

# A Predictive Model of Human Myelotoxicity Using Five Camptothecin Derivatives and the *In vitro* Colony-Forming Unit Granulocyte/Macrophage Assay

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## ABSTRACT

**Purpose:** Many promising anticancer drugs are limited by myelosuppression. It is difficult to evaluate human myelotoxicity before a Phase I study because of the susceptibility of humans and animals to hematotoxicity. The purpose of this study was to establish a reliable method to predict the human maximum tolerated dose (MTD) of five camptothecin derivatives: SN-38, DX-8951f, topotecan, 9-aminocamptothecin, and camptothecin.

**Experimental Design:** The myelotoxicity of SN-38 and DX-8951f were evaluated on bone marrow from mice, dogs, and humans using a 14-day colony-forming unit, granulocyte-macrophage (CFU-GM) assay to determine the 50%, 75%, and 90% inhibitory concentration values (IC<sub>50</sub>, IC<sub>75</sub>, and IC<sub>90</sub>, respectively).

**Results:** Species differences in myelotoxicity were observed for SN-38 and DX-8951f. Using human and murine IC<sub>90</sub>s for myelotoxicity of these compounds and other camptothecin compounds (topotecan, 9-aminocamptothecin, and camptothecin), *in vivo* toxicological data, and pharmacokinetic parameters (data referred to in the literature), human MTDs were predicted retrospectively. The mechanism-based prediction model that is proposed uses the *in vitro* camptothecin assay and *in vivo* parameters on the basis of free fraction of area under the concentration-curve at the MTD ( $r^2 = 0.887$ ) and suggests that the human MTDs were well predicted for the five camptothecin derivatives by this model rather than by other models.

**Conclusion:** The human MTDs of the camptothecin drugs were successfully predicted using the mechanism-based prediction model. The application of this model for *in*

*vitro* hematotoxicology could play an important role for the development of new anticancer agents.

## INTRODUCTION

Most anticancer and many anti-HIV compounds produce severe myelotoxicity that limits their clinical usefulness (1–5). Potential toxicity to hematopoietic tissue must be evaluated early in the development of such compounds. However, results from commonly used cancer models in animals (*i.e.*, murine or human tumor xenografts in nude mice) have provided little predictive value for clinical pharmacodynamics and pharmacokinetics in humans. Consequently, many promising anticancer drugs fail during Phase I clinical trials because of myelosuppression effects in patients. Selection of the starting dose for a Phase I trial [typically based on the maximum-tolerated dose (MTD) of the most sensitive species], is critical. The goal is to decrease the risk of a lethal overdose in the first cohort of patients, whereas effectively determining the MTD to achieve the maximum therapeutic effect of an anticancer drug. To achieve this, it is desirable to predict the human MTD for safe and efficient clinical dose escalation studies, while reducing the number of patients treated with an ineffective dose. When combined with efficacy data, preclinical evaluation of hematopoietic toxicity may identify the least toxic analogue with the best therapeutic index in humans. For almost all of the cytotoxic agents, clinical testing starts at a dose that kills 10% of the tested animals (LD<sub>10</sub>). The dose is then gradually increased in modified Fibonacci steps. *In vitro* hematotoxicity assessment using a colony-forming unit, granulocyte-macrophage (CFU-GM) as a surrogate marker of myelosuppression could play a key role in linking animal toxicology studies to clinical investigations. However, the predictive bone marrow MTD for the neutrophil lineage will equal the actual human MTD only when the myelopoietic tissue of bone marrow is a primary target of toxicity. Several methods of predicting the human MTD have been proposed for a number of anticancer drugs using *in vitro* myelotoxicity data (6–9). These predictions were made on the basis of the concentration that inhibited CFU-GM by 90% (IC<sub>90</sub>) as a more predictive end point for the MTD in animals and humans. However, because they did not consider the pharmacokinetics of the compounds, the *in vitro*–*in vivo* correlations were not evaluated quantitatively. Erickson-Miller *et al.* (7) showed the differential sensitivity between murine and human myelosuppression in three camptothecin analogues (*i.e.*, topotecan, 9-aminocamptothecin, and camptothecin) and tried to find *in vitro*–*in vivo* correlations. However, a correct prediction of human MTD was difficult except for topotecan. Pessina *et al.* (9) developed a standard operating procedure for optimizing the CFU-GM assay. Whereas the purpose of the CFU-GM assay is to predict the human MTD, it is also necessary to develop a mechanism-based model for accurate pharmacokinetic/pharma-

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codynamic analysis. Our objective was to validate a human MTD prediction model using five camptothecin derivatives.

CPT-11, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxy-camptothecin hydrochloride trihydrate, is metabolized to SN-38 (Fig. 1) in mice and humans. This active metabolite plays an important role during the antitumor effect of CPT-11 (10), which was approved by the United States Food and Drug Administration in 2000 as a first-line treatment for advanced colorectal carcinoma (11). CPT-11 is now widely used as a promising anticancer agent. The principal dose-limiting toxicity of CPT-11 is myelosuppression and diarrhea (2, 12, 13). SN-38 contributes to both the efficacy and toxicity of CPT-11. Consequently, SN-38 was used in the current *in vitro* study. DX-8951f, (1*S*,9*S*)-1-amino-9-ethyl-5-fluoro-1,2,3,9,12,15-hexahydro-9-hydroxy-4-methyl-10*H*,13*H*-benzo [*de*]pyrano[3',4':6,7]indolizino[1,2-*b*]-quinoline-10,13-dione, monomethanesulfonate dihydrate is also shown in Fig. 1. *In vitro*, DX-8951f has demonstrated stronger antitumor activity than other clinically relevant camptothecin analogues, such as SN-38, topotecan, or camptothecin itself (14). This increased antitumor activity of DX-8951f has been observed in nude mice, using gastric, pancreatic, colon, breast, ovarian, and lung tumors (14). DX-8951f is being evaluated currently in Phase III clinical trials. Myelosuppression, especially neutropenia, is the principal dose-limiting toxicity of the camptothecin derivatives, such as DX-8951f, topotecan (3, 15, 16), 9-aminocamptothecin (4, 17, 18), and camptothecin (19, 20). In clinical studies, camptothecin derivative-induced myelosuppression can be readily managed by administering granulocyte colony-stimulating factor. But if it were possible to predict the hematotoxicity of an anticancer agent and its maximum tolerated level, a predictive model would play an important role in the discovery and development of the new drug.

Thus, we investigated the myelotoxicity of SN-38 and DX-8951f to bone marrow samples from three species (*i.e.*, mouse, dog, and human). Subsequently, we correlated these *in vitro* data with available *in vivo* myelosuppression data of SN-

38, DX-8951f, topotecan, 9-aminocamptothecin, and camptothecin. On the basis of these findings, we have proposed a mechanism-based predictive model of the human MTD for camptothecin derivatives. This model may extend to other anticancer drugs.

## MATERIALS AND METHODS

**Bone Marrow Samples.** Seven to 10-week-old B6C3F1 mice were obtained from Taconic (Germantown, NY) and Charles River Laboratories (Raleigh, NC). Mice received standard laboratory diet and filtered tap water *ad libitum*. Animals were sacrificed by CO<sub>2</sub> asphyxiation, marrow was aseptically flushed from the femurs, and single-cell suspensions were prepared by gentle disruption. Cells were washed with medium and adjusted to the appropriate concentration. Medium consisted of Iscove's Modified Dulbecco's Medium (IMDM) containing 25 mmol/L HEPES buffer and 5% (v/v) fetal bovine serum, referred to as IMDM/5. The femoral marrow from 4 animals was pooled for each of the three experiments conducted in mice. Canine marrow samples were obtained from the University of Auburn Scott-Ritchey Research Center (Auburn, AL). The 3 dogs used in these studies were from 4 to 6 months old when their marrow was aspirated. Upon receipt, marrow was centrifuged over a Ficoll-Hypaque gradient ( $\rho = 1.083$ ) to obtain the mononuclear fraction. Cells were then washed in IMDM/5 and adjusted to the appropriate cell concentration. Human marrow samples were obtained from AllCells, LLC (Berkeley, CA) from donors that had given informed consent. The age of the 3 donors ranged from 20 to 45 years. Upon receipt, marrow was centrifuged over a Ficoll-Hypaque gradient ( $\rho = 1.077$ ) to obtain the mononuclear fraction. Cells were then washed in IMDM/5 and adjusted to the appropriate cell concentration.

**Test Articles.** The negative control for SN-38 and DX-8951f was 0.5% DMSO (as these compounds were dissolved in DMSO) and was obtained from Fisher Scientific (Fair Lawn,

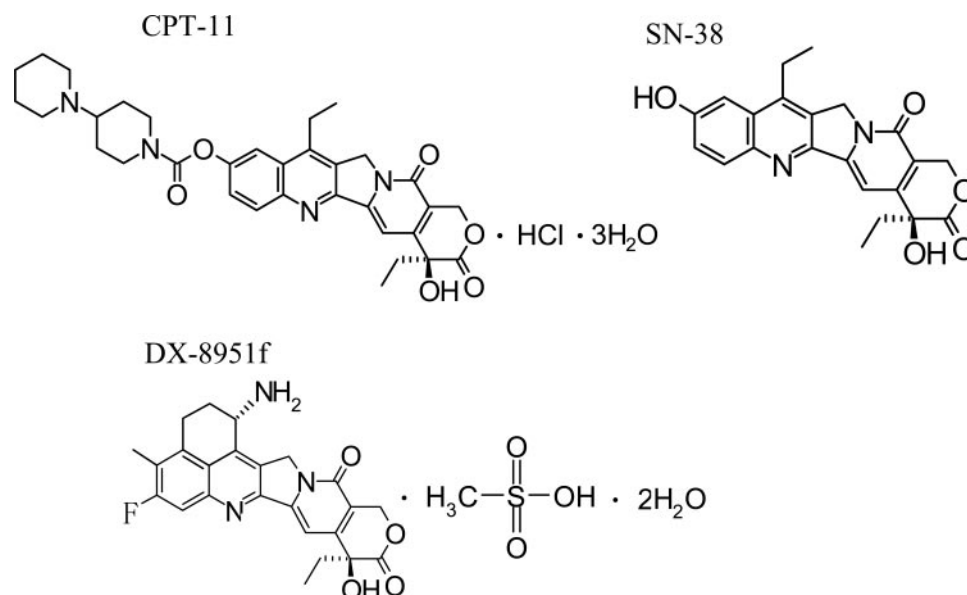


Fig. 1. Chemical structures of CPT-11, SN-38, and DX-8951f.

Table 1 Plasma-free fraction (%) of SN-38, DX-8951f, topotecan, 9-aminocamptothecin, and camptothecin in mouse and human

	Mouse	Human
SN-38	3.4 (25)	1.6 (25)
DX-8951f	2.0*	2.6*
topotecan	67 (34)	65 (35)
9-aminocamptothecin	12*	0.30 (40)
camptothecin	26 (43)	0.36 (19)

NOTE. The values in parenthesis show the reference numbers.

\* N. Masubuchi, unpublished observation.

NJ). SN-38 was synthesized by Yakult Honsha (Tokyo, Japan). DX-8951f was synthesized by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan). The compounds were weighed and dissolved in DMSO. Serial dilutions were made in DMSO for subsequent addition to tubes containing bone marrow cells and MethoCult hemopoietic culture mix. The final DMSO concentration in all of the cultures was 0.5%. Test articles were tested at final concentrations ranging from 16 pmol/L to 500 nmol/L (SN-38) and 3.2 pmol/L to 500 nmol/L (DX-8951f).

**In Vitro Progenitor Cell Assays.** CFU-GM were assayed in duplicate as described by Kuwata *et al.* (21), dishes containing bone marrow cells ( $5 \times 10^4$  for mouse,  $2 \times 10^5$  for dog, and  $1 \times 10^5$  for human). MethoCult GF M3534, GF+H4535, and GF H4534 methylcellulose media (StemCell Technologies, Vancouver, British Columbia, Canada) were used for mouse, dog, and human, respectively. The test compounds remained in contact with the cells for the entire culture period (14 days). CFU-GM colonies were scored using an inverted microscope with phase contrast. All of the studies were performed in accordance with United States Food and Drug Administration Good Laboratory Practice Regulations (21 CFR Part 58).

**Data Analyses.** The 50%, 75%, and 90% inhibitory concentration values ( $IC_{50}$ ,  $IC_{75}$ , and  $IC_{90}$ , respectively) were estimated from log-linear regression relating drug concentrations and CFU-GM inhibition data from individual experiments for these species.

**Prediction Models of Human MTD.** To predict the human MTD for acute neutropenia, three predictive models were applied using *in vitro*  $IC_{90}$  values for myelotoxicity, the murine MTD ( $LD_{10}$ ), area under the concentration-curve (AUC) of total (lactone + carboxylate) drug at the MTD, and the plasma unbound ratio for each compound. The actual murine MTD and *in vivo* pharmacokinetic parameters were taken from the following literature sources: SN-38 (2, 12, 13, 22–25), DX-8951f (26–30), topotecan (3, 15, 16, 31–35), 9-aminocamptothecin (4, 18, 36–40), and camptothecin (19, 20, 41–43).

Model A: Predicted human MTD = Actual murine MTD

$$\times \frac{IC_{90} \text{ human CFU-GM assay}}{IC_{90} \text{ murine CFU-GM assay}}$$

Model B: Predicted human AUC at MTD

= Actual murine AUC at MTD

$$\times \frac{IC_{90} \text{ human CFU-GM assay}}{IC_{90} \text{ murine CFU-GM assay}}$$

Model C: Predicted human free fraction of AUC at MTD

= Actual murine-free fraction of AUC at MTD

$$\times \frac{IC_{90} \text{ human CFU-GM assay}}{IC_{90} \text{ murine CFU-GM assay}}$$

where free fraction of AUC = plasma unbound ratio  $\times$  AUC. The plasma unbound ratios are referred to in Table 1.

**Statistical Analysis.** The observed and predicted MTDs on the basis of the above models were independently subjected to log-linear regression analysis (SAS version 8.02, SAS Institute Inc., Cary, NC) and led to the  $r^2$  values and  $P$ s reported for the predictive modeling.

## RESULTS

**CFU-GM assays of SN-38 and DX-8951f.** Representative results from the experiments in the three species are shown in Fig. 2. The data were normalized to “percent of control” values, using the number of CFU-GM in the 0.5% DMSO control group as 100% for both compounds. A summary of the inhibition caused by these compounds, showing  $IC_{50}$ ,  $IC_{75}$ , and  $IC_{90}$  values calculated, is shown in Table 2.

For mice,  $IC_{90}$  values with SN-38 ranged from 25.3 to 368 nmol/L with a mean  $\pm$  SD of  $191 \pm 172$  nmol/L, as shown in Fig. 2 and Table 2; the  $IC_{90}$  values with DX-8951f ranged from 9.5 to 16.8 nmol/L with a mean  $\pm$  SD of  $13.6 \pm 3.8$  nmol/L. For dogs,  $IC_{90}$  values with SN-38 ranged from 9.6 to 15.6 nmol/L with a mean  $\pm$  SD of  $13.4 \pm 3.3$  nmol/L;  $IC_{90}$  values with DX-8951f ranged from 1.5 to 8.3 nmol/L with a mean  $\pm$  SD of  $5.0 \pm 3.4$  nmol/L. For humans,  $IC_{90}$  values with SN-38 ranged from 15.8 to 18.7 nmol/L with a mean  $\pm$  SD of  $17.5 \pm 1.5$

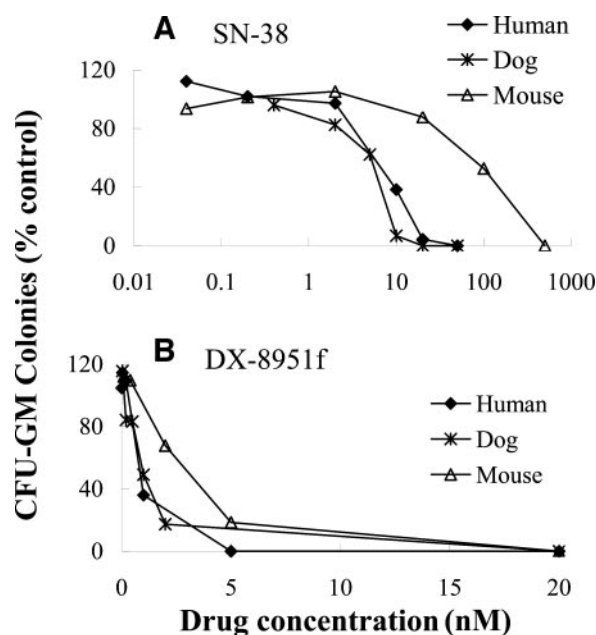


Fig. 2 *In vitro* myelotoxicity of SN-38 and DX-8951f to human, canine, and murine CFU-GM. The data shown are representative of three experiments performed in each species.

Table 2 Summary of inhibition of CFU-GM with SN-38 and DX-8951f in human, murine, and canine bone marrow

Compound	IC <sub>50</sub> (nM)			IC <sub>75</sub> (nM)			IC <sub>90</sub> (nM)		
	Human	Murine	Canine	Human	Murine	Canine	Human	Murine	Canine
SN-38	7.7 ± 2.1	53.6 ± 51	5.9 ± 1.4	12.9 ± 2.0	109 ± 112	9.3 ± 1.2	17.5 ± 1.5	191 ± 172	13.4 ± 3.3
DX-8951f	1.2 ± 0.80	5.1 ± 2.9	0.9 ± 0.5	3.1 ± 2.9	8.9 ± 4.3	1.6 ± 0.64	5.9 ± 6.0	13.6 ± 3.8	5.0 ± 3.4

nmol/L; IC<sub>90</sub> values with DX-8951f ranged from 1.6 to 12.7 nmol/L with a mean ± SD of 5.9 ± 6.0 nmol/L. Table 2 also shows the IC<sub>50</sub> and IC<sub>75</sub> values of all three of the species, all of which showed the same general trends that DX-8951f was more myelotoxic than SN-38 and that both compounds exhibited greater toxicity in dogs and humans than in mice. The IC<sub>90</sub> ratios of mouse/human for DX-8951f and SN-38 were 2.3 and 10.9, respectively (Table 2).

**Prediction of Human MTD for Camptothecin Derivatives.** To predict acute neutropenia from *in vitro* data and *in vivo* pharmacokinetic and toxicological data for the five camptothecin derivatives, DX-8951f, SN-38, topotecan, 9-aminocamptothecin, and camptothecin, predictive models A, B, and C (described in Materials and Methods) were applied. Model A predicts *in vivo* MTD in humans using *in vitro* CFU-GM data from mice and humans. Model B expands upon Model A incorporating AUC data. Model C builds on Model B by taking into account the free fraction of AUC at the *in vivo* MTD.

The human MTD values of the five camptothecin derivatives were estimated using the three models (Tables 3-7). For all of the compounds, several Phase I studies were conducted separately with different dosing schedules in several patient groups. Therefore, several MTD values were obtained, depending on the dosing schedule for each compound. These estimates in the human for predicted and observed MTD values of the compounds were plotted for each model (Fig. 3). The predicted MTDs from models A, B, and C were then subjected to log-linear regression analysis individually against the observed MTD using SAS version 8.02 software. As a result, model C ( $r^2 = 0.887$ ,  $P < 0.001$ ) was a more predictive model of human MTD than model A ( $r^2 = 0.628$ ,  $P < 0.001$ ) or model B ( $r^2 = 0.463$ ,  $P < 0.001$ ) for all of the dosing schedules of these camptothecin derivatives.

## DISCUSSION

Upon comparing the five camptothecin derivatives, the *in vitro* inhibitory values of hematotoxicity suggested that DX-8951f, 9-aminocamptothecin, and camptothecin were comparable in their myelotoxicity in all three of the species, whereas SN-38 and topotecan were less myelotoxic, particularly in mice (Table 8). All of the compounds exhibited greater toxicity in the dog and human than they did in mice, as mentioned for topotecan, 9-aminocamptothecin, and camptothecin by Erickson-Miller *et al.* (7). We used the IC<sub>90</sub> ratios of mouse/human to determine the relative toxicity coefficients for SN-38, DX-8951f, topotecan, 9-aminocamptothecin, and camptothecin. This yielded ratios of 10.9, 2.3, 9.8, 10.6, and 2.3, respectively; those of dog/human were 0.8, 0.9, 0.2, 1.2, and 0.2, respectively (Table 8). SN-38, topotecan, and 9-aminocamptothecin appeared to show greater susceptibility in human and mouse than

did DX-8951f and camptothecin. A similar sensitivity between canine and human CFU-GM was observed for all of the compounds, as opposed to murine and human CFU-GM. The IC<sub>50</sub> and IC<sub>90</sub> values of all of the compounds (except 9-aminocamptothecin) were lowest in dog, followed by those in human, and then in mouse, suggesting that the level of sensitivity to these drugs, in terms of *in vitro* myelotoxicity, was dog > human > mouse. For the 9-aminocamptothecin, this hierarchy of sensitivity was human > dog > mouse.

With regards to the prediction models, model A requires the murine MTD and the IC<sub>90</sub> ratio of human/mouse from *in vitro* CFU-GM assay data. It is possible to apply this model when a drug shows similar pharmacokinetic properties between mouse and human, as was the case for topotecan. However, this model should be applied cautiously, because species differences in pharmacokinetic properties may unexpectedly be observed, such as for DX-8951f and camptothecin [mean total body clearance: DX-8951f: 12 L/hour/m<sup>2</sup> in mice<sup>3</sup> and 1.8 L/hour/m<sup>2</sup> in humans (26–30); camptothecin: 20 L/hour/m<sup>2</sup> in mice (41, 42) and 1.1 L/hour/m<sup>2</sup> in humans (19)]. For such compounds, it may be difficult to predict the human MTD on the basis of the murine MTD and *in vitro* CFU-GM data alone (model A). For 9-aminocamptothecin and camptothecin, species differences of the plasma-free fraction were observed [9-aminocamptothecin: 12% in mice<sup>3</sup> and 0.30% in humans (40); camptothecin: 26% in mice (43) and 0.36% in humans (19)]. For such compounds, plasma-free fraction values should be considered in the prediction process. Therefore, models A and B would be inadvised, favoring model C on the basis of the free fraction of AUC at the MTD, which is useful in many cases, because model C integrates the plasma unbound fraction, as well as drug exposure and *in vivo* toxicological information. For models A and B, interspecies differences of pharmacokinetic parameters are not adequately considered; with these models the human MTD was sometimes predicted to be greater or extremely less than the actual MTD. Model C also supports individualized dose escalation, because the target AUC at the MTD can be determined for each patient. Furthermore, model C also contributes to accelerated dose-escalation. As observed with topotecan and 9-aminocamptothecin, model C is also appropriate for special populations, such as pediatric patients whose pharmacokinetics are different, in many cases, from those of adults. Pessina *et al.* (9) applied model A as the predictive model of human MTD on the basis of a prevalidation study. In doing so, they mentioned that the pharmacokinetic differences across species can contribute to as much as a 4-fold difference in the MTD, because their

<sup>3</sup> N. Masubuchi, unpublished observations.

Table 3 Predicted human MTD values of CPT-11 from *in vitro* CFU-GM assay data

<i>In vivo</i>								
Species	Schedule	CPT-11 MTD (mg/m <sup>2</sup> /cycle)	SN-38 AUC at MTD (ng·h/mL)	Free fraction of SN-38 AUC at MTD (ng·h/mL)	MTD ratio (mouse/human)	AUC ratio at MTD (mouse/human)	Free fraction of AUC ratio at MTD (mouse/human)	Ref.
Mouse	Single	330*	2549	87				23*
Human	Single	320	712	11	1.0	3.6	7.6	2
	Single	250	876	14	1.3	2.9	6.2	22
	Daily for 3 days	345	843	13	1.0	3.0	6.4	13
	Single	275	502	8.0	1.2	5.1	11	12
	c.i.v. over 5 days	150	960	15	2.2	2.7	5.6	24

Abbreviation: c.i.v. continuous intravenous infusion.

\* S. Ono, unpublished data.

Table 4 Predicted human MTD values of DX-8951f from *in vitro* CFU-GM assay data

<i>In vivo</i>								
Species	Schedule	MTD (mg/m <sup>2</sup> /cycle)	AUC at MTD (ng·h/mL)	Free fraction of AUC at MTD (ng·h/mL)	MTD ratio (mouse/human)	AUC ratio at MTD (mouse/human)	Free fraction of AUC ratio at MTD (mouse/human)	Ref.
Mouse	Single	193	16176	324				*
	Daily for 5 days	34	2696	54				*
Human	Single	5	2092	54	39	7.7	5.9	26
	Single	7.1	4817	125	27	3.4	2.6	27
	c.i.v. over 24 h	2.4	1846	48	14	1.5	1.1	28
	c.i.v. over 24 h	0.8	491	13	43	5.5	4.2	29
	Daily for 5 days	2.5	308	8.0	14	8.8	6.7	30

Abbreviation: c.i.v., continuous intravenous infusion.

\* T. Kajimura, unpublished data.

Table 5 Predicted human MTD values of topotecan from *in vitro* CFU-GM assay data

<i>In vivo</i>								
Species	Schedule	MTD (mg/m <sup>2</sup> cycle)	AUC at MTD (μg·h/mL)	Free fraction of AUC at MTD (ng·h/mL)	MTD ratio (mouse/human)	AUC ratio at MTD (mouse/human)	Free fraction of AUC ratio at MTD (mouse/human)	Ref.
Mouse	Single	75	3.76	2.52				3
	Daily for 5 days	70	3.51	2.35				
Human	Daily for 5 days	10	0.366	0.24	7.0	9.6	9.9	3
	Daily for 5 days	8.8	0.424	0.28	8.0	8.3	8.5	15
	Daily for 5 days	7.5	0.306†	0.20	9.3	12	12	16
	c.i.v. over 72 h*	3	0.462	0.30	23	7.6	7.8	31
	Daily for 5 days*	7	0.933	0.61	10	3.8	3.9	32
	c.i.v. over 24 h*	5.5	0.522	0.34	14	7.2	6.9	33

Abbreviation: c.i.v., continuous intravenous infusion.

\* Clinical study for pediatric people.

† Total AUC values were calculated from lactone AUC with the ratio of lactone/total = 0.17 (Ref. 15).

Table 3 Continued

<i>In vitro</i>			MTD prediction		
Human IC <sub>90</sub> (nM)	Mouse IC <sub>90</sub> (nM)	IC <sub>90</sub> ratio (mouse/human)	Model A	Model B	Model C
			Predicted human MTD by MTD ratio (mg/m <sup>2</sup> /cycle)	Predicted human MTD by AUC at MTD (mg/m <sup>2</sup> /cycle)	Predicted human MTD by free fraction of AUC at MTD (mg/m <sup>2</sup> /cycle)
17.5	191	10.9	30	105	222
			30	66	141
			30	95	203
			30	128	271
			30	36	77

Table 4 Continued

<i>In vitro</i>			MTD prediction		
Human IC <sub>90</sub> (nM)	Mouse IC <sub>90</sub> (nM)	IC <sub>90</sub> ratio (mouse/human)	Model A	Model B	Model C
			Predicted human MTD by MTD ratio (mg/m <sup>2</sup> /cycle)	Predicted human MTD by AUC at MTD (mg/m <sup>2</sup> /cycle)	Predicted human MTD by free fraction of AUC at MTD (mg/m <sup>2</sup> /cycle)
5.9	13.6	2.3	83	17	13
			83	10	7.9
			15	1.5	1.2
			15	1.9	1.4
			15	9.4	7.2

Table 5 Continued

<i>In vitro</i>			MTD prediction		
Human IC <sub>90</sub> (nM)	Mouse IC <sub>90</sub> (nM)	IC <sub>90</sub> ratio (mouse/human)	Model A	Model B	Model C
			Predicted human MTD by MTD ratio (mg/m <sup>2</sup> /cycle)	Predicted human MTD by AUC at MTD (mg/m <sup>2</sup> /cycle)	Predicted human MTD by free fraction of AUC at MTD (mg/m <sup>2</sup> /cycle)
39	381	9.8	7.2	9.8	10
			7.2	7.4	7.6
			7.2	8.8	9.1
			7.2	2.3	2.4
			7.2	2.7	2.8
			7.2	3.8	3.9

Table 6 Predicted human MTD values of 9-aminocamptothecin from *in vitro* CFU-GM assay data

<i>In vivo</i>								
Species	Schedule	Human MTD (mg/m <sup>2</sup> cycle)	Human AUC at MTD (ng·h/mL)	Free fraction of AUC at MTD (ng·h/mL)	MTD ratio (mouse/human)	AUC ratio at MTD (mouse/human)	Free fraction of AUC ratio at MTD (mouse/human)	Ref.
Mouse	Multiple	19.5	471	57				36
Human	c.i.v. over 72 h	3.4	1805*	5.4	5.8	0.3	10	4
	c.i.v. over 24 h	1.7	1357	4.1	12	0.3	14	37
	c.i.v. over 72 h†	3.7	1913	5.7	5.3	0.2	10	38
	c.i.v. over 120 h‡	3.0	4442	13	6.5	0.1	4.2	18
	c.i.v. over 120 h§	2.4	5588	17	8.1	0.1	3.4	18
	c.i.v. over 72 h	3.9	1648	4.9	5.0	0.3	11	39

Abbreviation: c.i.v., continuous intravenous infusion.

\* Total AUC values were calculated from lactone AUC with the ratio of lactone/total = 0.07 (Ref. 37).

† Clinical study for pediatric people.

‡ Colloidal dispersion formulation.

§ Dimethylacetamide/polyethylene glycol formulation.

Table 7 Predicted human MTD values of camptothecin from *in vitro* CFU-GM assay data

<i>In vivo</i>								
Species	Schedule	MTD (mg/m <sup>2</sup> /cycle)	AUC at MTD (µg·h/mL)	Free fraction of AUC at MTD (ng·h/mL)	MTD ratio (mouse/human)	AUC ratio at MTD (mouse/human)	Free fraction of AUC ratio at MTD (mouse/human)	Ref.
Mouse	Single	24	1.2	0.31				41, 42
Human	Daily for 5 days	75	66	0.24	0.32	0.02	1.3	19
	Single (weekly)	44	39*	0.14	0.55	0.03	2.2	20

\* AUC was calculated with the clearance value referring from Ref. 19.

prediction model did not incorporate the interspecies differences of total body clearance. This model is valuable when a prediction is needed before a clinical study and human pharmacokinetic information is not yet available. However, considering the accuracy of its prediction, the model would be better served by including pharmacokinetic factors, such as clearance and the plasma protein-binding ratio in each species. Our present retrospective analysis with each population group and several dosing schedules suggested that a prediction model requires both the free fraction of the target AUC at the MTD in terms of pharmacokinetic/pharmacodynamic analysis associated with the interspecies differences. The incorporation of model C may be beneficial to the pharmacokinetically guided drug development program, which is proposed by Collins *et al.* (44). After a careful collection and fitting of Model C to the preclinical pharmacokinetic/pharmacodynamic information obtained from *in vitro* and *in vivo* studies, the human MTD could be estimated using the preliminary pharmacokinetic data obtained from the first group of patients in the starting dose level of a Phase I study. Subsequent dose escalations could be made in an attempt to target the predicted AUC in a series of graded steps in subsequent patient cohorts. Therefore, the construction of a mechanism-based model plays an important role in reducing patient numbers to reach an MTD rather than the use of the

traditional dose-escalation system. An acceleration of dose escalation is also available, based on this theoretically accurate model.

For SN-38 (the active metabolite of CPT-11, which contributes to bone marrow suppression as the dose-limiting toxicity in Phase I studies), the model allowed for a successful prediction of the human MTD. Therefore, the prediction of the MTD with model C is applicable to the prodrug of an anticancer agent such as CPT-11. As seen in these studies, it is important to evaluate pharmacokinetic parameters such as AUC at the MTD and the plasma protein-binding ratio of the corresponding active metabolite. The applicability of the approach is limited to agents that have myelosuppression as their dose limiting toxicity and for those that have relatively well-behaved pharmacokinetic/pharmacodynamic relationships. In other words, the pharmacokinetics have to be generally predictable, and the pharmacodynamic response has to be correlated with the plasma drug exposures (or free fraction drug exposure). Further improvements in these correlations might be achieved in the case of the camptothecins by accounting for the degree of plasma lactone stability that can differ for various camptothecin derivatives (45). However, increasing the complexity of the predictive model may or may not additionally improve things. The approach of the construction of the predicting model should be

Table 6 Continued

<i>In vitro</i>			MTD prediction		
Human IC <sub>90</sub> (nM)	Mouse IC <sub>90</sub> (nM)	IC <sub>90</sub> ratio (mouse/human)	Model A	Model B	Model C
			Predicted human MTD by MTD ratio (mg/m <sup>2</sup> /cycle)	Predicted human MTD by AUC at MTD (mg/m <sup>2</sup> /cycle)	Predicted human MTD by free fraction of AUC at MTD (mg/m <sup>2</sup> /cycle)
6.2	66	10.6	1.8	0.1	3.3
			1.8	0.1	2.2
			1.8	0.1	3.4
			1.8	0.03	1.2
			1.8	0.02	0.8
			1.8	0.11	4.2

Table 7 Continued

<i>In vitro</i>			MTD prediction		
Human IC <sub>90</sub> (nM)	Mouse IC <sub>90</sub> (nM)	IC <sub>90</sub> ratio (mouse/human)	Model A	Model B	Model C
			Predicted human MTD by MTD ratio (mg/m <sup>2</sup> /cycle)	Predicted human MTD by total AUC at MTD (mg/m <sup>2</sup> /cycle)	Predicted human MTD by free fraction of AUC at MTD (mg/m <sup>2</sup> /cycle)
29	67	2.3	10	1	43
			10	1	42

broadly applicable throughout drug development in cancer chemotherapy.

In conclusion, for this family of camptothecin derivatives, model C appeared to be superior to the other models of human MTD prediction, because it takes into account *in vivo* toxicity

and pharmacokinetic data such as AUC at the MTD and plasma protein binding in human and experimental animals, as well as *in vitro* data of hematopoietic progenitor cells. By identifying the safety margins of these compounds in humans at an earlier stage, it should be possible to conduct dose escalation safely and

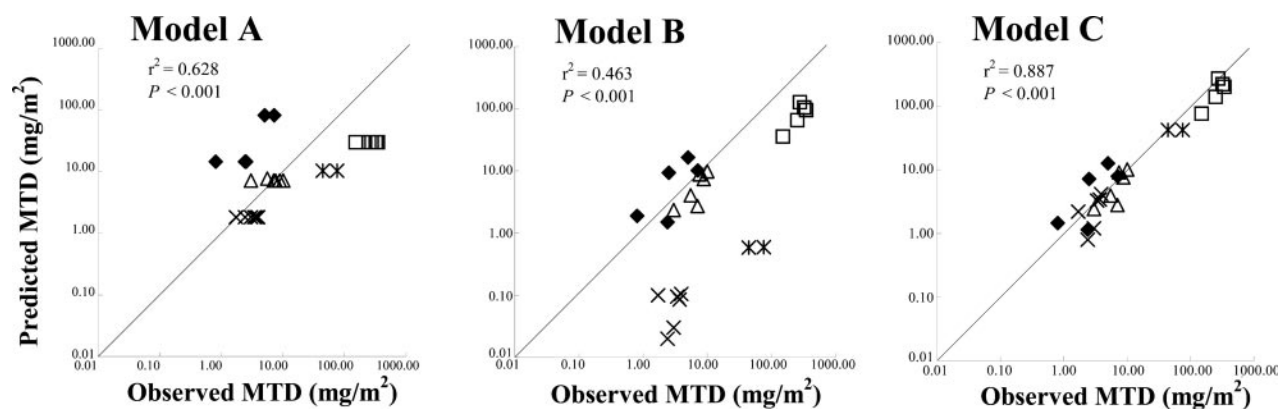


Fig. 3 Prediction of human MTD from murine MTD and *in vitro* CFU-GM assay for camptothecin derivatives. ◆, DX-8951f; □, CPT-11; △, TPT; ×, 9AC; ✕, CAM.

Table 8 Summary of inhibition of CFU-GM with SN-38, DX-8951f, and other camptothecin derivatives using human, murine, and canine bone marrow

Compound	IC <sub>50</sub> (nM)			IC <sub>90</sub> (nM)			Ref.
	Human	Murine	Canine	Human	Murine	Canine	
SN-38	7.7	54	5.9	17.5	191	13.4	This work
DX-8951f	1.2	5.1	0.93	5.9	13.6	5.0	This work
CAM lactone*	1.7	18	0.5	16	42	7.6	(7)
CAM†	ND	17	ND	29	67	6.9	(7)
TPT	2.8	128	1.7	39	381	7.6	(7)
9-AC	0.6	20	0.3	6.2	66	7.6	(7)

Abbreviation: ND, no data.

\* CAM lactone, NSC-94600.

† CAM, NSC-100880.

efficiently in clinical studies of new anticancer drugs and, thus, reduce the number of patients treated at significantly lower doses than potentially therapeutic doses.

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