ALKL1196M. Therefore, we could expect that the second-generation ALK inhibitors would potentially have a better antitumor efficacy than crizotinib in the clinic, provided their unbound exposures reached efficacious levels.

When comparing in vivo potencies of the second-generation ALK inhibitors between oral administration and subcutaneous infusion, the $EC_{50,\text{in vivo}}$ estimates for PF06463922 and PF06471402 were 3 to 4-fold lower in oral-dose studies (36 and 20 nM, respectively) than in subcutaneous infusion studies (100 and 69 nM, respectively). These $EC_{50,\text{in vivo}}$ estimates were 2 to 15-fold higher than the corresponding $EC_{50,\text{in vitro}}$ values (Table 5). Establishing an in vitro-in vivo correlation of ALK inhibition potency would thus appear to be difficult, since the $EC_{50,\text{in vivo}}$ estimates depended upon the pharmacokinetic profiles following different administration routes. The $Tsc$ estimates for PF06463922 and PF06471402 in oral-dose studies (51 and 27 nM, respectively) were also lower than those estimated in subcutaneous infusion studies (116 and 70 nM, respectively). Thus, different ALK inhibitors with different pharmacokinetic profiles yielded different PKPD relationships from their pharmacokinetic profiles to target modulation (inhibition of ALK phosphorylation), as shown by the different $EC_{50,\text{in vivo}}$ estimates for ALK inhibition. These differences in $EC_{50,\text{in vivo}}$ estimates could ultimately result in the different $Tsc$ between the different ALK inhibitors and between the different pharmacokinetic profiles. The reason why oral administration was more effective on target modulation and antitumor efficacy than subcutaneous infusion remains unclear. One of the potential reasons could be due to the biological signaling mechanism and the degree of network feedback, as network signals can quickly undergo potential adaptive
changes (Riely et al., 2007; Soria et al., 2012; Rosell et al., 2013). In the present study, significant ALK inhibition (e.g., near 100% inhibition at the higher doses) was clearly achieved immediately after oral administration of PF06463922 and PF06471402, even though ALK rebounds were observed prior to each dose. To inhibit ALK phosphorylation significantly and effectively, ultimately leading to more robust antitumor efficacy, the maximal ALK inhibition corresponding to the maximal plasma concentrations after oral administration might be more relevant than the constant ALK inhibition achieved by maintaining steady-state plasma concentrations during subcutaneous infusion. Despite ALK responses having returned to near or above baseline prior to each dose, a clear dose-dependent tumor growth inhibition by PF06463922 and PF06471402 was consistently observed throughout the oral-dose treatment periods. A dose-dependent tumor growth inhibition by both inhibitors was also observed during subcutaneous infusion periods. These findings suggested that the effect of ALK rebounds on antitumor efficacy could be negligible in ALK-xenograft models.

To summarize, the PKPD relationships of systemic exposures of PF06463922 and PF06471402 to pharmacodynamic biomarker (i.e., inhibition of ALK phosphorylation in tumor) and pharmacological efficacy (i.e., tumor growth inhibition) in ALK-xenograft models were characterized in a quantitative manner using a mathematical modeling approach (Fig. 6). The present study demonstrated that the $EC_{50-60, in vivo}$ estimates for PF06463922- and PF06471402-mediated ALK inhibition consistently corresponded to the estimated $T_{sc}$ in all studies, suggesting that the mechanistic pharmacodynamic relationships could ultimately be consistent not only between different ALK inhibitors, but also between different pharmacokinetic profiles. Moreover, these findings suggest
that any differences in the characteristics of ALK inhibitors, such as physicochemical property, ALK inhibition potency and pharmacokinetic profiles, could have negligible effects on the pharmacodynamic relationships of target modulation to antitumor efficacy. Thus, a given target’s PKPD relationship could possibly be translatable from nonclinical models to patients and also patients to patients even if pharmacokinetic profiles were considerably different between nonclinical models and patients or among patients. Based on the PKPD results in oral-dose studies, we have previously proposed that the \( EC_{60,\text{in vivo}} \) estimates for ALK inhibition could be considered a minimal target efficacious concentration of PF06463922 in cancer patients with EML4-ALK rearrangements (Yamazaki et al., 2014). That being said, given the extent of target modulation differed between pharmacokinetic profiles, a study with one administration route might be an overly simplistic approach to project a target efficacious concentration of molecularly targeted agents (e.g., \( EC_{60,\text{in vivo}} \) for ALK inhibition corresponding to \( Tsc \)). As we have remarked earlier, the pharmacokinetic profiles could influence a degree of target modulation, resulting in the different \( EC_{50,\text{in vivo}} \) estimates; therefore, accounting for these effects on PKPD relationships via a quantitative dynamic modeling approach would be central to properly understand the PKPD relationships relevant to translational pharmacology.

Ideally, clinical PKPD relationships of antitumor agents’ exposures to target modulation (or its surrogate biomarker response) could be established in phase I trials, e.g., an expanded cohort with selected patients. However, it is generally difficult to obtain tumor biopsy samples from patients, especially serial samples of individual tumor to measure its time-courses. In fact, only 20% of ~2500 phase I trials submitted to the
American Society of Clinical Oncology incorporated biomarker assessments (Goulart et al., 2007). An alternative course of action would be a modeling approach to predict time-courses of target modulation in relation to the observed degree of antitumor efficacy in a patient population (based on nonclinical PKPD modeling results) as soon as pharmacokinetic profiles are available in the clinic. A clinically recommended dose for molecularly targeted agents should be established as a pharmacologically active dose based on an expected degree of target modulation in tumor (or surrogate biomarker response), rather than a maximal tolerated dose based on dose-limiting toxicity as traditionally conducted for cytotoxic agents. The pharmacologically active dose approach has been relatively rare in oncology field, but it has potential to maximize benefits for cancer patients and minimize possible risks of molecularly targeted agents (Plummer et al., 2008; Le Tourneau et al., 2009; Stroh et al., 2014). This approach would also be valuable to enable subsequent drug candidates (e.g., second-generation inhibitors) to conduct phase I trials safely and effectively. We believe that the consistent pharmacodynamic relationships demonstrated in the present study could contribute, in part, to making this approach successful in the clinic. We also believe that quantitative PKPD understanding would be a key asset for translational pharmacology and could ultimately increase the success rate of molecularly targeted therapies in the clinic.
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AUTHORSHIP CONTRIBUTION

Participated in research design: Lam, Wang, Yamazaki and Zou

Conduct experiments: Wang and Zou

Contributed new reagents or analytic tools: na

Performed data analysis: Lam, Vicini, Yamazaki and Zou

Wrote or contribute to the writing of the manuscript: Lam, Smeal, Vicini, Wang, Yamazaki and Zou
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FOOTNOTES

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LEGENDS FOR FIGURES

Fig. 1. Chemical structures of PF06463922 (R = CH) and PF06471402 (R = N).

Fig. 2. One-compartment model-fitted and observed plasma concentrations of PF06463922 (S1 and S2) and PF06471402 (S3 and S4) in athymic mice implanted with H3122 NSCLC cells expressing EML4-ALKL1196M following repeated oral administration (A) or subcutaneous infusion (B). Animals received twice-daily oral doses of PF06463922 at 0.6, 2, 6, 20 and 40 mg/kg/day for 13 days in study 1 (S1), subcutaneous infusion doses of PF06463922 at 0.5, 1.5, 5, 15 and 40 mg/kg/day for 13 days in study 2 (S2), twice-daily oral doses of PF06471402 at 1.2, 4, 12, 40 and 60 mg/kg/day for 11 days in study 3 (S3), and subcutaneous infusion doses of PF06471402 at 0.5, 1.5, 5, 15 and 50 mg/kg/day for 12 days in study 4 (S4). The x-axis represents the time after dosing on the last dosing day in hours (A) or the subcutaneous infusion period in days (B) and the y-axis represents the observed plasma concentrations of PF06463922 and PF06471402 (OBS) with the model-fitted individual (IPRED) and typical (PRED) profiles in nanograms per milliliter on a logarithmic scale.

Fig. 3. Precursor model-fitted and observed ALK inhibition by PF06463922 (S1 and S2) and PF06471402 (S3 and S4) in athymic mice implanted with H3122 NSCLC cells expressing EML4-ALKL1196M following repeated oral administration (A) or subcutaneous infusion (B). Animals received twice-daily oral doses of PF06463922 at 0.6, 2, 6, 20 and 40 mg/kg/day for 13 days in study 1 (S1), subcutaneous infusion doses of PF06463922 at 0.5, 1.5, 5, 15 and 40 mg/kg/day for 13 days in study 2 (S2), twice-
daily oral doses of PF06471402 at 1.2, 4, 12, 40 and 60 mg/kg/day for 11 days in study 3 (S3), and subcutaneous infusion doses of PF06471402 at 0.5, 1.5, 5, 15 and 50 mg/kg/day for 12 days in study 4 (S4). The x-axis represents the time after dosing on the last dosing day in hours (A) or the subcutaneous infusion period in days (B). The left side of the y-axis represents the observed (ALK OBS) and model-fitted (ALK PRED) ALK inhibition in the ratio to the mean value of control animal data, and the right side of y-axis represents the model-predicted plasma concentrations of PF06463922 and PF06471402 (CP PRED) in nanograms per milliliter on a logarithmic scale.

Fig. 4. Drug-disease model-fitted tumor growth curves and observed tumor volumes in athymic mice implanted with H3122 NSCLC cells expressing EML4-ALK<sup>L1196M</sup> during repeated oral administration (A) or subcutaneous infusion (B) of PF06463922 (S1 and S2) and PF06471402 (S3 and S4). Animals received twice-daily oral doses of PF06463922 at 0.6, 2, 6, 20 and 40 mg/kg/day for 13 days in study 1 (S1), subcutaneous infusion doses of PF06463922 at 0.5, 1.5, 5, 15 and 40 mg/kg/day for 13 days in study 2 (S2), twice-daily oral doses of PF06471402 at 1.2, 4, 12, 40 and 60 mg/kg/day for 11 days in study 3 (S3), and subcutaneous infusion doses of PF06471402 at 0.5, 1.5, 5, 15 and 50 mg/kg/day for 12 days in study 4 (S4). The x-axis represents the treatment period in days and the y-axis represents the model-fitted typical tumor growth curves (PRED) with the observed individual tumor volumes (OBS) in cubic millimeters.
Fig. 5. Comparison of plasma concentration-response curves of PF06463922 (A and B) and PF06471402 (C and D) for target modulation and antitumor efficacy in athymic mice implanted with H3122 NSCLC cells expressing EML4-ALK<sup>L1196M</sup>. Concentration-response curves for ALK inhibition and tumor growth inhibition were simulated at the concentration range of 1 to 10,000 ng/mL using the pharmacodynamic parameters obtained from ALK-xenograft models. PF06463922 and PF06471402 were orally administered to animals twice daily, 7-hour apart, in studies 1 (A) and 3 (C), respectively, or continuously administered to animals via subcutaneous ALZET osmotic pumps in studies 2 (B) and 4 (D), respectively. The estimated $EC_{50-60, \text{in vivo}}$ for ALK inhibition and tumor stasis concentration ($Tsc$) are indicated as red dashed line. The x-axis represents the plasma concentrations of PF06463922 or PF06471402 in nanograms per milliliter on a logarithmic scale, the left y-axis represents tumor growth inhibition (TGI) as percent inhibition and the right y-axis represents ALK inhibition (ALK) as percent inhibition.

Fig. 6. PKPD summary of ALK inhibitors in EML4-ALK-driven NCSLC xenograft models. $Cp$, plasma concentration; $t$, treatment period; TGI (%); percent of tumor growth inhibition; ALK (%), percent inhibition of ALK phosphorylation; $Tsc$, tumor stasis concentration.
<table>
<thead>
<tr>
<th>ALK Inhibitor</th>
<th>Study#</th>
<th>Dosing Route</th>
<th>Dosing Regimen</th>
<th>Dose Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF06463922</td>
<td>1</td>
<td>PO</td>
<td>twice daily, 7-h apart</td>
<td>0.6 – 40</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>SC</td>
<td>constant infusion</td>
<td>0.5 – 40</td>
</tr>
<tr>
<td>PF06471402</td>
<td>3</td>
<td>PO</td>
<td>twice daily, 7-h apart</td>
<td>1.2 – 60</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>SC</td>
<td>constant infusion</td>
<td>0.5 – 40</td>
</tr>
</tbody>
</table>

The results of study 1 are reported in the previous report (Yamazaki et al., 2014). PO, oral administration by gavage; SC, subcutaneous constant infusion via ALZET osmotic pumps at the infusion rate of 0.5 μL/h.
<table>
<thead>
<tr>
<th>ALK Inhibitor</th>
<th>Study</th>
<th>$CL/F$</th>
<th>$V/F$</th>
<th>$k_a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L/h/kg</td>
<td>L/kg</td>
<td>h⁻¹</td>
</tr>
<tr>
<td>PF06463922</td>
<td>1</td>
<td>1.1</td>
<td>7.0</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.2)</td>
<td>(0.9)</td>
<td>(0.1)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.84</td>
<td>13</td>
<td>0.062</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.04)</td>
<td>(1)</td>
<td>(0.006)</td>
</tr>
<tr>
<td>PF06471402</td>
<td>3</td>
<td>3.3</td>
<td>14</td>
<td>7.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.7)</td>
<td>(4)</td>
<td>(0.7)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>1.7</td>
<td>32</td>
<td>0.036</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.4)</td>
<td>(7)</td>
<td>(0.005)</td>
</tr>
</tbody>
</table>

Precision of the estimates is expressed as S.E. in parentheses. a The values in study 1 are cited from the previous report (Yamazaki et al., 2014). PF06463922 and PF06471402 were administered to animals orally twice daily, 7-hour apart (studies 1 and 3) or continuously via subcutaneous infusion with ALZET osmotic pumps (studies 2 and 4).
## TABLE 3
Pharmacodynamic parameter estimates of PF06463922 and PF06471402 for ALK phosphorylation by the precursor model in athymic mice implanted with H3122 NSCLC cells expressing EML4-ALK<sup>L1196M</sup>

<table>
<thead>
<tr>
<th>ALK Inhibitor</th>
<th>Study</th>
<th>$EC_{50}$ (ng/mL)</th>
<th>$k_{out}$ (h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>$k_{md}$ (h&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>$\gamma$</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF06463922</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>58</td>
<td>1.8</td>
<td>0.021</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14)</td>
<td>(0.4)</td>
<td>(0.003)</td>
<td>(0.07)</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>162</td>
<td>0.010</td>
<td>0.011</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(44)</td>
<td>(0.0003)</td>
<td>(0.003)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>PF06471402</td>
<td>3</td>
<td>40</td>
<td>5.0</td>
<td>0.012</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td>(0.1)</td>
<td>(0.001)</td>
<td>(0.01)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>140</td>
<td>0.040</td>
<td>0.0062</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(92)</td>
<td>(0.003)</td>
<td>(0.0015)</td>
<td>(0.3)</td>
</tr>
</tbody>
</table>

Precision of the estimates is expressed as S.E. in parentheses. $E_{max}$ was fixed at unity in all studies. <sup>a</sup>The values in study 1 are cited from the previous report (Yamazaki et al., 2014). PF06463922 and PF06471402 were administered to animals orally twice daily, 7-hour apart (studies 1 and 3) or continuously via subcutaneous infusion with ALZET osmotic pumps (studies 2 and 4).
### TABLE 4

Pharmacodynamic parameter estimates of PF06463922 and PF06471402 for tumor growth inhibition in athymic mice implanted with H3122 NSCLC cells expressing EML4-ALK<sup>L1196M</sup>

<table>
<thead>
<tr>
<th>ALK Inhibitor</th>
<th>Study</th>
<th>(KC_{50}) ng/mL</th>
<th>(K_{max}) h&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>(k_{ng}) h&lt;sup&gt;-1&lt;/sup&gt;</th>
<th>(T_{ss}) mm&lt;sup&gt;3&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>PF06463922</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33</td>
<td>0.011</td>
<td>0.0094</td>
<td>1530</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(14)</td>
<td>(0.001)</td>
<td>(0.0012)</td>
<td>(201)</td>
</tr>
<tr>
<td>PF06471402</td>
<td>2</td>
<td>151</td>
<td>0.011</td>
<td>0.0073</td>
<td>2140</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(48)</td>
<td>(0.001)</td>
<td>(0.0029)</td>
<td>(325)</td>
</tr>
<tr>
<td>PF06463922</td>
<td>3</td>
<td>24</td>
<td>0.0091</td>
<td>0.0084</td>
<td>1260</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(6)</td>
<td>(0.0005)</td>
<td>(0.0011)</td>
<td>(33)</td>
</tr>
<tr>
<td>PF06471402</td>
<td>4</td>
<td>117</td>
<td>0.023</td>
<td>0.0162</td>
<td>1190</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(25)</td>
<td>(0.001)</td>
<td>(0.0081)</td>
<td>(85)</td>
</tr>
</tbody>
</table>

Precision of the estimates is expressed as S.E. in parentheses. Hill coefficient (\(\gamma\)) was fixed at unity in all studies. <sup>a</sup> The values in study 1 are cited from the previous report (Yamazaki et al., 2014). PF06463922 and PF06471402 were administered to animals orally twice daily, 7-hour apart (studies 1 and 3) or continuously via subcutaneous infusion with ALZET osmotic pumps (studies 2 and 4).
## TABLE 5

Summary of pharmacodynamic parameter estimates for target modulation and antitumor efficacy by PF06463922 and PF06471402 in athymic mice implanted with H3122 NSCLC cells expressing EML4-ALK

<table>
<thead>
<tr>
<th>ALK Inhibitor</th>
<th>Study</th>
<th>Target Modulation</th>
<th>Antitumor Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$EC_{50, in vitro}$</td>
<td>$EC_{50, in vivo}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>nM free</td>
<td>nM free</td>
</tr>
<tr>
<td>PF06463922</td>
<td>1 $^a$</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>PF06471402</td>
<td>3</td>
<td>5.6</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td></td>
<td>69</td>
</tr>
</tbody>
</table>

$^a$ The values are cited from the previous report (Yamazaki et al., 2014). PF06463922 and PF06471402 were administered to animals orally twice daily, 7-hour apart (studies 1 and 3) or continuously via subcutaneous infusion with ALZET osmotic pumps (studies 2 and 4).
Figure 2: Graphs showing plasma concentration (ng/mL) over time after dosing (h) and treatment period (day) for different groups. Each group is represented by distinct line styles and markers, indicating observed (OBS), intermediate predicted (IPRED), and population predicted (PRED) concentrations.
**PKPD Study**

**Different ALK Inhibitors**
- Oral Dose (Twice Daily)
- ALK-driven NSCLC Xenograft Models
- Subcutaneous Constant Infusion

**Different PK Profiles**

**PKPD Outcome**

**Consistent PD Relationship**

\[ T_{sc} \approx ALK\ EC_{50-60} \]