Bridging the Gap between Preclinical and Clinical Studies Using Pharmacokinetic-Pharmacodynamic (PK-PD) Modeling: An Analysis of GDC-0973, a MEK Inhibitor

Harvey Wong1, Laurent Vernillet2, Amy Peterson3, Joseph A Ware2, Lillian Lee5, Jean-Francois Martini5, Peiwen Yu5, Congfen Li5, Geoffrey Del Rosario5, Edna F Choo1, Klaus P Hoeflich4, Yongchang Shi5, Blake T Aftab5, Ron Aoyama5, Scott Lam5, Marcia Belvin4 and John Prescott3

Departments of Drug Metabolism and Pharmacokinetics1, Clinical Pharmacology2, Exploratory Clinical Development3, Cell Signaling Pathways4, Genentech Inc, South San Francisco, CA, 94080 and Exelixis5, South San Francisco, CA,

RUNNING TITLE: PK-PD Analysis of a MEK Inhibitor

KEYWORDS: GDC-0973, MEK inhibitor, PK-PD, Pharmacodynamics, Xenografts

CORRESPONDING AUTHOR: Dr. Harvey Wong, Drug Metabolism and Pharmacokinetics, Genentech, Inc., 1 DNA Way, MS 412a, South San Francisco, CA, 94080. E-mail: wong.harvey@gene.com
STATEMENT OF TRANSLATIONAL RELEVANCE:

The current work applies PK-PD modeling to characterize tumor disposition and pharmacodynamics of a MEK inhibitor, GDC-0973, in preclinical tumor models. PK-PD modeling was used to translate preclinical tumor accumulation characteristics and identify a minimum target plasma concentration in patients. Predictions of active doses were based upon ≥ 80% suppression of the RAF/MEK/ERK pathway and compared well with reported clinical responses. Using an integrated PK-PD-efficacy model, the relationship between pathway modulation and efficacy was defined. Our analysis suggests a pathway suppression threshold beyond which anti-tumor activity switches on. Our observations are consistent with clinical data reported for PD-0325901, another MEK inhibitor, and vemurafenib, a B-RAF inhibitor, both of which target the RAF/MEK/ERK pathway. This work illustrates how PK-PD modeling can be used to improve the predictive value of preclinical data in the Phase 1 setting.
**ABSTRACT**

**Purpose:** GDC-0973 is a potent and selective MEK inhibitor. Pharmacokinetic-pharmacodynamic (PK-PD) modeling was used to relate GDC-0973 plasma and tumor concentrations, tumor pharmacodynamics (PD) and anti-tumor efficacy in order to establish pharmacokinetic endpoints and predict active doses in the clinic.

**Experimental Design:** A PK-PD model was used to characterize GDC-0973 tumor disposition and in vivo potency in WM-266-4 xenograft mice. Simulations were performed using the PK-PD model along with human pharmacokinetics to identify a target plasma concentration and predict active doses. In vivo potency and anti-tumor efficacy was characterized in A375 melanoma xenograft mice, and a population based integrated PK-PD-efficacy model was used to relate tumor PD (%pERK decrease) to anti-tumor activity.

**Results:** GDC-0973 showed a sustained tumor PD response due to longer residence in tumor than plasma. Following single doses of GDC-0973, estimated in vivo IC$_{50}$ of %pERK decrease based on tumor concentrations in xenograft mice was 0.78 (WM-266-4) and 0.52 µM (A375). Following multiple doses of GDC-0973, the estimated in vivo IC$_{50}$ in WM-266-4 increased (3.89 µM). Human simulations predicted a minimum target plasma concentration of 83 nM and an active dose range of 28-112 mg. The steep relationship between tumor PD (%pERK decrease) and anti-tumor efficacy suggests a pathway modulation threshold beyond which anti-tumor efficacy switches on.

**Conclusions:** Clinical observations of %pERK decrease and anti-tumor activity were consistent with model predictions. This manuscript illustrates how PK-PD modeling can improve the translation of preclinical data to man by providing a means to integrate preclinical and early clinical data.
INTRODUCTION

The RAF/MEK/ERK signaling pathway is highly conserved and plays an important role in cell proliferation, survival, migration, cell cycle regulation, and angiogenesis (1-4). Activating mutations in B-RAF have been frequently observed in several tumor types, including 50 to 70% of malignant melanomas, 30% of papillary thyroid cancer, and 10 to 15% of colorectal and ovarian cancers, among others (1,5-7). The majority of these mutations are in exon 15, which results in a Val600 Glu (V600E) amino acid substitution, leading to constitutive kinase activation (8). Cancer cells harboring the V600E B-RAF mutation have been shown to be particularly sensitive to inhibition of mitogen-activated protein kinase kinase (MEK) (9) making MEK an attractive target for small molecule inhibitors.

More than ten MEK inhibitors have been evaluated in the clinic (4). Despite the activity around this target and signs of clinical efficacy (10,11), none have yet been approved for clinical use. However, the recently approved B-RAF inhibitor, vemurafenib (PLX4032), has been reported to be very effective in melanoma patients with mutated B-RAF causing robust complete or partial tumor regression (12). The vemurafenib study highlights the importance of the RAF/MEK/ERK signaling pathway in cancers with B-RAF mutations. Therefore, it can be expected that there will be continued interest in MEK inhibitors as an alternative means of targeting the RAF/MEK/ERK pathway. Finally, MEK inhibitors given in combination with B-RAF inhibitors can provide a means to prevent the emergence of acquired drug resistance in tumors (13,14).

GDC-0973 (also known as XL518), (S)-[3,4-difluoro-2-(2-fluoro-4-iodophenylamino)phenyl][3-hydroxy-3-(piperidin-2-yl)azetidin-1-yl]methanone,
(Supplementary Figure S1) is a novel potent, selective MEK1 inhibitor with an IC$_{50}$ estimate of 4.2 nM in an in vitro biochemical assay against purified MEK1 enzyme (15). GDC-0973 showed >100 fold selectively for MEK1 over MEK2 and demonstrated no significant inhibition when tested against a panel of >100 of serine-threonine and tyrosine kinases. Preliminary studies performed in xenograft mice suggested that the duration of tumor phosphorylated ERK1/2 (pERK) inhibition, the predominant downstream measure of MEK inhibition, outlasted the presence of GDC-0973 in plasma, and was consistent with the residence time of GDC-0973 in tumors (data not shown). This observed disconnect between GDC-0973 plasma concentrations and tumor pharmacodynamic (PD) response suggested that additional investigation into the pharmacokinetic-pharmacodynamic relationship of GDC-0973 in tumor was warranted.

Preclinical pharmacokinetic-pharmacodynamic (PK-PD) modeling can play an important role in the drug discovery and development process by providing an integrated understanding of relationships between compound plasma concentrations, PD marker response, and efficacy. In addition, PK-PD modeling is a useful tool that can facilitate the translation of preclinical data to humans. The objectives of the current studies were 1) to characterize the tumor disposition and in vivo potency of GDC-0973 in xenograft mice, 2) to estimate the minimum target GDC-0973 plasma concentrations in humans, and 3) to characterize the relationship between RAF/MEK/ERK signaling pathway inhibition and anti-tumor efficacy. Our studies with GDC-0973 illustrate how the incorporation of early Phase 1 clinical data into existing preclinical PK-PD models can serve to not only improve our understanding of GDC-0973’s behavior in humans, but also help to prospectively predict responses in the clinic.
MATERIALS AND METHODS

GDC-0973 (also known as XL518; Figure S1) was provided by Exelixis (South San Francisco, CA, USA). All studies were performed using the di-hydrochloride salt of GDC-0973. Solvents used for analysis were of analytical or HPLC grade (Fisher Scientific, Pittsburgh, PA, USA). WM-266-4 and A375 human melanoma cells were purchased from ATCC (Manassas, VA, USA). All other reagents or material used in this study were purchased from Sigma-Aldrich (St Louis, MO) unless otherwise stated. In vivo studies were performed at Exelixis and the Exelixis Institutional Animal Care and Use Committee approved all procedures in animals.

IN VIVO PK-PD STUDY IN WM-266-4 XENOGRAFTS

Briefly, five million WM-266-4 melanoma cells were resuspended in Hank’s Balanced Salt Solution and implanted intradermally into the hind flank of female NCR nude mice (Taconic, Germantown, NY, USA). On day 11 or 13 after the implantation, xenograft mice with tumor volumes of approximately 100-120 mm$^3$ were randomly assigned to eight groups (n=27 per group), 4 single dose groups and 4 multiple dose groups. One day after randomization and group assignment, mice in the single dose groups were given a single oral dose of vehicle (water for injection USP), 1, 3 or 10 mg/kg of GDC-0973 (expressed as free base equivalents). Mice in the multiple dose groups were given daily oral doses of vehicle (water for injection USP), 1, 3 or 10 mg/kg of GDC-0973 for 14 days. Plasma and tumor samples (n=3 per timepoint) were collected from euthanized mice pre-dose and at 2, 4, 8, 16, 24, 72, 120, and 168 hours post-dose on Day 1 (single dose groups) or Day 14 (multiple dose groups). Samples were stored at -80°C until analysis. GDC-0973 concentrations in plasma and tumor lysates were determined using
LC/MS/MS. The dynamic range of the assay was 0.004 – 35 µM. ERK1/2 phosphorylation was determined by Western blot analysis.

**IN VIVO TARGET MODULATION IN A375 XENOGRAFTS**

A375 xenograft-bearing mice were established as described above for WM-266-4 xenograft-bearing mice. A375 xenograft-bearing mice were given a single oral dose of 0.3, 1, 3, 10 or 30 mg/kg of GDC-0973 (expressed as free base equivalents). Plasma and tumor GDC-0973 concentrations, and tumor pERK inhibition were assessed at 24 (all doses), 48 (10 and 30 mg/kg only), and 72 (10 and 30 mg/kg only) hours post-dose (n=5 animals per assessment). Quantitation of GDC-0973 concentrations in plasma and tumor, and tumor %pERK decrease was performed as described for the WM-266-4 studies.

Equation 4 was fit to GDC-0973 tumor concentrations and tumor %pERK decrease data from A375 xenografts using GraphPad Prism V4.02 (GraphPad Software Inc., San Diego, CA). The hill coefficient \((h)\) was fixed to 1 for this analysis. The use of this model (Equation 4) is explained in more detail in the Pharmacokinetic-Pharmacodynamic (PK-PD) Modeling Section below.

**IN VIVO EFFICACY STUDY WITH A375 XENOGRAFTS**

The GDC-0973 in vivo efficacy study was performed with A375 xenograft-bearing mice. Tumor volumes were measured in two dimensions (length and width) using Ultra Cal-IV calipers (Model 54-10-111, Fred V. Fowler Company, Inc., Newton, MA). The following formula was used with Excel v11.2 (Microsoft Corporation, Redmond, WA) to calculate tumor volume (TV): 

\[
TV \ (\text{mm}^3) = (\text{length} \times \text{width}^2) \times 0.5
\]

Tumors were
monitored until they reached a mean volume of approximately 100-120 mm$^3$ at which time the mice were assigned to each dosing group (n=10 animals per group) such that mean tumor volume was similar for each group. Mice in each group received oral doses of either vehicle (water for injection USP) once daily (QD), 0.3 mg/kg QD, 1 mg/kg QD, 3 mg/kg QD, 10 mg/kg QD, 30 mg/kg every second day (Q2D), or 30 mg/kg every third day (Q3D) of GDC-0973 free base equivalents for 14 days. Tumor sizes and body weights were recorded twice weekly, and the mice were regularly observed over the course of the study. Mice were euthanized if their tumor volume exceeded 2000 mm$^3$ or if their body weight dropped by more than 20% of the starting weight.

PHARMACOKINETIC-PHARMACODYNAMIC (PK-PD) MODELING

WM-266-4 Xenograft PK-PD Model:

PK-PD modeling for the WM-266-4 xenograft studies was performed using SAAM II (Saam Institute, University of Washington, Seattle, WA). Briefly, equations 1-3 and Figure 1A describe the PK model that was simultaneously fit to mean plasma and tumor GDC-0973 concentrations from single and multiple dose WM-266-4 xenograft mice studies. Equations 1-3 are described as follows:

$$\frac{dX_0}{dt} = -k_a X_0 \quad \text{(Equation 1)}$$

$$\frac{dC_p}{dt} = k_a X_0 + Cl_{tp} C_t - Cl_{pt} C_p - CIC_p \quad \text{(Equation 2)}$$

$$\frac{dC_t}{dt} = Cl_{pt} C_p - Cl_{tp} C_t \quad \text{(Equation 3)}$$
\( X_0 \) (\( \mu \text{mole} \)) is the amount of GDC-0973 in the oral compartment, \( t \) (day) is time, \( k_a \) (day\(^{-1}\)) is the absorption rate constant, \( C_p \) (\( \mu \text{M} \)) is the plasma GDC-0973 concentration, \( C_t \) (\( \mu \text{M} \)) is the tumor GDC-0973 concentration, \( Cl \) (L/day/kg) is the plasma clearance, \( Cl_{pt} \) (L/day/kg) is the inter-compartmental clearance from the plasma to the tumor compartment and \( Cl_{tp} \) (L/day/kg) is the inter-compartmental clearance from tumor to plasma compartment.

The pharmacodynamic effect (%pERK decrease) was related to GDC-0973 tumor concentrations using the following equation.

\[
\% \text{pERK}_{\text{decrease}} = \frac{I_{\text{max}} \times C_t^h}{IC_{50}^h + C_t^h}
\]  

(Equation 4)

\( \% \text{pERK}_{\text{decrease}} \) (%) is the percent decrease of \( pERK \), \( I_{\text{max}} \) (%) is the maximum % decrease of \( pERK \), \( h \) is the hill coefficient, and \( IC_{50} \) (\( \mu \text{M} \)) is the tumor concentration at which the \( \% \text{pERK}_{\text{decrease}} \) is \( 1/2 \) of \( I_{\text{max}} \). Parameter estimates from the PK model described by Equations 1-3 were fixed prior to estimating the pharmacodynamic parameters shown in Equation 4. The PK-PD model (Equations 1-4 and Figure 1B) was fit to \%pERK decrease data from single and multiple dose studies separately as there were apparent changes in the tumor \%pERK response to GDC-0973 following multiple days of dosing. PK and PD parameters estimates are presented as the estimate followed by the \%SE in parentheses. Finally, the rationale for using the PK-PD model described by Equations 1-4 and Figure 1B stemmed from observations from preliminary studies that the duration of
tumor %pERK decrease was substantially longer than the presence of GDC-0973 in plasma and was consistent with the longer residence time of GDC-0973 in tumors.

**Human Simulations:**

Patients in the Phase 1 clinical trial were given daily oral doses of GDC-0973 continuously for 21 days over a 28 day cycle (16). Mean GDC-0973 concentration-time data were obtained from three patients from cohort 1 that were given 0.05 mg/kg daily oral doses of GDC-0973 (16). In these 3 patients, plasma samples for pharmacokinetic evaluation were collected on day 1 and day 21 at predose, 0.5, 1, 2, 4, 8, 24, 48 (day 21 only) and 192 (day 21 only) hours postdose. The WM-266-4 PK model accounting for tumor disposition described by Equations 1-3 and Figure 1A was fit to mean human GDC-0973 concentration-time data in order to estimate $Cl$, $k_a$, and $V/F$ (apparent volume of distribution in L/kg). Tumor disposition was assumed to be similar between humans and WM-266-4 xenografts, thus $Cl_{tp}$ and $Cl_{pt}$ were fixed during the PK model fitting process. Human PK parameters are presented as the estimate followed by the %SE in parentheses. Human PK parameter estimates along with PD parameters ($I_{max}$, $IC_{50}$ and $h$) from the single and multiple dose WM-266-4 xenografts studies were used in the PK-PD model (Figure 1B) to perform human simulations in order to provide a range of response.

**Integrated PK-PD-Efficacy Modeling:**

In order to relate pathway modulation (expressed as tumor %pERK decrease) to anti-tumor effect, an integrated population based PK-PD-efficacy model (Figure 1C) was used
to fit individual longitudinal tumor data from A375 melanoma xenograft efficacy studies. The following equations describe the model used.

\[
\frac{d(TV)}{dt} = k_{ng}(TV) - K(TV) \tag{Equation 5}
\]

where

\[
K = \frac{K_{\text{max}} \times (\text{%pERK}_{\text{decrease}})^n}{K(\%I)_{50} + (\text{%pERK}_{\text{decrease}})^n}
\]

\(TV\ (\text{mm}^3)\) is defined as the tumor volume, \(k_{ng}\ (\text{day}^{-1})\) is the net growth rate constant, \(K\ (\text{day}^{-1})\) is the rate constant describing the anti-tumor effect of GDC-0973, \(K_{\text{max}}\ (\text{day}^{-1})\) is the apparent maximum value of \(K\), \(K(\%I)_{50}\) is defined as the \%pERK decrease where \(K\) is 50\% of \(K_{\text{max}}\), and \(n\) is the hill coefficient.

Briefly, \%pERK decrease in tumor was simulated for all dose levels and schedules of the A375 xenograft efficacy study during fitting process using the WM-266-4 xenograft PK-PD model and estimated PD parameters from the in vivo target modulation studies with A375 xenografts (see Figure 4A). The S-ADAPT program, an augmented version of ADAPT II with population analysis capabilities (17,18) was used to fit individual tumor volumes from all dose levels of the A375 xenograft efficacy study simultaneously. Inter-subject variability was assumed to be log-normally distributed and fitted using an exponential variance model. Residual variability error was modeled using a proportional-additive error model. In cases where inter-subject variance was small and could not be estimated reliably, the inter-subject variance was fixed to 0.00001.

Population PD parameter estimates are presented as the estimate followed by the \%SE in parentheses.
RESULTS

STUDIES IN WM-266-4 XENOGRAFTS

PK-PD model adequately characterizes GDC-0973 tumor accumulation and tumor PD response in WM-266-4 xenograft mice

The objective of this study was to characterize the concentration of GDC-0973 in plasma and tumor tissue, the PD response in tumor tissue, and the associated PK-PD relationships over time. WM-266-4 melanoma cells are PTEN deficient and harbor a V600D B-RAF mutation conferring activation of the RAF/MEK/ERK pathway that is functionally similar to the V600E B-RAF mutation (5). WM-266-4 xenografts were chosen for this study because they are moderately responsive to MEK inhibition allowing for enough tumor tissue to remain after 14 days of dosing to evaluate both GDC-0973 concentrations and PD response in tumor. Little or no tumor tissue remains following 14 days of dosing in xenograft mice bearing more sensitive melanoma tumors such as A375. The plasma and tumor concentration-time profiles and tumor %pERK decrease for the WM-266-4 xenografts given a single or multiple daily oral doses of 1, 3, and 10 mg/kg of GDC-0973 are presented in Figure 2A and 2B, respectively. Following single oral doses, GDC-0973 concentrations and PD response is dose dependent (Figure 2A; Figure S2). GDC-0973 concentrations in tumor tissue are both higher and more sustained compared to plasma. PD response mirrored tumor concentrations with %pERK decrease in tumor observed well beyond the timeframe that GDC-0973 was observed in plasma. Overall, a similar situation was observed following fourteen days of daily GDC-0973 dosing. However, despite higher GDC-0973 concentrations in plasma and tumor due to drug
accumulation compared to the single dose study, the magnitude of tumor \( \%pERK \) decrease was less at later timepoints (Figure 2B).

Figure 2C and D show plots of observed vs predicted plasma and tumor GDC-0973 concentrations, respectively, following simultaneous fitting of the PK Model (Figure 1A) to single and multiple dose PK data from WM-266-4 xenografts. Estimated pharmacokinetic parameters are presented in Table 1. The higher and sustained tumor concentrations observed in WM-266-4 xenografts was reflected in the estimate of inter-compartmental clearance from the plasma to tumor compartment \( (Cl_{pt}) \) being approximately 40 fold higher than the inter-compartmental clearance from the tumor to plasma compartment \( (Cl_{tp}) \). Pharmacodynamic parameters describing pERK reduction were also estimated separately for single and multiple dose experiments using the PK-PD model (Figure 1B). Plots showing observed and model-predicted \( \%pERK \) decrease in tumor are presented in Figure 2E and F for single and multiple dose studies, respectively. No evidence of hysteresis indicative of time-delays in onset of PD response was observed in these plots. Associated pharmacodynamic parameter estimates for single and multiple dose studies are presented in Table 1. Based upon the pharmacodynamic parameters presented in Table 1, there appeared to be a shift in the in vivo potency of GDC-0973 following multiple days of dosing with the \( \%pERK \) decrease IC\(_{50}\) estimate increasing from 0.78 to 3.89 \( \mu \)M. Pharmacokinetic and pharmacodynamic parameters were estimated with good precision with percent standard error for all parameters shown in Table 1 being < 30\%. Overall, the proposed PK-PD model adequately characterized the plasma and tumor concentrations, and tumor pERK inhibition in WM-266-4 xenografts.
Human simulations identify minimum GDC-0973 target plasma concentrations and predict tumor PD response and active doses

One of the primary objectives of the WM-266-4 xenograft studies was to characterize the relationship between plasma concentrations and tumor PD modulation in order to identify a minimum target plasma concentration required in patients. Early reports of the Phase 1 trial for the MEK inhibitor PD-0325901 suggested a trend towards progression free survival at \( \%pERK \) decreases of \( >60\% \) (19). In addition, signs of activity were observed in the Phase 1 clinical trial for CI-1040, a first generation MEK1/2 inhibitor, at doses associated with a median tumor \( \%pERK \) decrease of 73\% (20). Based on these data, we set \( \geq 80\%\) pERK decrease at steady-state as our target for tumor PD modulation.

The PK model used to characterize tumor disposition in WM-266-4 xenograft mice (Figure 1A) was fit to mean GDC-0973 plasma-concentration time data from the first cohort of patients on the GDC-0973 Phase I clinical trial (16). An assumption was made that tumor disposition is similar in humans and xenograft-bearing mice; therefore, \( \text{Cl}_{tp} \) and \( \text{Cl}_{pt} \) were fixed to estimates from WM-266-4 xenograft mice during the fitting process. The estimated human PK parameters are presented in Supplementary Table S1, and the plot of observed vs predicted GDC-0973 plasma concentrations following the model fitting is shown in Supplementary Figure S3. Simulations were performed using human PK and the PD parameters from the WM-266-4 single dose study. The PD parameters from the single dose WM-266-4 study were chosen for this simulation since we wanted to identify the minimum target plasma concentration, and the in vivo IC\textsubscript{50} following multiple doses of GDC-0973 was approximately 5-fold higher (see Table 1). In addition, the in vivo IC\textsubscript{50} estimate from the single dose WM-266-4 study was
comparable to the in vivo IC_{50} estimate from V600E B-RAF mutant A375 melanoma xenograft mice (see below). Based on the human simulation shown in Figure 3A, the minimum GDC-0973 plasma concentration at trough (24 hours post-dose) required for ≥ 80% tumor pERK decrease at steady-state is 83 nM. No adjustments were made to account for potential differences in free drug concentrations since there is no evidence of GDC-0973 protein binding differences in mouse and human plasma (both ~ 95% bound, data not shown).

In order to assess the cohort at which the target of ≥ 80% tumor pERK decrease could be achieved, we performed prospective human simulations of the Phase 1 clinical trial. Figure 3B and 3C show prospective human trial simulations mimicking the planned doses in the Phase I clinical trial. Since we would not know the B-RAF mutation status in patients during the dose escalation portion of the Phase 1 clinical trial, we performed these prospective human simulations using PD parameters from both the single and multiple dose GDC-0973 studies in WM-266-4 xenograft mice in order to provide a range of doses where we might see anti-tumor activity. Based on our simulations, the goal of reducing pERK by ≥ 80% could be achieved at a daily oral dose of 28 mg (0.4 mg/kg; shown in Figure 3B) to 112 mg (1.6 mg/kg; shown in Figure 3C) of GDC-0973 in cohorts 4 to 6 of the clinical trial.

During the course of the Phase 1 clinical trial, tumor biopsies were collected for biomarker assessments. In order to test the performance of our PK-PD model, we performed a retrospective simulation to compare the modeled decrease in tumor pERK with the observed IHC-assessed decrease in one patient from the 0.2 mg/kg dose cohort for whom we had evaluable pre- and post-dosing matched tumor biopsies. Two tumor
biopsies were collected from this patient at 4 hours post-dose on day 20 of dosing.

Human simulations at the same dose (0.2 mg/kg) using PD parameters from both single and multiple dose WM-266-4 studies resulted in a mean tumor pERK decrease of 51% (29% using multiple dose PD parameters and 73% using single dose PD parameters) comparable the observed mean decrease of 41% (29% and 52% in independent metastatic lesions, unpublished data).

**STUDIES IN A375 XENOGRAFTS**

**Relationship between tumor %pERK decrease and GDC-0973 tumor concentrations in A375 xenograft mice**

In order to understand the relationship between GDC-0973 concentration and PD response in an additional B-RAF mutant xenograft tumor, equation 4 was fit to GDC-0973 tumor concentration and tumor pERK data from A375 xenograft-bearing mice. A375 melanoma cells harbor the B-RAF V600E mutation conferring activation of the RAF/MEK/ERK pathway (5, 21). This V600E mutant melanoma cell line is relevant for understanding the effect of MEK inhibition on human melanoma patients with this common B-RAF mutation. The in vivo IC$_{50}$ estimated in A375 xenograft mice (0.52 µM; see Figure 4A) was comparable to the in vivo IC$_{50}$ estimated from the single dose WM-266-4 xenograft study (i.e. 0.78 µM, see Table 1 and Figure 2E).

**GDC-0973 shows dose dependent anti-tumor efficacy in A375 xenograft mice**

The objective of the A375 xenograft efficacy study was to characterize the anti-tumor activity of GDC-0973 in xenograft mice bearing melanoma tumors with the V600E B-
RAF mutation. GDC-0973 showed dose-dependent inhibition of tumor growth following oral administration over a broad dose range (0.3 to 30 mg/kg) and using varying schedules (QD, Q2D and Q3D) (see Figure 4B). All three schedules provided significant anti-tumor activity in this model, consistent with published reports for GDC-0973 (15).

**Integrated PK-PD-Efficacy Analysis in A375 Xenografts**

An integrated PK-PD Efficacy analysis was performed on the A375 xenograft efficacy study in order to understand the relationship between modulation of the RAF/MEK/ERK signaling pathway and anti-tumor efficacy in a B-RAF V600E melanoma tumor model. All 70 xenograft mice (10 per dose group) were included in the analysis. The process is described by Figure 4C and utilizes the integrated PK-PD-efficacy model shown in Figure 1C. In brief, a PK-PD model (Figure 1B) built using the PK parameters from WM-266-4 xenografts (Table 1) and PD parameters from A375 xenografts (Figure 4A) was used to simulate tumor %pERK decreases for all dosing regimens in the A375 xenograft efficacy study. Individual longitudinal tumor volume data from all A375 xenograft-bearing mice were fit simultaneously to an integrated PK-PD efficacy model (Figure 1C) which involved the addition of an indirect response model (Equation 5), where tumor growth inhibition is driven by tumor %pERK decrease, to the PK-PD model described. Figure 4D is a plot of the individual observed versus individual predicted tumor volumes of the resulting population model fit suggesting that the model adequately captured the growth characteristics of the tumor data from the efficacy study. Estimated PD parameters from this integrated PK-PD-efficacy analysis are presented in Table 2. PD parameters along with the inter- and intra-individual variability were estimated with
acceptable precision with % standard error of all estimates being ≤ 38%. The maximum anti-tumor effect (\(K_{\text{max}}\)) was approximately 2 fold larger than the estimated net growth rate constant (\(k_{\text{ng}}\)) consistent with regression of A375 xenograft tumors in response to GDC-0973 treatment.

Using the estimated PD parameters, a plot of the relationship between RAF/MEK/ERK pathway knockdown (i.e. %pERK decrease) and efficacy (i.e. \(K\) - rate constant describing the anti-tumor effect of GDC-0973) was generated and is shown in Figure 4E. The steep relationship between pathway inhibition and efficacy is characterized by a hill slope (\(n\)) of approximately 11 (Figure 4E, Table 2) and suggests that there is a pathway suppression threshold beyond which anti-tumor activity “switches on”. In addition, the plot shown in Figure 4E suggests that for maximal anti-tumor activity, > 60-70% %pERK decrease is required in A375 xenografts.
DISCUSSION

GDC-0973 is a potent and selective MEK1 inhibitor that is currently in Phase I clinical trials as a potential anti-tumor agent. The current study shows that GDC-0973 causes potent inhibition of phosphorylated ERK in two preclinical models of B-RAF mutant melanoma, WM-266-4 and A375 xenograft mice. A375 xenografts bear melanoma tumors that harbor a V600E B-RAF mutation that is sensitive to MEK inhibition and represents a common mutation in human melanoma patients (5,21). In contrast, WM-266-4 (V600D B-RAF mutation and PTEN deficient) xenografts are only moderately responsive to MEK inhibition but allow for sufficient tumor tissue after multiple days of GDC-0973 dosing required to characterize GDC-0973 tumor disposition. Our studies with WM-266-4 xenografts show that GDC-0973 is not only present at higher concentrations in tumor relative to plasma, but also resides in the tumor for a longer duration. In vivo IC$_{50}$s based on GDC-0973 concentrations in tumor tissue (A375 and WM-266-4 xenografts) ranged from 0.52 to 3.89 µM, and the pharmacodynamic response was shown to be directly correlated with tumor concentrations in both xenograft models examined. These data are consistent with our observations from preliminary studies in other xenograft mice (data not shown).

Mechanistic PK-PD modeling serves as an important tool to bridge the gap between preclinical and clinical data (22-24). Our studies used WM-266-4 xenograft-bearing mice as an experimental system to characterize the GDC-0973 concentration in tumor tissue over time. The incorporation of human pharmacokinetics into the PK-PD model built using WM-266-4 xenograft mice studies allows for normalization of known species.
differences in pharmacokinetics (25). We chose a sustained pERK reduction target of ≥80% at steady-state as the PD endpoint for these simulations based on early published reports on MEK inhibitors in the clinic (19,20). Our simulations using this “human PK-PD model” identified a minimum plasma concentration of 83 nM where we anticipated ≥80% pERK decrease would be maintained at steady-state in tumor tissues. Prospective simulations of a human dose escalation mimicking the Phase 1 trial design predicted that doses of 28 to 112 mg once daily would be sufficient to meet this target. Anti-tumor activity in melanoma patients with B-RAF mutations has now been reported at daily doses of 60 and 100 mg in the clinic (16), consistent with predictions based on the described PK-PD model.

One interesting observation was that the tumor GDC-0973 concentration required to reduce pERK in WM-266-4 tumor tissue increased during the two week dosing period. The reasons for this shift in sensitivity over time are not known, but could be explained by subpopulations of less sensitive tumor cells escaping MEK inhibition, or the emergence of resistant cells in response to continuous MEK inhibition. Both mechanisms of resistance have been reported for B-RAF and MEK inhibitors (26-28).

As a final part of our analysis, we wanted to quantify the relationship between RAF/MEK/ERK pathway modulation (%pERK decrease) and tumor growth inhibition through the use of an integrated PK-PD-efficacy model (Figure 1C). A “pathway modulation-response curve” showing the relationship between %pERK decrease and K (a measure of anti-tumor effect of GDC-0973) was sigmoidal and described by a Hill
coefficient of ~11 (Figure 4E). This steep relationship between pathway modulation and anti-tumor effect is suggestive of a threshold of pERK inhibition beyond which anti-tumor activity “turns on”. The observed “switch-like” behavior is consistent with reports of ultrasensitive stimulus-response curves observed for mitogen-activated protein kinase cascades (29). “Switch-like” behavior has also been recently reported in similar analyses performed with other kinase inhibitors such as GDC-0879, a B-RAF kinase inhibitor (30) and GDC-0834, a Bruton’s Tyrosine Kinase inhibitor (31), and the hedgehog signaling pathway inhibitor, vismodegib (32).

Robust clinical activity has been reported for vemurafenib (PLX4032) in B-RAF mutant melanoma patients (12,33). Interestingly, no clinical responses were reported in melanoma patients with cytoplasmic reductions of pERK in tumor of less than ~60%, while pERK reductions were typically >80% in patients that responded to vemurafenib (33). Similarly, Phase 1 data for PD-0325901, a MEK inhibitor, showed signs of clinical activity in B-RAF V600E melanoma patients at pERK decreases of 76% or greater (10). Based on human simulations presented in this manuscript, the %pERK decrease in tumor tissue is anticipated to be > 80% at clinical doses of 28-112 mg/day GDC-0973, consistent with the observed clinical activity in B-RAF mutant melanoma patients at daily doses of 60 and 100 mg (16). Taken together, available clinical data suggest that a high degree of suppression of the RAF/MEK/ERK pathway, above a certain threshold, is required for robust anti-tumor activity.
Preclinical PK-PD modeling of anti-cancer therapeutics is associated with certain assumptions and caveats. Xenograft mouse models are the most common preclinical in vivo efficacy models used to evaluate and select new anti-cancer therapies for clinical development (34, 35). An important assumption when translating xenograft data to humans is that drug concentrations required for PD modulation and anti-tumor activity are the same in xenograft-bearing mice and human cancer patients. This assumes that drug distribution is similar, despite reported differences in tumor vasculature and transport in xenograft versus human tumors (36). Also, differences in growth rate of human and xenograft tumors complicate the interpretation of xenograft data. Finally, the selection of relevant tumor types that are reflective of the disease that is being targeted is challenging. Despite these challenges, the present GDC-0973 study illustrates how PK-PD modeling can be utilized to improve clinical translation of preclinical data to humans through integration of all available information. Through our PK-PD analysis using combined preclinical and limited early clinical data we characterized the relationship between GDC-0973 plasma and tumor concentrations, tumor PD response and anti-tumor activity. Using this knowledge, we were able to successfully prospectively predict active doses of GDC-0973 in V600E melanoma patients and roughly identify the number of dose escalations in the Phase 1 clinical trial required to reach active doses.
ACKNOWLEDGEMENTS

The authors thank colleagues at Exelixis and Genentech for their contributions in generating data for this study. The authors also thank Drs. Stephen E. Gould, Mark Merchant, O. Helen Chan, Lichuan Liu and Cornelis Hop for their helpful comments and discussion.
REFERENCES


daily in patients with advanced solid tumors. AACR 102nd Annual Meeting.

2011; Minisymposium Abstract # 4716.


growth inhibition to an orally available cMet kinase inhibitor in human tumor xenograft mouse models. Drug Metab Dispos. 2008; 36:1267-1274


30. Wong H, Belvin M, Herter S, Hoeflich KP, Murray LJ, Wong L, et al. Pharmacodynamics of 2-\{4-[(1E)-1-(Hydroxyimino)-2,3-dihydro-1H-inden-5-yl]-3-(pyridine-4-yl)-1H-pyrazol-1-yl}\ethanol (GDC-0879), a Potent and Selective


## Tables

### Table 1 - Summary of pharmacokinetic and pharmacodynamic parameters estimated from WM-266-4 xenograft studies.

<table>
<thead>
<tr>
<th>Pharmacokinetic Parameter</th>
<th>Estimate (%SE) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (hr$^{-1}$)</td>
<td>1.08 (5.6)</td>
</tr>
<tr>
<td>$CL$ (L/hr/kg)</td>
<td>3.13 (2.1)</td>
</tr>
<tr>
<td>$CL_{pt}$ (L/hr/kg)</td>
<td>1.74 (4.5)</td>
</tr>
<tr>
<td>$CL_{ip}$ (L/hr/kg)</td>
<td>0.040 (5.8)</td>
</tr>
<tr>
<td>$V/F$ (L/kg)</td>
<td>40.5 (1.5)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pharmacodynamic Parameter for %pERK Decrease</th>
<th>Single Dose Estimate (%SE) a</th>
<th>Multiple Dose Estimate (%SE) a</th>
</tr>
</thead>
<tbody>
<tr>
<td>$IC_{50}$ (µM)</td>
<td>0.78 (26.4)</td>
<td>3.89 (7.7)</td>
</tr>
<tr>
<td>$I_{max}$ (%)</td>
<td>97 (10.4)</td>
<td>100 (fixed)</td>
</tr>
<tr>
<td>$h$</td>
<td>1 (fixed)</td>
<td>1.85 (13.8)</td>
</tr>
</tbody>
</table>

*Fitted parameters are expressed as estimate followed by the % standard error in parentheses.*
Table 2 – A375 melanoma xenograft pharmacodynamic parameter estimates from integrated population PK-PD-efficacy model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Population mean (%SE)</th>
<th>Interindividual variance (%SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{ng}$ (day$^{-1}$)</td>
<td>0.0828 (10.7)</td>
<td>0.426 (26.9)</td>
</tr>
<tr>
<td>$K_{max}$ (day$^{-1}$)</td>
<td>0.201 (0.4)</td>
<td>fixed</td>
</tr>
<tr>
<td>$K(%I)_{50}$ (%pERK decrease)</td>
<td>50.2 (0.7)</td>
<td>fixed</td>
</tr>
<tr>
<td>$n$</td>
<td>11.5 (0.4)</td>
<td>fixed</td>
</tr>
<tr>
<td>Initial Tumor Volume (mm$^3$)</td>
<td>117 (3.1)</td>
<td>0.024 (37.6)</td>
</tr>
</tbody>
</table>

**Residual Variability**

- Proportional Error $\sigma_{prop}$ (%) 0.113 (19.3) -
- Additive Error $\sigma_{add}$ (mm$^3$) 15.2 (14.8) -
LEGENDS FOR FIGURES

Figure 1 Models used in the analyses performed in this manuscript are described in Figure 1. Figure 1A is the pharmacokinetic (PK) model describing tumor disposition of GDC-0973. Figure 1B is the pharmacokinetic-pharmacodynamic (PK-PD) model linking %pERK decrease in tumor to GDC-0973 tumor concentrations. Figure 1C is an integrated PK-PD-efficacy model where the anti-tumor efficacy is driven by %pERK decrease in tumor.

Figure 2 GDC-0973 plasma and tumor concentration and tumor %pERK decrease profiles in WM-266-4 xenografts following oral administration of a single (2A) or multiple (2B) oral doses of GDC-0973 at 1, 3 and 10 mg/kg (mpk). For Figure 2A, the time on the X-axis refers to time after the first does. For Figure 2B, the time on the X-axis refers to the time after the last (14th) dose. Each point in Figures 2A and 2B represents the mean of three animals. Plots of the observed vs predicted GDC-0973 plasma (2C) and tumor (2D) concentrations following fitting of WM-266-4 xenograft data to the PK model presented in Figure 1A. In Figures 2C and 2D, the dashed line is the line of identity and the solid line is a regression line. Plots of the relationship between GDC-0973 tumor concentration and tumor %pERK decrease from single (2E) or multiple dose (2F) studies of GDC-0973 in WM-266-4 xenograft mice. In Figures 2E and 2F, the solid line is predicted tumor %pERK decrease from the PK-PD model shown in Figure 1B.

Figure 3 Human simulation of GDC-0973 plasma and tumor concentrations and tumor %pERK decrease at a dose (0.33 mg/kg) where tumor %pERK decrease is ≥80% at steady-state is shown in Figure 3A. Figure 3B and Figure 3C show human simulations of tumor %pERK decrease for escalating daily doses of GDC-0973 using single dose (3B) or multiple dose (3C) PD parameters from
WM-266-4 xenograft studies. The horizontal black dashed line in Figures 3A, 3B, and 3C serve as a reference line for 80% pERK decrease in tumor.

**Figure 4** A plot of the relationship between GDC-0973 tumor concentration and tumor %pERK decrease following oral administration of a single dose of GDC-0973 to A375 xenografts is presented in Figure 4A. The solid line in Figure 4A is the model predicted %pERK decrease, and model estimates of $I_{\text{max}}$ and $IC_{50}$ are presented. In vivo efficacy of GDC-0973 in A375 xenografts is presented in Figure 4B. In Figure 4B, QD represents once daily, Q2D represents once every 2 days and Q3D represents once every 3 days. Figure 4C describes the integrated PK-PD-efficacy analysis used to relate pathway modulation to anti-tumor efficacy. The analysis involves first linking plasma to tumor concentrations and then tumor concentrations to tumor %pERK decrease in A375 xenograft mice using the model described in Figure 1B such that tumor %pERK decrease can be simulated for all dosing regimens in the xenograft efficacy study. The final step of the analysis involves understanding the relationship between tumor %pERK decrease and anti-tumor efficacy using the full integrated PK-PD-efficacy model shown in Figure 1C where tumor %pERK decrease drives anti-tumor effect. Figure 4D is a plot of individual observed versus individual predicted tumor volumes from the A375 xenograft efficacy study following fitting the integrated PK-PD-efficacy model to tumor volume data. Figure 4E is a plot showing the relationship between %pERK decrease in tumor and rate constant (K) describing the anti-tumor effect of GDC-0973 in A375 xenograft mice. Parameters used to simulate Figure 4E are as follows: $K_{\text{max}} = 0.201 \text{ day}^{-1}$, $K(\%)_50 = 50.2$ % pERK decrease, and $n = 11.5$. 
Figure 1
Figure 2
Figure 3
Figure 4
Clinical Cancer Research

Bridging the Gap between Preclinical and Clinical Studies Using Pharmacokinetic-Pharmacodynamic (PK-PD) Modeling: An Analysis of GDC-0973, a MEK Inhibitor

Harvey Wong, Laurent Vernillet, Amy Peterson, et al.

Clin Cancer Res  Published OnlineFirst April 10, 2012.

Updated version
Access the most recent version of this article at:
doi:10.1158/1078-0432.CCR-12-0445

Supplementary Material
Access the most recent supplemental material at:
http://clincancerres.aacrjournals.org/content/suppl/2012/04/10/1078-0432.CCR-12-0445.DC1

Author Manuscript
Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.