Dogs Are More Sensitive to Antagonists of Inhibitor of Apoptosis Proteins Than Rats and Humans: A Translational Toxicokinetic/Toxicodynamic Analysis

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Inhibitor of apoptosis (IAP) proteins suppress apoptosis and are overexpressed in a variety of cancers. GDC-0152 is a potent and selective IAP antagonist being developed as an anticancer agent. In preclinical safety studies, dogs were particularly sensitive to GDC-0152 showing adverse signs of a tumor necrosis factor alpha (TNF-α) driven systemic inflammatory response, related to cellular IAP degradation and activation of NFκB signaling, at lower exposures compared with rat. In addition, downstream increases in systemic levels of cytokines and chemokines, such as monocyte chemotactic protein-1 (MCP-1), were observed. A semimechanistic population toxicokinetic/toxicodynamic (TK/TD) model incorporating transit compartments was used to fit MCP-1 plasma concentrations from rats or dogs given iv GDC-0152 doses. Estimated TD parameters inferred that lower GDC-0152 plasma concentrations triggered more severe increases in plasma MCP-1 in dogs compared with rats. Human simulations performed using dog TD parameters and human pharmacokinetics predicted 300–2400% increases of MCP-1 in humans at iv doses from 0.76 to 1.48 mg/kg. Similar simulations using rat TD parameters suggest little or no change. Patients given iv doses of GDC-0152 up to 1.48 mg/kg iv showed no substantial increases in systemic MCP-1 or signs of a severe TNF-α driven systemic inflammatory response. Emerging clinical data reported for other IAP antagonists are consistent with our observations. Taken together, the data suggest dogs are more sensitive to IAP antagonists compared with humans and rats. This study illustrates how TK/TD analysis can be utilized to quantitatively translate and context an identified preclinical safety risk in dogs to humans.

Key Words: TK/TD; PK/PD; pharmacokinetic-pharmacodynamic; toxicodynamics; toxicokinetics; IAP.

Inhibitor of apoptosis (IAP) proteins are involved in the regulation of programmed cell death or apoptosis. They were first identified in baculoviruses where they function to suppress host cell death responses during viral infection (Flygare and Fairbrother, 2010; Varfolomeev and Vucic, 2011). IAP proteins suppress apoptosis, in part, by inhibiting activated cytosolic cysteine/aspartate-specific proteases (caspases) that are critical for its execution (Flygare and Fairbrother, 2010; Varfolomeev and Vucic, 2011). The mammalian family of IAP proteins includes X-linked IAP (XIAP), cellular IAP 1 and 2 (cIAP1 and cIAP2), and melanoma IAP (ML-IAP). Of the IAP proteins, only XIAP directly inhibits effector caspases-3 and -7, which execute the apoptotic death program. XIAP also indirectly inhibits effector-caspase activity via inhibition of the initiator caspase-9. ML-IAP and cIAPs directly antagonize second mitochondria-derived activator of caspase/direct IAP-binding protein with low pi (Smac/DIABLO), an endogenous IAP antagonist that acts by blocking XIAP-mediated inhibition of caspases. cIAPs have an additional role of preventing death receptor (e.g., tumor necrosis factor alpha [TNF-α], Fas, DR4, and DR5) mediated activation of initiator caspase-8, thereby stopping downstream activation of effector caspases-3 and -7. Finally, cIAPs positively regulate canonical NF-κB signaling and negatively regulate noncanonical NF-κB signaling and induction of NF-κB target genes, including TNF-α. IAP proteins are frequently overexpressed in cancer cells where they serve as regulators of cancer cell survival and are often indicators of poor prognosis (Vucic and Fairbrother, 2007). Conversely, inhibition of IAP proteins has been shown to sensitize cancer cells to proapoptotic anticancer agents. Thus,
the development of IAP antagonists as potential anticancer agents has been of interest.

GDC-0152 (Fig. 1) is a potent pan inhibitor of IAP proteins with binding affinities to the BIR3 domains of X-IAP and cIAP1/2, and the BIR domain of ML-IAP in the low nanomolar range ($K_c < 50 \text{nM}$) using a fluorescence polarization-based competition assay (Flygare et al., 2012). GDC-0152 caused a decrease in cell viability in MDA-MB-231 human mammary epithelial cells while having no effect on normal human mammary epithelial cells. In MDA-MB-231 xenograft mice studies, GDC-0152 showed robust single-agent activity resulting in significant tumor growth inhibition at weekly oral doses of 10, 50, or 100 mg/kg. GDC-0152 was evaluated in rat safety studies following iv doses of 3–45 mg/kg given once every 2 weeks for 6 weeks resulting in daily exposures (i.e., area under the plasma concentration-time curve [AUC]$_{0-\infty}$) ranging from 3.51 to 113 M·h. Dog safety studies were performed at lower iv doses ranging from 0.3 to 10 mg/kg which accordingly resulted in lower GDC-0152 exposures compared with rat ranging from 0.723 to 16.5 µM·h (Erickson et al., 2011). In both rat and dog safety studies, in-life observations included elevated plasma cytokines and chemokines (including TNF-α and monocyte chemotactic protein-1 [MCP-1]), an inflammatory leukogram, and increased liver transaminases, in addition to histopathological findings of inflammatory infiltrates and apoptosis and/or necrosis in multiple tissues (Erickson et al., 2011). In particular, dogs appeared more sensitive to TNF-α driven inflammatory response-associated effects of IAP inhibition with some dogs showing transient clinical signs of fever, tremors, and hypotension that were not as observed in rats (Erickson, personal communication). From these studies, MCP-1 was identified to be the most sensitive downstream marker of TNF-α upregulation and NF-κB activation.

The objective of this study was to characterize species differences in sensitivity to inhibition of IAP proteins using plasma MCP-1, an NF-κB target gene that is downstream of TNF-α, as a marker of a systemic TNF-α driven inflammatory response. More importantly, we utilize toxicokinetic/toxicodynamic (TK/TD) modeling and human data from a GDC-0152 clinical trial to understand how preclinical safety findings translate to humans. We found that dogs were extremely sensitive to IAP inhibition compared with humans and rats. This study shows an approach utilizing TK/TD modeling to quantitatively understand and properly context preclinical toxicity findings and will aid in the interpretation of human safety risks of IAP antagonists in general.

**MATERIALS AND METHODS**

**Rat studies.** Female Sprague Dawley rats (weighting 230–250g; Charles River, Wilmington, MA) were given an iv dose of 20 ($n = 4$), 40 ($n = 4$), 80 ($n = 4$), or 120 ($n = 4$) mg/kg of GDC-0152 (Genentech Inc., South San Francisco, CA) in 10% hydroxypropyl-β-cyclodextrin and 20mM sodium succinate (pH 5.5) via a femoral vein cannula. Blood samples (~0.2 ml per sample) were collected from each animal via jugular vein cannulae at the following timepoints: predose, 0.083, 0.25, 1, 3, 6, 9, 12, 24, 36, and 48h postdose. All samples were collected into tubes containing potassium EDTA as an anticoagulant. Blood samples were centrifuged within 30 min of collection and plasma was harvested. Plasma samples were stored at ~70°C until analysis for GDC-0152 concentrations by a liquid chromatographic-tandem mass spectrometric (LC/MS/MS) assay method. Plasma MCP-1 concentrations were determined by multiplexed sandwich immunoassay using Luminex Rodent Multi-Analyte Profiling (MAP) platform (Rules-Based Medicine, San Antonio, TX).

**Dog studies.** Male and female beagle dogs (weighing 7–12 kg; Covance Research Products, Cumberland, VA) were given 30-min iv infusions of 0.3 ($n = 8$), 0.75 ($n = 8$), 1.5 ($n = 6$), 5 ($n = 6$), 10 ($n = 6$), and 15 mg/kg ($n = 6$) of GDC-0152 in 15% hydroxypropyl-β-cyclodextrin and 20mM succinic acid in 5% dextrose. Blood samples (~0.8 ml per sample) were collected from each animal via jugular vein cannulae at the following timepoints: predose, 0.083, 0.17, 0.5, 1, 1.5, 3, 6, 12, and 24h postdose. All samples were collected into tubes containing potassium EDTA as an anticoagulant and kept on ice. Blood samples were centrifuged within 1h of collection and plasma was harvested. Plasma samples were stored at ~70°C until analysis for GDC-0152 concentrations by LC/MS/MS. MCP-1 levels in plasma were measured using an immunoassay (Duoset Canine CCL2 [JE; MCP-1]) ELISA Development Kit (R&D Systems, Minneapolis, MN).

**Human clinical study.** The human pharmacokinetic (PK) parameters were determined using data from a phase 1a study in cancer patients as described by Flygare et al. (2012). The protocol for the phase 1a study was approved by the Institutional Review Boards at participating institutions. Written informed consent was obtained from all trial participants. Briefly, doses ranging from 0.049 to 1.48 mg/kg were administered by a 0.5-h iv infusion once every 14 days to cancer patients. Plasma samples were collected at predose, 0.2, 0.4, 0.8, 1.5, 3, 4.5, 6.5, 9.5, 12.5, 18.5, and 24.5 h postdose. Plasma samples were stored at ~70°C until analysis for GDC-0152 concentrations by LC/MS/MS. Plasma MCP-1 concentrations were determined using a multiplexed sandwich immunoassay using Luminex Human MAP platform (Rules Based Medicine).

**TK analysis.** Standard TK parameters (presented in Tables 1 and 2) were calculated by noncompartmental methods as described by Gilbaldi and Perrier (1982). TK parameters are reported as the mean ± SD.

**TK/TD modeling.** A two-compartment model as described by Equations 1 and 2 (Fig. 2A) was used to characterize the toxicokinetics of GDC-0152 in dogs.

\[
\frac{dX_c}{dt} = k_p X_r - k_c X_c - k_c X_c
\]

\[
\frac{dX_r}{dt} = k_p X_c - k_c X_r
\]

where $X_c$ is the amount of GDC-0152 in the central compartment, $X_r$ the amount of GDC-0152 in the peripheral compartment, $t$ the time, $k_p$ the rate constant
TK/TD ANALYSIS OF MCP-1

from central compartment to peripheral compartment, $k_{pc1}$ the rate constant from peripheral compartment to central compartment, and $k_c$ the elimination rate constant.

Because GDC-0152 toxicokinetics in rats is nonlinear, a two-compartment model described by Equations 2 and 3 was used to characterize the toxicokinetics of GDC-0152.

$$\frac{dX}{dt} = k_p X_p - k_{pc1} X_p - \frac{V_m}{V_k + X_p} X_p$$

(3)

where $V_m$ is the maximum rate of GDC-0152 elimination and $K_m$ (Michaelis-Menten constant) the concentration associated with ½ $V_m$.

A semimechanistic population TK/TD model incorporating a series of transit compartments was used to characterize the MCP-1 response to GDC-0152 and is described by Equations 4–8 (Fig. 2B).

$$\frac{dX}{dt} = k (X_p - T_i)$$

(4)

$$\frac{dT_i}{dt} = k (T_i - T_p)$$

(5)

$$\frac{dT_p}{dt} = k (T_p - T_c)$$

(6)

$$\frac{d(MCP-1)}{dt} = k_p (1 + E \frac{T_p}{V}) - k_{se} (MCP-1)$$

(7)

$$k_{se} = k_n R_n$$

(8)

where $T_i, T_p, and T_c$ represent transit compartments delaying the effect of GDC-0152 on MCP-1, $c$ the MCP-1 plasma concentration (pg/ml), $k$ the transit rate constant, $E$ a constant relating GDC-0152 concentrations to its effect on the formation rate of MCP-1, $V$ the volume of distribution of the central compartment, $T_p/V$, the GDC-0152 concentration following the delay by the transit compartments, $n$ the exponent of the power function "$E(T_p/V)^n"$ that represents the effect of GDC-0152 on the formation rate of MCP-1, $k_{se}$ the rate constant describing the decrease of MCP-1, and $R$ the initial or baseline value of MCP-1.

Population TK/TD modeling was performed using S-ADAPT II, an augmented version of ADAPT II with population analysis capabilities (Bauer and Guzy, 2004; D’Argenio and Schumitzky, 1997). The described TK/TD model was fit to GDC-0152 and MCP-1 plasma concentrations simultaneously for all individual dogs or rats at all dose levels. Interspecies variability was assumed to be log-normally distributed and fitted using an exponential variance model. Residual variability error was modeled using an additive and proportional error model for PK and a proportional error model for MCP-1. Population parameter estimates are presented as the estimate followed by the ±%SE within parentheses.

Pharmacokinetics of GDC-0152 in humans. Human PK parameters were estimated by fitting a three-compartment model, as described by Equations 9–11, to mean human plasma-concentration-time data from cancer patients.

$$\frac{dX}{dt} = k_p X_p + k_{pc2} X_p - (k_p + k_{se2}) X_p$$

(9)

$$\frac{dX}{dt} = k_p X_p - k_{pc3} X_p$$

(10)

$$\frac{dX}{dt} = k_{pc3} X_p - k_{se2} X_p$$

(11)

where $X_p, X_p$, and $k_{pc}$ are as defined previously. $X_p$ is the amount of GDC-0152 in a second peripheral compartment, $k_{pc}$ the rate constant from central to the second peripheral compartment, and $k_{se2}$ the rate constant from the second peripheral compartment to the central compartment.

RESULTS

Toxicokinetics of GDC-0152 in Rats and Dogs

In order to assess the exposure in the rat and dog studies, GDC-0152 toxicokinetics for rats and dogs were assessed and are summarized in Tables 1 and 2, respectively. Plasma clearance (CL) was nonlinear in rats ranging from high (approximately rat hepatic blood flow) at a 20-mg/kg dose to moderate (~36% of rat hepatic blood flow) at a 120-mg/kg dose (Table 1). Accordingly, increases in AUC$_{0-\infty}$ in rat trended toward being greater than dose proportional, and the half life ($t_{1/2}$) progressively increased with increasing dose. The volume of distribution at steady-state ($V$) in rats was moderate to high at ~5 l/kg at all doses. In contrast to the rat, the toxicokinetics of GDC-0152 in dogs was reasonably linear, and AUC$_{0-\infty}$ estimates were dose proportional over the dose range tested (0.3–15 mg/kg). CL was moderate in dogs being ~30–50% of hepatic blood flow (Davies and Morris, 1993). $V$ in dogs was low to moderate ranging from 0.66 to 1.50 l/kg over the dose range tested. Overall, the AUC$_{0-\infty}$ range tested in dogs (12.6–206 μM·h) was higher than the AUC$_{0-\infty}$ range tested in rats (0.85–34.9 μM·h). Finally, the AUC$_{0-\infty}$ range observed in rats and dogs generally covered the AUC$_{0-\infty}$ range evaluated in previous preclinical safety studies.

TK/TD Modeling

In order to understand the relationship between GDC-0152 plasma concentrations and MCP-1 response in rats and dogs, a population TK/TD model shown in Figure 2 was simultaneously fit to GDC-0152 and MCP-1 plasma concentration data from rats or dogs. The described nonlinearity in rat toxicokinetics necessitated the use of a TK model (Fig. 2A) with nonlinear elimination of GDC-0152. Figures 3A and D show representative concentration-time plots for GDC-0152 and MCP-1 in rat and dog, respectively. A system of transit compartments were used to characterize the delayed MCP-1 response to GDC-0152 observed in both species. Finally, despite higher GDC-0152 concentrations in the rat, the MCP-1 response was substantially higher in the dog (Figures 3A and D).

Plots of observed versus individual predicted GDC-0152 and MCP-1 plasma concentrations are shown for the rat (Figs. 3B and C) and dog (Figs. 3E and F). As mentioned, representative concentration-time plots of observed GDC-0152 and MCP-1...
plasma concentrations and model predictions for a representative rat and dog are presented in Figures 3A and D, respectively. Overall, the described integrated TK/TD model adequately described the observed GDC-0152 and MCP-1 plasma concentration data for both preclinical species.

TK and TD parameter estimates from rats and dogs following fitting of the integrated TK/TD model are presented in Tables 3 and 4. TK and TD parameters and inter- and intrasubject variances were estimated with good precision for both rats and dogs with standard errors being ≤ 45%. The parameters E and n relate GDC-0152 plasma concentrations to MCP-1 response. The effect of GDC-0152 plasma concentrations on increasing the formation rate of MCP-1 is described in Equation 7 where this effect is equal to “E(T3/Vc)n” where “T3/Vc” represents GDC-0152 plasma concentrations following the delay created by the transit compartments. The estimate of the typical value of E is ~30-fold higher in dogs compared with rats (Table 4). In addition, the estimate of “n” is greater than twofold higher in the dog. Figure 4 shows a plot describing the relationship between GDC-0152 plasma concentrations and MCP-1 response. The plot clearly illustrates the MCP-1 response in dogs is more sensitive to IAP inhibition by GDC-0152.

**Human Translational MCP-1 TK/TD Modeling**

In order to investigate MCP-1 sensitivity to GDC-0152 in humans, the rat and dog TK/TD models and human pharmacokinetics were used to simulate predicted MCP-1 responses in humans. PK parameters for humans were estimated by fitting a three-compartment model to individual plasma concentration-time information from a phase 1a study in cancer patients (Flygare et al., 2012). PK parameters are presented in Table 5. A plot of observed versus individual predicted GDC-0152 concentrations for this fit is presented in Supplementary figure S1. TD parameters were fixed to values estimated for rat or dog, and simulations were performed using the GDC-0152 PK model for humans to drive the MCP-1 response. Figure 5 shows anticipated percent changes in MCP-1 response in humans for the 0.76, 1.06, and 1.48 mg/kg iv doses based upon rat (Fig. 5A) or dog (Fig. 5B) TD parameters. Based upon rat TD parameters, no dramatic change in MCP-1 was anticipated in humans with a maximum predicted change of < 50% over the dose range evaluated. In contrast, simulations using dog TD parameters suggested a maximum dose-dependent change ranging from ~300 to 2400%.

In cancer patients, no dramatic changes in MCP-1 response or signs of a systemic inflammatory response were observed following GDC-0152 administration. Measured maximum percent changes of MCP-1 from the human clinical trial are presented in Figure 6. Based upon the variability observed in MCP-1, there was no significant dose-dependent increase in MCP-1 response to GDC-0152 in humans. These data suggest that human MCP-1 response to IAP inhibition is not as sensitive as observed for dogs and is more in line with observations in the rat.

**DISCUSSION**

Efforts to discover selective inhibitors of IAP proteins in recent years have been accompanied by elucidation of the mechanisms of their antitumor activity, both as single agents and
in combination with other anticancer agents. IAP antagonists induce cIAP protein E3 ubiquitin ligase activity that results in ubiquitination of RIP1, as well as rapid autoubiquitination and proteasomal degradation of the cIAP proteins (Dueber et al., 2011; Varfolomeev et al., 2007; Vince et al., 2007). RIP1 ubiquitination induces activation of the canonical NF-κB pathway. The cIAP proteins also regulate the noncanonical NF-κB pathway through ubiquitination of NIK—their loss therefore results in activation of the noncanonical pathway via NIK stabilization. NF-κB pathway activation leads to induction of NF-κB target genes which include TNF-α and subsequent downstream cytokine and chemokines. In the absence of cIAP proteins, TNF-α binds to TNFR1 resulting in caspase-8 activation and initiation of apoptosis (Varfolomeev et al., 2007; Vince et al., 2007). Thus, the single-agent activity of IAP antagonists is dependent on TNF-α. More recent data suggest that inhibition

![Image](https://academic.oup.com/toxsci/article-abstract/130/1/205/1667839/FIG.3代表了GDC-0152或MCP-1的血浆浓度-时间图，对于接受120-mg/kg iv给药的rat (A)和狗 (D)。观察值与个体预测的GDC-0152血液浓度在rat (B)和狗 (E)。观察值与个体预测的MCP-1血液浓度在rat (C)和狗 (F)。)

![Image](https://academic.oup.com/toxsci/article-abstract/130/1/205/1667839/Table3 TK Parameter Estimates From MCP-1 Population TK/PD Model for Rat and Dog)

<table>
<thead>
<tr>
<th>TK parameters</th>
<th>Rat (n = 16)</th>
<th>Dog (n = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_p$ (h⁻¹)</td>
<td>0.195 (44.2)</td>
<td>2.89 (0.706)</td>
</tr>
<tr>
<td>$k_c$ (h⁻¹)</td>
<td>0.365 (9.30)</td>
<td>0.0879 (13.1)</td>
</tr>
<tr>
<td>$V_c$ (l/kg)</td>
<td>3.73 (6.22)</td>
<td>0.0460 (19.3)</td>
</tr>
<tr>
<td>$k_e$ (h⁻¹)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>$V_{max}$ (µmol/h/kg)</td>
<td>23.5 (14.5)</td>
<td>0.278 (9.13)</td>
</tr>
<tr>
<td>$K_m$ (µM)</td>
<td>5.57 (23.5)</td>
<td>0.724 (7.41)</td>
</tr>
</tbody>
</table>

Residual variability

- Proportional error ($\sigma_{prop}$) | 0.201 (7.92) | 0.242 (5.64) |
- Additive error ($\sigma_{add}$) | 0.00533 (22.7) | 0.00222 (14.2) |
of both cIAP and XIAP proteins is necessary for more efficient single-agent activity of IAP antagonists (Ndubaku et al., 2009). In contrast to single-agent activity, combination activity of IAP antagonists with proapoptotic receptor antagonists appears to be related to the inhibition of XIAP and can be independent of TNF-α signaling (Varfolomeev et al., 2009).

Prior to testing new molecular entities in humans, a thorough preclinical safety evaluation is conducted in a rodent and a non-rodent species. A safe starting dose for first in human studies is determined based on this evaluation of preclinical safety (DeGeorge et al., 1998; ICH, 2010). The first in human starting dose is usually determined using the most sensitive preclinical species with the safety of human subjects participating in the clinical trial being of the utmost importance. Based upon this paradigm, toxicological evaluations in preclinical species that are particularly sensitive to a new molecular entity’s pharmacology or off-target toxicity can result in an unnecessarily low starting dose. In the phase 1 oncology setting, this is less than ideal because more patients will be exposed to subtherapeutic doses, and cancer patients primarily participate in such clinical trials with the hope of clinical benefit. Therefore, the appropriate translation of preclinical safety risks to humans requires not only an understanding of the relevance of the preclinical safety findings, but also an understanding of the sensitivity of the preclinical species to the particular toxicity identified in relation to man.

Our previous work characterizing the toxicology profile of the pan IAP antagonist, GDC-0152, described the link between TNF-α pharmacology and adverse findings in rats and dogs (Erickson et al., 2011). Dogs exhibited transient increases in TNF-α, MCP-1, IL-6, and IFNγ at relatively low doses compared with rat. These alterations in cytokine and chemokine profiles were consistent with clinical signs (e.g., fever) in dogs and dose-dependent acute systemic inflammatory responses in both rats and dogs. Furthermore, liver injury characterized by dose-dependent increases in serum liver enzymes and/or total bilirubin levels and microscopic findings of hepatic apoptosis/necrosis were observed in both species. Finally, the dog appeared to be much more sensitive to IAP antagonism with comparable adverse effects being observed at doses that were 10- to 15-fold lower than those in the rat. Our current work investigating concentration-effect relationships between GDC-0152 and MCP-1 suggests that dogs are > 30-fold more sensitive to IAP inhibition compared with rats based on the higher estimated value of $E$ and $n$ for the dog (Table 4), and the resulting steeper relationship between GDC-0152 concentration and MCP-1 response in dogs compared with rats (Fig. 4).

Overall, the preclinical toxicology findings were consistent with the described single-agent mechanism of action and the associated NF-κB activation and subsequent upregulation of

### Table 4
TD Parameter Estimates from MCP-1 Population TK/PD Model for Rat and Dog

<table>
<thead>
<tr>
<th>TD parameters</th>
<th>Population mean (%SE)</th>
<th>Interindividual variance (%SE)</th>
<th>Population mean (%SE)</th>
<th>Interindividual variance (%SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K$ (h$^{-1}$)</td>
<td>0.727 (7.45)</td>
<td>0.0511 (33.4)</td>
<td>0.584 (3.59)</td>
<td>0.0256 (37.4)</td>
</tr>
<tr>
<td>$E$ (µM$^{-1}$)</td>
<td>1.05 (37.0)</td>
<td>1.86 (13.2)</td>
<td>30.5 (34.9)</td>
<td>4.57 (28.0)</td>
</tr>
<tr>
<td>$k_c$ (pg*mL$^{-1}$h$^{-1}$)</td>
<td>14.2 (12.4)</td>
<td>0.122 (45.3)</td>
<td>125 (26.1)</td>
<td>2.21 (27.1)</td>
</tr>
<tr>
<td>$R_c$ (pg/ml)</td>
<td>128 (4.19)</td>
<td>0.0224 (43.4)</td>
<td>132 (16.2)</td>
<td>0.899 (24.6)</td>
</tr>
<tr>
<td>$n$</td>
<td>1.26 (18.7)</td>
<td>0.533 (1.84)</td>
<td>3.02 (11.1)</td>
<td>0.183 (41.5)</td>
</tr>
<tr>
<td>Residual variability</td>
<td>Proportional error ($\sigma_{\text{res}}$)</td>
<td>0.245 (6.24)</td>
<td>—</td>
<td>0.334 (6.75)</td>
</tr>
</tbody>
</table>

### Table 5
PK Parameter Estimates for GDC-0152 in Human (n = 36)

<table>
<thead>
<tr>
<th>PK parameters</th>
<th>Population mean (%SE)</th>
<th>Interindividual variance (%SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_c$ (h$^{-1}$)</td>
<td>1.06 (7.25)</td>
<td>—</td>
</tr>
<tr>
<td>$k_c$ (h$^{-1}$)</td>
<td>2.52 (5.48)</td>
<td>0.00263 (56.7)</td>
</tr>
<tr>
<td>$k_c$ (h$^{-2}$)</td>
<td>0.159 (9.43)</td>
<td>—</td>
</tr>
<tr>
<td>$k_c$ (h$^{-3}$)</td>
<td>0.228 (16.9)</td>
<td>0.129 (35.8)</td>
</tr>
<tr>
<td>$V_0$ (l)</td>
<td>8.49 (9.69)</td>
<td>0.151 (53.5)</td>
</tr>
<tr>
<td>$k_c$ (h$^{-1}$)</td>
<td>5.26 (6.73)</td>
<td>0.0573 (45.7)</td>
</tr>
<tr>
<td>Residual variability Proportional error ($\sigma_{\text{res}}$)</td>
<td>0.0813 (16.9)</td>
<td>—</td>
</tr>
</tbody>
</table>
TNF-α. Cancer cells have been shown to frequently overexpress IAP proteins where they serve as regulators of cancer cell survival and are often indicators of poor prognosis (Vucic and Fairbrother, 2007). An increase in local TNF-α in cancer tumor cells overexpressing IAP proteins would be essential for any single-agent antitumor effects of IAP antagonists. However, normal cells are also subject to the effects of IAP antagonism and widespread NF-κB activation can result in adverse events due to a TNF-α driven inflammatory response. In clinical trials, TNF-α driven adverse events ranging from hypotension, thrombocytopenia, leukopenia, neurotoxicity, fever, and nausea/vomiting to more serious events such as hypotensive shock proved to be limiting in the exploration of recombinant TNF-α as an anticancer agent (Roberts et al., 2011).

From a therapeutic standpoint, for IAP antagonists to succeed as anticancer agents, local upregulation of TNF-α must occur in cancer cells before significant systemic TNF-α upregulation occurs. Systemic MCP-1, a chemokine that plays a critical role in recruitment of monocytes during the body’s response to inflammation or infection (Yadav et al., 2010), was identified as the most sensitive downstream marker of TNF-α upregulation and NF-κB activation in toxicology studies with GDC-0152.

FIG. 5. Simulations of % change of MCP-1 vs. time using human pharmacokinetics and rat (A) or dog (B) TD parameters at iv doses of 0.76, 1.06, and 1.48 mg/kg. These doses represent the doses in the last three cohorts of reported data from the GDC-0152 clinical trial.

FIG. 6. The maximum % change in MCP-1 observed in each cohort of the clinical trial in cancer patients. Maximum % change was observed from 4.5 to 18.5 h postdose in cancer patient samples.
(Erickson et al., 2011). In our studies, MCP-1 served as a marker of widespread systemic TNF-α upregulation.

TK-TD modeling offers a tool that improves the translatability of preclinical safety findings to humans. It provides a more mechanistic and robust quantitative assessment of concentration/toxicity relationships beyond more traditional evaluations of dose or exposure (i.e., AUC/toxicity relationships and also provides a means to account for species differences in pharmacokinetics (Wong et al., 2012a,b). Our work with MCP-1 shows significant species differences in sensitivity to the TNF-α driven systemic inflammatory response as a consequence of IAP antagonism. The difference in the steepness of the relationship between GDC-0152 plasma concentration and the percent increase in MCP-1 formation rate between rat and dog illustrates a dramatic species difference in the systemic inflammatory response to IAP inhibition, with the dog being clearly more sensitive (Fig. 4). More importantly, we utilize existing clinical data to assess the risk of IAP antagonism in humans. Based upon simulations of MCP-1 response using human pharmacokinetics from the phase 1a clinical trial, the sensitivity of the TNF-α driven inflammatory response exhibited by dogs does not appear to be duplicated in humans. The simulations suggest up to ~850–2400% increase in MCP-1 levels in humans at the two highest doses tested (i.e., 1.06 and 1.48 mg/kg) if human responded similarly to dogs (Fig. 5). The observed maximum changes in MCP-1 levels in cancer patients at these doses were considerably less and were considered within the normal variability observed in patients.

Our findings with GDC-0152 are consistent with literature describing the dog’s sensitivity to endotoxin-induced shock, a process that is also mediated by TNF-α and results in inflammatory and acute-phase protein responses and hepatic injury. The dog is ~100-fold more sensitive to endotoxin compared with the rat (Hamano et al., 2002). Compared with monkeys, dogs exhibit markedly enhanced effects of endotoxin on portal and systemic arterial pressures, which are rapidly increased and decreased, respectively (Brobmann et al., 1970). This unique sensitivity to endotoxin observed in dogs has been partially attributed to larger increases in Kupffer cell-mediated release of cytokines (IL-1β and IL-6) in dogs following endotoxin stimulation (Hamano et al., 2002). Also of note, dogs have large amounts of smooth muscle in sphincter-like configurations around the hepatic vein which contract in response to vasoactive cytokines, resulting in congestive hepatopathy and ischemic liver injury (Aydinli and Bayraktar, 2007; Legare and Lautt, 1987). This unique feature of dog anatomy may in part explain the observed vasodynamic differences between canine and primate species. In terms of relevance to humans, responses to endotoxin-induced shock in monkeys appear to be more representative of humans (Cavanagh et al., 1970).

Our conclusions that dogs are much more sensitive than humans to IAP antagonism are consistent with emerging clinical data on IAP antagonists, LCL161, HGS1029, and TL32711 (Graham et al., 2011; Infante et al., 2010; Sikic et al., 2011). All compounds have been reported to be well tolerated thus far in clinical trials. TL32711 and LCL161 have reached anticipated target efficacious exposures. Administration of LCL161 and HGS1029 has resulted in cytokine and chemokine upregulation with minimal adverse effects and no reported signs of a severe TNF-α driven systemic inflammatory response. Taken together, these data to date suggest that the systemic inflammatory response to IAP inhibition in humans is more similar to that observed in rats rather than in dogs. In rats, TNF-α driven systemic inflammatory response (as assessed by MCP-1 changes) appears to increase relatively gradually with increasing concentrations of IAP antagonist compared with the dog. Therefore, the expectation is that therapeutic concentrations of IAP antagonists can be achieved in humans prior to observations of dose-limiting adverse events associated with upregulation of TNF-α and other inflammatory cytokines and chemokines. In summary, our work on GDC-0152 presents for the first time, direct evidence based on MCP-1 changes in preclinical species and humans that dogs are more sensitive than humans and rats to IAP antagonism. Finally, our work presents a means of utilizing existing preclinical and clinical data and TK/TD modeling in order to provide a more quantitative assessment of the safety risk of IAP antagonists to humans and properly context the adverse findings observed in dogs.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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