Pharmacokinetic–Pharmacodynamic Analysis of Vismodegib in Preclinical Models of Mutational and Ligand-Dependent Hedgehog Pathway Activation

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Abstract

**Purpose:** Vismodegib (GDC-0449) is a potent and selective inhibitor of the Hedgehog (Hh) pathway that shows antitumor activity in preclinical models driven by mutational or ligand-dependent activation of the Hh pathway. We wished to characterize the pharmacokinetic–pharmacodynamic (PK/PD) relationship of vismodegib in both model systems to guide optimal dose and schedule for vismodegib in the clinic.

**Experimental Design:** Preclinical efficacy and PK/PD studies were carried out with vismodegib in a Ptch¹⁻/⁻ allograft model of medulloblastoma exhibiting mutational activation of the Hh pathway and patient-derived colorectal cancer (CRC) xenograft models exhibiting ligand-dependent pathway activation. Inhibition of the hedgehog pathway was related to vismodegib levels in plasma and to antitumor efficacy using an integrated population-based PK/PD model.

**Results:** Oral dosing of vismodegib caused tumor regressions in the Ptch¹⁻/⁻ allograft model of medulloblastoma at doses ≥25 mg/kg and tumor growth inhibition at doses up to 92 mg/kg dosed twice daily in two ligand-dependent CRC models, D5123, and 1040830. Analysis of Hh pathway activity and PK/PD modeling reveals that vismodegib inhibits Gli1 with a similar IC₅₀ in both the medulloblastoma and D5123 models (0.165 μmol/L ±11.5% and 0.267 μmol/L ±4.83%, respectively). Pathway modulation was linked to efficacy using an integrated PK/PD model revealing a steep relationship where > 50% of the activity of vismodegib is associated with >80% repression of the Hh pathway.

**Conclusions:** These results suggest that even small reductions in vismodegib exposure can lead to large changes in antitumor activity and will help guide proper dose selection for vismodegib in the clinic. *Clin Cancer Res; 17(14); 4682–92. ©2011 AACR.*

Introduction

The hedgehog (Hh) pathway has been implicated in a number of cancers including basal cell carcinoma (BCC) (1, 2), medulloblastoma (3, 4), colorectal, pancreatic (5), prostate (6), and lymphoma (7). Hh pathway activation can occur through mutational activation in the case of BCC and medulloblastoma where aberrant proliferation is driven by ligand-independent cell autonomous Hh signaling. These cancers driven by mutational activation of the Hh pathway have been referred to as type-1 cancers whereas the larger set of cancers, driven by paracrine Hh signaling between tumor cells and the surrounding stroma, have been referred to as ligand-dependent or type-3 cancers (8).

In the latter cases Hh ligands (Sonic, Indian, and Desert) bind to a twelve-pass transmembrane receptor called patched (PTCH) which, in the absence of ligand, inhibits a seven-pass transmembrane protein named smoothened (SMO). In the presence of Hh ligand the repressive action of PTCH on SMO is relieved and SMO initiates signal transduction through the GLI family of transcription factors leading to induction of downstream targets which include GLI1 and PTCH forming both positive and negative feedback loops, respectively. Additional transcriptional targets include the cell-cycle regulator cyclin D2 (9, 10), the anti-apoptotic protein BCL-2 (11, 12), and the proangiogenic factors VEGF and angiopoietin (13). In the case of BCC approximately 90% of sporadic cases have been associated with inactivating mutations of the negative regulator PTCH, which leads to constitutive activation of the pathway (14) and up to 10% contain activating mutations in the positive regulator SMO (15, 16). Sporadic cases of medulloblastoma have also been associated with inactivating mutations in PTCH albeit at a lower frequency of approximately 30% of cases (17). Taken together these findings have spurred interest in the development of inhibitors of this pathway as potential antitumor therapeutics the most advanced of which is vismodegib (GDC-0449), currently in late-phase clinical development (8, 18).
Translational Relevance

The current work defines the pharmacokinetic–pharmacodynamic (PK/PD) relationship of the Hedgehog pathway inhibitor (HPI) vismodegib (GDC-0449) in preclinical tumor models driven by either mutational activation or ligand-dependent activation of the Hedgehog pathway. Cancers that contain mutational activation of the Hh pathway, such as basal cell carcinoma and medulloblastoma, display a cell-autonomous dependency on Hh-signaling whereas cancers such as colorectal and pancreatic adenocarcinoma depend on Hh ligand-dependent paracrine signaling between tumor and the surrounding stroma. Surprisingly, despite these differences, we find similar PK/PD relationships amongst these two types of models. Using an integrated population-based PK/PD model we define here the interaction between Hh pathway suppression and antitumor response. We find a steep PD response–effect relationship that operates like an on–off switch where even a small reduction in pathway suppression can lead to dramatic reductions in efficacy. These observations are pertinent to all HPI being tested in the clinic and will help guide proper dose and schedule to achieve maximal clinical benefit.

Vismodegib is a potent, selective Hedgehog pathway inhibitor (HPI) that targets SMO with an IC50 of 2.8 nmol/L on a human palatal mesenchymal cell line (HEPM) stably expressing a GLI-responsive luciferase reporter gene (19–21). Vismodegib shows favorable pharmacokinetics in preclinical species characterized by moderate clearance in monkeys and low to very low clearance in mouse, rat, and dog (20). Based on the data presented to date, the pharmacokinetics of vismodegib is consistent with it being a very low clearance compound in humans (22). Vismodegib has shown dramatic antitumor activity in clinical trials treating autonomous-driven, or humans (22). Vismodegib was formulated as a suspension in 0.5% methylcellulose, 0.2% tween-80 (MCT), and Tween 80 (MCT). Blood samples (1 mL) were collected via direct implantation of surgical material obtained from the Coop- erative Human Tissue Network funded by the National Cancer Institute into female CD-1 nu/nu mice 6 to 8 weeks of age (Charles River Laboratories) and propagated in vivo as described (5). Mice were housed and maintained according to the animal use guidelines of Genentech, Inc, conforming to California State legal and ethical practices. Medulloblastoma allografts were established from direct transplantation of spontaneous central nervous system (CNS) tumors that formed in Pch+/− mice (27) via direct transplantation into female CD-1 nu/nu mice. Allograft tumors were serially transplanted via dissection of the tumor, mechanical disaggregation, and subcutaneous inoculation of 5 to 10 million cells. Calu6 xenograft tumors were established through inoculation of 4 × 10⁶ viable cells (ATCC) subcutaneously in the hind flank of female CD-1 nu/nu mice 6 to 8 weeks of age (Charles River Laboratories) using a 1 cc syringe fitted with a 22-gauge needle as a 50:50 suspension in growth factor depleted Matrigel (No. 354230, BD Biosciences). Tumor-bearing mice were distributed into tumor volume-matched cohorts when the tumors reached between 200 and 350 mm³. The vismodegib-resistant medulloblastoma allograft, sg274, was developed by intermittent suboptimal dosing of a Pch+/−, p53+/− medulloblastoma allograft as described previously (21). Vismodegib was formulated as a suspension in 0.5% methylcellulose, 0.2% tween-80 (MCT), and was administered orally. Tumor volumes were determined using digital calipers (Fred V. Fowler Company, Inc.) using the formula (L × W × W)/2. Tumor growth inhibition (%TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that %TGI = 100 × 1−(AUCtreatment/day)/(AUCvehicle/day). Curve fitting was applied to Log₂ transformed individual tumor volume data using a linear mixed-effects model using the R package nlme, version 3.1–97 in R v2.12.0.

Materials and Methods

Subcutaneous xenograft and allograft models in mice

Patient-derived colorectal cancer xenograft tumor models (D5123 and 1040830) were established from direct implantation of surgical material obtained from the Cooperative Human Tissue Network funded by the National Cancer Institute into female CD-1 nu/nu mice 6 to 8 weeks of age (Charles River Laboratories) and propagated in vivo as described (5). Mice were housed and maintained according to the animal use guidelines of Genentech, Inc, conforming to California State legal and ethical practices. Medulloblastoma allografts were established from direct transplantation of spontaneous central nervous system (CNS) tumors that formed in Pch+/− mice (27) via direct transplantation into female CD-1 nu/nu mice. Allograft tumors were serially transplanted via dissection of the tumor, mechanical disaggregation, and subcutaneous inoculation of 5 to 10 million cells. Calu6 xenograft tumors were established through inoculation of 4 × 10⁶ viable cells (ATCC) subcutaneously in the hind flank of female CD-1 nu/nu mice 6 to 8 weeks of age (Charles River Laboratories) using a 1 cc syringe fitted with a 22-gauge needle as a 50:50 suspension in growth factor depleted Matrigel (No. 354230, BD Biosciences). Tumor-bearing mice were distributed into tumor volume-matched cohorts when the tumors reached between 200 and 350 mm³. The vismodegib-resistant medulloblastoma allograft, sg274, was developed by intermittent suboptimal dosing of a Pch+/−, p53+/− medulloblastoma allograft as described previously (21). Vismodegib was formulated as a suspension in 0.5% methylcellulose, 0.2% tween-80 (MCT), and was administered orally. Tumor volumes were determined using digital calipers (Fred V. Fowler Company, Inc.) using the formula (L × W × W)/2. Tumor growth inhibition (%TGI) was calculated as the percentage of the area under the fitted curve (AUC) for the respective dose group per day in relation to the vehicle, such that %TGI = 100 × 1−(AUCtreatment/day)/(AUCvehicle/day). Curve fitting was applied to Log₂ transformed individual tumor volume data using a linear mixed-effects model using the R package nlme, version 3.1–97 in R v2.12.0.

Pharmacokinetic studies

Female CD-1 nude mice (weighing 25–28 g) (Charles River Laboratories) were administered oral doses of 5, 15, 50, and 100 mg/kg (free base equivalent) of vismodegib hydrochloride salt in 0.5% methylcellulose/0.2% Tween 80 (MCT). Blood samples (~1 mL) were collected...
Pharmacodynamic studies in mice

Tumor-bearing mice were treated with vismodegib in 0.5% methylcellulose/0.2% Tween 80 (MCT) either as a single dose (medulloblastoma model), or as a series of 5 consecutive doses given on a twice-daily schedule [D5123 CRC, Calu6 non–small cell lung carcinoma (NSCLC) models]. Tumors were harvested at various times following the last dose and total RNA extracted for qPCR using the Qiagen RNeasy Mini Kit and RNA samples treated with Ambion rDNAse according to the manufacturer’s protocol (Ambion). qPCR was carried out on an Applied Biosystems 7500 thermocycler (Applied Biosystems), using 100 ng RNA/reaction. Relative gene expression was calculated using the following equation: 2-δct×1000. Data are presented either as relative expression or as fold change under the concentration-time profile from time zero to the last measurable timepoint. Relative gene expression was calculated using Ambion rDNAse according to the manufacturer’s protocol (Ambion). qPCR was carried out on an Applied Biosystems 7500 thermocycler (Applied Biosystems), using 100 ng RNA/reaction. Relative gene expression was calculated using the following equation: 2-δct×1000. Data are presented either as relative expression or as fold change from untreated tumors using RPL19 as a loading control, as indicated. Primers and probes sequences were; Gli1, F 5’-AGA AGG TGA CCT GGA-3’, R 5’-TGA TAC ATA TGG CGG TCA ATC T-3’, P 5’-CTC TCC AGG CAG AGC CCC CAG C-3’, RPL19 F 5’-AGA AGG TGA CCT GGA-3’, Gli1 mRNA inhibition studies in medulloblastoma allografts and D5123 xenografts

The PK/PD relationship of vismodegib plasma concentrations to Gli1 mRNA inhibition was characterized using an indirect response model (29) where vismodegib concentrations inhibit the formation of Gli1 mRNA as described by the following equation:

\[
\frac{d}{dt} \left( \frac{Gli1}{C0} \right) = k_{in} \left( 1 - \frac{C}{IC_{50} + C} \right) - k_{out} \left( Gli1 \right)
\]  

\[ \text{IC}_{50} \] (PD units) is defined as the % normalized Gli1 mRNA, \( t \) (hr) is the time, \( k_{in} \) (PD units/hr) is the formation rate of Gli1 mRNA, \( C \) (μmol/L) is the concentration of vismodegib. IC\(_{50}\) (μmol/L) is the vismodegib concentration where there is 50% inhibition of Gli1 mRNA, \( k_{out} \) (hr\(^{-1}\)) is the rate constant describing the loss of Gli1 mRNA. At steady state, \( k_{in} = k_{out} \) (Gli1); therefore \( k_{out} \) was replaced by \( k_{out}(Gli1)_{\text{initial}} \) where \( (Gli1)_{\text{initial}} \) is the control (predose) value of Gli1 mRNA. Plasma and tumor Gli1 mRNA were fit simultaneously using SAAM II. Pharmacodynamic parameters are presented as the estimate followed by the %SE (percent standard error of the estimate = (standard error of the estimate/parameter estimate) × 100) in parentheses. Free IC\(_{50}\) and IC\(_{95}\) for both medulloblastoma allografts and CRC xenografts were calculated using a mean vismodegib unbound fraction of 1.34 ± 0.17% determined ex vivo by equilibrium dialysis from plasma collected from these pharmacodynamic studies.

Integrated PK/PD-efficacy model

To understand the relationship between vismodegib plasma concentrations, Gli1 mRNA inhibition and tumor growth inhibition, an integrated PK/PD-efficacy model was constructed by adding an antitumor efficacy component to the PK/PD model used to characterize the relationship between vismodegib concentrations and Gli1 mRNA inhibition described above (Supplementary Fig. S1). Briefly, the PK/PD model describing Gli1 mRNA inhibition (Eq. A) was used to simulate Gli1 mRNA inhibition for all xenograft/allograft dose groups. The following equations describe the model used in the fitting process to relate Gli1 mRNA inhibition to tumor volume:

\[
\frac{d}{dt}(TV) = k_{log}(TV) - K(TV)
\]

where

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

TV (mm\(^3\)) is defined as the tumor volume, \( t \) (day) is time, \( k_{log} \) (day\(^{-1}\)) is the net growth rate constant, \( K \) (day\(^{-1}\)) is the
rate constant describing the antitumor tumor effect of vismodegib, $K_{\text{max}}$ (day$^{-1}$) is the maximum value of $K$, $n$ is the Hill coefficient, and $K$ ($\% I_{50}$) is defined as the %I where $K$ is 50% of $K_{\text{max}}$. $\% I$ is defined as the percent inhibition of $Gli1$ mRNA and was calculated as follows:

$$\% I = \left( \frac{(Gli1)_{\text{initial}} - (Gli1)}{(Gli1)_{\text{initial}}} \right) \times 100\% \quad (D)$$

Concentrations of vismodegib in mice were simulated based on the pharmacokinetic parameters obtained from the pharmacokinetic studies. The S-ADAPT program, an augmented version of ADAPT II with population analysis capabilities (30, 31) was used to fit individual tumor volumes from all dose levels simultaneously. Intersubject variability was assumed to be log-normally distributed and fitted using an exponential-variance model. Residual variability was modeled using a proportional error model. In cases where intersubject variance was small and could not be estimated reliably, the intersubject variance was fixed to 0.00001. Population parameters estimates are presented as the estimate followed by the %SE in parentheses.

The described integrated PK/PD-efficacy model was fit simultaneously to tumor volumes from three medulloblastoma allograft studies. For study 1, medulloblastoma allograft mice were given vehicle and daily doses of 0.3 to 75 mg/kg vismodegib; for study 2, allograft mice were given vehicle and daily doses of 10 or 75 mg/kg vismodegib (data not shown); and for study 3, allograft mice were given vehicle and daily doses of 1 to 175 mg/kg vismodegib (data not shown). Of these medulloblastoma allograft studies, study 1 best captures the dose response relationship based on the doses selected (Fig. 1A and B) and serves as the representative study. This integrated PK/PD-efficacy model was also used to fit tumor volumes from the D5123 study where xenograft mice were given twice daily doses of 46 to 92 mg/kg vismodegib (Fig. 1C).

### Results

**Vismodegib is highly efficacious in medulloblastoma allograft tumors**

We first characterized the dose response of vismodegib in the subcutaneous murine $Pch^{+/−}$ medulloblastoma-allograft model. Tumor growth in this model is driven by mutational inactivation of the tumor suppressor $Pch$, and therefore directly tests the dependency of tumor growth on autocrine Hh pathway activation (27). Mice were implanted subcutaneously with serially passaged allograft tumor fragments originally derived from a spontaneous medulloblastoma tumor that formed in a $Pch^{+/−}$ mouse. Daily oral dosing with 0.3 to 75 mg/kg of vismodegib for 14 days leads to dose responsive antitumor activity with tumor regressions occurring at or above 25 mg/kg (Fig. 1A, B). Tumor regressions occur rapidly with the bulk of the activity observed within a week of initiating treatment. Antitumor activity reached a plateau above 25 mg/kg with little increase in activity seen with higher doses suggesting the effect was saturated (Fig. 1B). The pharmacokinetics study of vismodegib showed a dose-dependent increase in exposure (AUC) at doses above 15 mg/kg, and therefore the saturation of efficacy was not due to dose-limiting exposure (Fig. 1E; Table 1).

**Vismodegib causes growth delay in patient-derived colorectal xenografts**

Unlike in medulloblastoma where Hh pathway activity is driven by mutational activation of the Hh pathway, pathway activity is driven by paracrine ligand signaling in colorectal, pancreatic, and ovarian cancers (5). In this paracrine model of Hh-dependent tumorigenesis, ligand produced by epithelial-derived tumors signals to the surrounding stromal cells which respond by providing direct growth signals and or by promoting a favorable environment for tumor growth. Early passage patient-derived colorectal xenografts were implanted subcutaneously into the flanks of nude mice and treated twice daily with 23 to 92 mg/kg vismodegib for 18 to 21 days. Two independent models (D5123 and 1040830) revealed that vismodegib at doses > 46 mg/kg twice daily caused tumor growth delay (52 and 69% TGI, respectively, Fig. 1C, D).

**PK/PD relationship of vismodegib in medulloblastoma allograft tumors**

We next wished to explore the PK/PD relationship in the medulloblastoma allograft model using $Gli1$ mRNA levels as a direct readout of Hh pathway activity. We dosed mice bearing subcutaneous $Pch^{+/−}$ allograft tumors with 0, 1, 10, and 50 mg/kg vismodegib and assayed pathway modulation in tumors via qPCR from 2 to 32 hours following a single dose to capture the dynamics of vismodegib activity. A fully efficacious dose of 50 mg/kg of vismodegib (119% TGI) suppressed $Gli1$ for greater than 12 hours with $Gli1$ levels increasing from 16 to 24 hours (Fig. 2A). This is in contrast to the partially efficacious dose of 10 mg/kg (85% TGI) which failed to inhibit $Gli1$ to the same extent and which was associated with increasing levels of $Gli1$ by 8 hours (Fig. 2A). Little, if any, $Gli1$ inhibition was observed in response to 1 mg/kg of vismodegib, consistent with the lack of antitumor activity observed at this dose (Fig. 1A; 14% TGI). Plasma was also collected and analyzed for vismodegib levels (Fig. 2D). Maximal repression of the pathway was associated with 3.2–13 μmol/L vismodegib during the first 12 hours whereas levels below 0.1 μmol/L failed to inhibit pathway activity relative to the vehicle treatment (Fig. 2A and D).

**PK/PD relationship of vismodegib in ligand-driven xenograft tumors**

As with the mutational driven tumors we wished to explore the PK/PD relationship in ligand-dependent tumors using $Gli1$ mRNA levels as the readout of Hh pathway activity. However, unlike in the medulloblastoma model where Hh pathway activation occurs within tumor cells, $Gli1$ activity was monitored in the tumor stroma to
detect paracrine Hh signaling between the xenografted tumor and infiltrating stroma using PCR primers and probes that specifically detect murine Gli1 (5). Two separate models were chosen to characterize the PK/PD relationship, a model that responds to vismodegib treatment by exhibiting growth delay (D5123) and second model (Calu6) that is insensitive to vismodegib treatment. Tumor-bearing mice were dosed orally with 11.5 to 92 mg/kg vismodegib bid for a total of 5 doses to achieve steady state levels in plasma and to mimic the dosing paradigm used in efficacy studies. In both cases, doses of 46 mg/kg or higher of vismodegib were required to cause reduction in stromal Gli1 expression over vehicle treated animals 12 hours following dosing (Fig. 2B, C). No changes in human GLI1 were detected within the tumors in either model showing that vismodegib was inhibiting paracrine Hh-signaling in these tumors [data not shown, (5)]. Significant inhibition of Gli1 at 12 hours was associated with 7.2 to 10.1 μmol/L of vismodegib in plasma (Fig. 2E, F).

**Single-dose pharmacokinetic assessment of vismodegib in mice**

To fully characterize the PK parameters of vismodegib following oral administration in mice we administered a single dose of vismodegib at 5, 15, 50, and 100 mg/kg in MCT and analyzed vismodegib levels in plasma for more than 24 hours. Estimated oral pharmacokinetic parameters are presented in Table 1. Exposure (AUC) increased with increasing dose over the dose range tested. However, this increase in AUC was greater than dose proportional consistent with nonlinear elimination. Half-life also increased with increasing dose, consistent with nonlinear elimination. For modeling and simulation purposes, the following
PK parameters were estimated by fitting an oral one-compartment model with nonlinear elimination to vismodegib concentration-time profiles from all doses simultaneously: \( k_a = 4.04 \text{ hr}^{-1}, V_{\text{max}} = 1.94 \text{ } \mu\text{mol/hr/kg hr}^{-1}, K_m = 4.22 \text{ } \mu\text{mol/L}, \) and \( V/F = 4.43 \text{ L/kg} \) (Fig. 1E and Table 1). These estimated parameters were used to simulate vismodegib plasma concentrations when fitting tumor-volume data from efficacy studies.

**Indirect response PK/PD model characterizing the vismodegib-Gli1 relationship**

To more fully characterize the PK/PD relationship of vismodegib in plasma to Gli1 mRNA levels in both the medulloblastoma allograft and D5123 CRC xenograft models we fitted an indirect response model to the vismodegib plasma concentration and Gli1 mRNA data (29). In this model, the concentration of vismodegib in plasma (C) influences the rate of Gli1 mRNA production (\( k_{\text{in}} \)) according to Eq. A such that steady state Gli1 levels are determined by a balance of production and degradation (\( k_{\text{out}} \)). We used an indirect response model as vismodegib binds to and inhibits SMO, which then allows accumulation of a cleaved form of GLI3, a repressor of Hh target gene transcription, including Gli1 (32). Using this approach we find that vismodegib inhibits Gli1 with a similar IC\(_{50}\) in both the medulloblastoma allograft model as well as the CRC xenograft model D5123 (0.165 and 0.267 \( \mu\text{mol/L} \), respectively; Table 2, Fig. 3) despite the different cellular compartments targeted (tumor vs. stroma for medulloblastoma and

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**Table 1. Oral pharmacokinetics of vismodegib in CD-1 nude mice**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>AUC (( \mu\text{mol/L} \times \text{hr} ))</th>
<th>( C_{\text{max}} ) (( \mu\text{mol/L} ))</th>
<th>( t_{\text{max}} ) (hr)</th>
<th>( t_{1/2} ) (hr)</th>
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<td>15</td>
<td>33.3</td>
<td>8.08</td>
<td>0.5</td>
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</tr>
<tr>
<td>50</td>
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<td>100</td>
<td>413</td>
<td>34.7</td>
<td>1</td>
<td>25.3</td>
</tr>
</tbody>
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Figure 2. Hedgehog pathway modulation in \( \text{Ptch}^{+}\text{+} \) medulloblastoma allograft tumors following a single dose of vismodegib (A). Animals (\( n = 3 \)) were given a single dose of 1, 10, and 50 mg/kg vismodegib and tumors harvested for analysis from 2–32 hours following drug administration. Hedgehog pathway activity was assayed through quantitative PCR of murine Gli1 mRNA. Data are plotted relative to vehicle treated tumors following normalization to GAPDH. D, levels of vismodegib were assayed in the plasma at each time point and dose level. Maximal repression of the pathway was associated with 3.2–13 \( \mu\text{mol/L} \) vismodegib during the first 12 hours whereas levels below 0.1 \( \mu\text{mol/L} \) failed to significantly alter pathway activity. Hedgehog pathway modulation in stroma from a patient-derived colorectal tumor (B, D5123) or a NSCLC xenograft (C, Calu6) following five doses of vismodegib dosed twice daily. Animals (\( n = 3 \)) were given a single dose of 11.5–92 mg/kg vismodegib and tumors harvested for analysis at either 1, 6, 12, 18, or 24 hours following the final dose of vismodegib. Hedgehog pathway activity was assayed through quantitative PCR of murine Gli1 using species-specific primers and probes. Data are plotted relative to vehicle treated tumors following normalization to murine Rpl19. E and F, levels of vismodegib were assayed in the plasma at each time point and dose level in the D5123 (E) and Calu6 models (F).
Table 2. Pharmacodynamic parameter estimates from Gli1 mRNA inhibition studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Medulloblastoma (%SE)</th>
<th>D5123 Model (%SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_0) (PD units/hr)</td>
<td>47.2 (4.0)</td>
<td>12.2 (5.73)</td>
</tr>
<tr>
<td>IC(_{50}) (umol/L)</td>
<td>0.165 (11.5)</td>
<td>0.267 (4.83)</td>
</tr>
<tr>
<td>Free IC(_{50}) (nmol/L)</td>
<td>2.21</td>
<td>3.58</td>
</tr>
<tr>
<td>Free IC(_{90}) (nmol/L)</td>
<td>42.0</td>
<td>68.1</td>
</tr>
</tbody>
</table>

D5123, respectively) and the different mechanisms of pathway activation (loss of PTCH function vs Hh-ligand expression).

**Integrated population pharmacokinetic/pharmacodynamic–efficacy model**

We next wished to understand the relationship between vismodegib plasma concentrations, Gli1 mRNA inhibition, and tumor-growth inhibition to determine the degree of pathway suppression associated with tumor-growth inhibition in both tumor models. The PK/PD model used to characterize the relationship between vismodegib concentrations and Gli1 mRNA inhibition described above was combined with an indirect response population model and this integrated PK/PD model was fit to the individual tumor volume data from the efficacy studies (see Material and Methods, Supplementary Fig. S1). The integrated model adequately characterizes the tumor volume data from the efficacy studies as evident in plots of predicted vs. observed tumor volume presented in Figs. 4A and B. Estimated pharmacodynamic parameters are presented in Table 3. The estimated pharmacodynamic parameters along with the inter- and intra-individual variability were estimated with good precision with % standard error of all estimates being ≤30%. The integrated PK/PD-efﬁcacy model predicts that in both mutation- and ligand-driven tumors 50% of the maximum antitumor effect is achieved at, or above, approximately 82% of pathway inhibition (Table 3, Fig. 4C). In addition, the relationship between Gli1 inhibition and tumor growth is characterized by a steep Hill slope (n) observed for both the medulloblastoma and D5123 models (8.7 and 8.9, respectively; Table 3, Fig. 4C). Such steep Hill slopes are indicative of on-off switches whereby narrow changes in pathway modulation may have disproportionately large impacts on physiologic outcome and have been described for several pathways that control binary cell fate decisions including Hh (33, 34).

The net growth rate constant of the medulloblastoma-allograft model was approximately 2-fold higher than for the D5123 xenograft model (\(k_{\text{reg}} = 0.154\) and 0.0810 day\(^{-1}\), respectively). The maximum value of the rate constant describing the antitumor effect of vismodegib (\(K_{\text{max}}\)) differed between the two models with \(K_{\text{max}}\) being approximately 11-fold greater in the medulloblastoma-allograft model compared with the D5123 CRC model (0.589 vs. 0.0524 day\(^{-1}\), respectively; Table 3, Fig. 4D). These differences in \(K_{\text{max}}\) are apparent from the maximal tumor growth inhibition noted in the efficacy studies with a maximal 52% tumor growth inhibition (TGI) observed in the D5123 CRC xenograft model and tumor regression (125% TGI) being observed in the medulloblastoma-allograft model (Fig. 1A, C).

**Pharmacokinetic/Pharmacodynamic relationship of vismodegib in a resistant model of medulloblastoma**

To further explore the prediction that small decreases in Gli1 inhibition can lead to disproportionately large effects on efficacy we characterized the PK/PD relationship of vismodegib in a model (sg274) of medulloblastoma that contains a SMO mutant (D477G) that is largely resistant to vismodegib (21). Treatment of established allografts of the sg274 model with 100 mg/kg bid of vismodegib led to 52% tumor growth inhibition whereas a considerably lower dose of vismodegib (25 mg/kg bid) was required to regress SMO\(^{WT}\) sensitive tumors (Fig. 1A). In the same study a second HPI, Hh-Antag, at 100 mg/kg bid led to tumor regression in the sg274 model. Tumor-growth inhibition was associated with 76% inhibition of the Hh pathway in response to vismodegib and 99% inhibition in response to Hh-Antag (Fig. 4E, F). These observations are consistent with our predictions based on the integrated PK/PD model that small decreases in the inhibition of Gli1 can result in large losses of antitumor activity.

**Discussion**

The development of rationally targeted agents for the treatment of cancer requires a full understanding of the pharmacokinetic–pharmacodynamic relationships of the investigational agent (35, 36). This information can provide useful pharmacokinetic exposure targets as well as pharmacodynamic endpoints that can be used as gates for further clinical development. Vismodegib is a potent and selective HPI currently in clinical development. We, and others have previously shown that two different modes of Hh-signaling can drive tumorigenesis: (i) ligand-independent mutational activation of the Hh pathway in the case of BCC and medulloblastoma, and (ii) paracrine ligand-dependent signaling observed in colorectal, pancreatic, and ovarian cancer (5, 6, 26). We have used preclinical models of both modes of Hh pathway activation to characterize the relationship of vismodegib in plasma to pathway inhibition, and pathway inhibition to efficacy with the aim of identifying any differences between Hh ligand-independent and Hh ligand-dependent cancers and to establish exposure targets for vismodegib in clinical trials.
We have found that vismodegib is a potent inhibitor of mutation-driven tumorigenesis leading to tumor regression in an allograft model of medulloblastoma driven by loss of function of PTCH with an approximate ED$_{50}$ of 6 mg/kg. In contrast, vismodegib at doses up to 92 mg/kg twice daily caused moderate tumor growth delay in two patient-derived CRC xenograft models that exhibit paracrine Hh-signaling. These more moderate effects on tumor-growth inhibition seen in the CRC models reported here are typical of the level of antitumor activity seen in additional paracrine models such as patient-derived and cell-line xenografts treated with HPIs including signal-blocking antibodies. These differences in responsiveness may reflect the nature of pathway activation between these two types of tumor. Medulloblastoma allografts are driven by constitutive activation of the Hh pathway owing to their loss of the tumor suppressor PTCH and are likely dependent on this activity for growth and survival. On the contrary, the patient-derived xenografts, which exhibit paracrine Hh-signaling between the tumor cells and surrounding stroma do not have a direct requirement for pathway activity. In this case the Hh pathway is not the oncogenic driver but Hh-signaling to the stroma leads indirectly to increased tumor growth presumably due to induction of signals that directly or indirectly promote tumor growth.

Despite the differences noted in the antitumor effects between the two types of models we found a very similar relationship between the IC$_{50}$ for vismodegib in plasma and pathway suppression as measured by Gli1 mRNA in tumor or stroma in the case of medulloblastoma allografts (0.165 μmol/L) and CRC patient-derived xenografts (0.267 μmol/L), respectively. These observations are consistent with vismodegib acting at the level of SMO which lies.
Figure 4. Integrated population PK/PD-efficacy model of both the D5123 xenograft and medulloblastoma allograft models. A and B, plots of predicted tumor volumes derived from the integrated population PK/PD-efficacy model versus the observed values for the D5123 (A) and medulloblastoma models (B) showing that model nicely characterizes tumor volume data for both models. C, relative maximal inhibition-response curves for D5123 (dashed) and medulloblastoma (solid) models showing a steep relationship characterized by Hill slopes of 8.9 and 8.7, respectively. D, the same data in (C) are plotted as absolute values showing the approximately 11-fold higher antitumor rate constant (K) seen in the medulloblastoma model versus the D5123 model. E, Treatment of established sg274 allografts with either 100 mg/kg bid of vismodegib (open triangles) or 100 mg/kg Hh-Antag bid (filled circles) led to 52% tumor growth inhibition or tumor regression, respectively. F, qRT-PCR from tumors treated in (E) 6 hours following a final dose of 100 mg/kg of either vismodegib or Hh-Antag revealing 76% inhibition of the Hh pathway in response to vismodegib and 99% inhibition in response to Hh-Antag. Error bars represent +/- SEM.

Table 3. Pharmacodynamic parameter estimates from the integrated population PK/PD-efficacy model

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Medulloblastoma</th>
<th>D5123 colorectal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Population mean (%SE)</td>
<td>Interindividual variance (%SE)</td>
</tr>
<tr>
<td>$k_{ng}$ (day$^{-1}$)</td>
<td>0.154 (4.4)</td>
<td>0.095 (21.4)</td>
</tr>
<tr>
<td>$K_{max}$ (day$^{-1}$)</td>
<td>0.589 (4.3)</td>
<td>0.046 (30.3)</td>
</tr>
<tr>
<td>$K(Gli1)_{50}$ (% Gli1 mRNA inhibition)</td>
<td>81.5 (0.3)</td>
<td>fixed</td>
</tr>
<tr>
<td>$n$</td>
<td>8.68 (0.4)</td>
<td>fixed</td>
</tr>
<tr>
<td>Initial tumor volume (mm$^3$)</td>
<td>290 (3.1)</td>
<td>0.078 (18.0)</td>
</tr>
<tr>
<td>Residual variability ($\sigma$)</td>
<td>0.254 (3.6)</td>
<td></td>
</tr>
</tbody>
</table>
downstream of the signaling defect present in the medulloblastoma model (loss of PTCH), inhibition of which should lead to similar suppression of the downstream transcriptional target Gli1. This also suggests that the difference in potency of vismodegib observed in the two systems is not due to differences in pathway dynamics but rather varying dependencies on the Hh pathway itself. Equal pathway inhibition leads to regression in one instance and tumor-growth delay in the other.

Using an integrated PK/PD-efficacy model we were able to characterize the relationship between Gli1 inhibition and efficacy in both mutation-driven and paracrine-driven models. Of great interest was the observed steep relationship between Gli1 inhibition and the rate constant describing the measure of antitumor effect (K). The Hill slope observed for both the D5123 and medulloblastoma model were ~9 suggesting that the pathway is acting as an on-off switch in both systems. During embryonic development the Hh pathway plays a fundamental role in specifying a variety of cell-fate choices that require precise temporal and spatial control of pathway activity. Hh pathway activity is regulated by both positive (GLI1) and negative (PTCH) feedback loops that help to restrict activity to cells in a narrow range of Hh ligand expression. Pathways characterized by the presence of both positive and negative feedback loops have been reported to exhibit switch-like behavior consistent with what we have observed (33). Based on the shapes and $K(\%I)_{50}$ of the response effect curves, it is clear that a high degree of inhibition of the pathway is needed to achieve maximal antitumor response, regardless of the mechanism of pathway activation. A similar requirement for near maximal repression of pathway signaling has been recently reported for the B-RAF inhibitor PLX4032 in melanoma patients where >80% of pERK inhibition was associated with tumor regression (37). In addition, an earlier report described a steep Hill slope for the response effect curve of a second B-RAF inhibitor, GDC-0879, in xenograft models (38) consistent with empirical modeling of the MEK-ERK pathway that suggested a switch-like behavior (39). The steep slope of the response effect curve seen with vismodegib also predicts that even small decreases in drug levels, and hence pathway suppression, can have disproportionately large effects on efficacy because the switch operates over a narrow range of Gli1 inhibition. This hypothesis is supported by the large difference in efficacy observed with Hh-Antag (complete tumor regression, 125% tumor growth inhibition) and vismodegib (52% tumor growth inhibition) in the sg274 resistance model despite the more modest differences noted in pathway suppression between the two compounds (99% and 76%, respectively). This requirement for near maximal pathway suppression has important implications for the clinical development of HPI’s as well as other agents that target pathways characterized by a similar steep response effect curve. To achieve maximal clinical benefit one must achieve near complete pathway inhibition and therefore the target needs to be nonessential for normal tissues to enable a favorable therapeutic index. Based on the data in this study and to account for potential species differences in protein binding, we prospectively set a target plasma concentration for vismodegib in our clinical trials to meet, or exceed, the free IC$_{50}$ for Gli1 inhibition (42 to 68 nmol/L, Table 2) to ensure maximal clinical benefit. Recent reports suggest that at the recommended dose and frequency of vismodegib (150 mg once daily) patients exceed these targets for unbound drug (109 nmol/L ± 0.058 SEM) (40).

Disclosure of Potential Conflicts of Interest

All of the authors are employees of Genentech, Inc.

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References


