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ORIGINAL ARTICLE

Preclinical pharmacokinetic/pharmacodynamic models to predict synergistic effects of co-administered anti-cancer agents

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Abstract
Purpose Pharmacokinetic/pharmacodynamic (PK/PD) models have been shown to be useful in predicting tumor growth rates in mouse xenografts. We applied novel PK/PD models to the published anticancer combination therapies of tumor growth inhibition to simulate synergistic changes in tumor growth rates. The parameters from the PK/PD model were further used to estimate clinical doses of the combination.
Methods A PK/PD model was built that linked the dosing regimen of a compound to the inhibition of tumor growth in mouse xenograft models. Two subsequent PK/PD models were developed to simulate the published tumor growth profiles of combination treatments. Model I predicts the tumor growth curve assuming that the effect of two anticancer drugs, AZD7762 and irinotecan, is synergistic when given in combination. Model II predicts the tumor growth curve assuming that the effect of co-administering flavopiridol and irinotecan is maximally synergistic when dosed at an optimal interval.
Results Model I was able to account for the synergistic effects of AZD7762 following the administration of irinotecan. When Model II was applied to the antitumor activity of irinotecan and flavopiridol combination therapy, the modeling was able to reproduce the optimal dosing interval between administrations of the compounds. Furthermore, Model II was able to estimate the biologically active dose of flavopiridol recommended for phase II studies.
Conclusions The timing of clinical combination therapy doses is often selected empirically. PK/PD models provide a theoretical structure useful in the design of the optimal clinical dose, frequency of administration and the optimal timing of administration between anticancer agents to maximize tumor suppression.

Keywords Xenograft mice · PK–PD · Combination therapy · Tumor growth

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Introduction

In vivo evaluation of pharmacological activity in a preclinical disease model is a key driver for a candidate drug to advance from drug discovery to development. In oncology, the predominant model of preclinical antitumor efficacy is the xenograft model. Traditional approaches to

measurement of efficacy have been based on the ratio of the tumor volumes/weight in treated and control animals at a specific end point. Recently, pharmacokinetic–pharmacodynamic (PK/PD) mathematical models have been proposed [10, 13, 14, 17] that provide time-dependent quantitative estimates of the suppression of tumor growth rates of a single compound. These models are capable of predicting the antitumor effect of a single compound as a function of the dosing regimen. The model-derived parameters describing the potency of the tested compounds coupled with PK parameters measured in phase I trials have been shown to predict efficacious doses for standard chemotherapies [14].

Many, if not most, oncology drugs are used in combination with multiple other agents [16, 21, 22]. Therefore, the development of PK/PD models that predict the antitumor effects of combination therapies is critical. There is a great deal of interest in the concurrent use of a checkpoint kinase (Chk, CHEK) inhibitor with DNA-damaging agents [21] to potentiate their chemo-therapeutic effect [8, 9, 21]. In response to DNA damage, the two phoshatidylinositol 3-kinases family members (ATM and ATR) activate Chk1. Chk1-mediated signaling then leads to S phase or G2/M cell-cycle arrest allowing for DNA repair prior to the next cell division. A Chk1 inhibitor would consequently permit a cell with damaged DNA to progress through the cell-cycle ultimately leading to mitotic catastrophe and/or apoptosis. One such proposed combination therapy combines the Chk inhibitor AZD7762 with the topoisomerase I inhibitor irinotecan. A second potentially useful combination therapy that has been demonstrated to be effective both in a mouse xenograft model and in a clinical setting is co-administering flavopiridol (a Cdk inhibitor) and irinotecan (a topoisomerase I inhibitor) [15, 16]. For this combination to work effectively, it was shown that the interval of flavopiridol administration following irinotecan (or CPT11) dosing is critical for the maximal suppression of the tumors in xenografts [11]. Therefore, there is interest not only in understanding the impact of combination therapy on tumor growth rates in xenografts but also understanding the optimal interval of dosing between agents in combination therapies. This information could then be used to determine schedules to be tested clinically.

The monotherapy-based PK/PD mathematical models currently available in the literature have limited power to predict the tumor growth rates in xenograft models being treated with combination therapies. This communication describes the development of models using tumor growth rates from published preclinical in vivo combination studies of irinotecan-AZD7762 and irinotecan-flavopiridol to simulate changes in tumor growth rates following administration of two anticancer agents. The goal of this work was to demonstrate that empirical PK/PD mathematical models could be used to predict the synergistic effects of specific combinations and to optimize dosing intervals of these particular drug combinations; a more ambitious goal of developing a general mechanistic model of combination therapies remains to be addressed. The PK/PD models can serve as a guide to aid the selection of clinical doses and the optimal timing of administration between these anticancer agents to maximize tumor suppression.

Materials and methods

Chemicals

For the PK studies in this manuscript, flavopiridol was purchased from Sigma–Aldrich (St. Louis, MO, USA) and the checkpoint kinase inhibitor AZ7762 was synthesized by AstraZeneca discovery chemistry (Waltham, MA, USA). All commercially available reagents and solvents were of either analytical or high-performance liquid chromatography grade and were purchased from Sigma–Aldrich.

Pharmacodynamic studies

Detailed in vivo PK/PD studies for irinotecan in combination with a checkpoint kinase inhibitor (AZD7762) in mouse xenografts were reported previously by Zabludoff et al. [21]. According to the published study, SW620 tumor cells (American Type Culture Collection) (3×10^6–6×10^6 cells) were implanted subcutaneously into the right flank of mice. Tumors were allowed to grow to $\sim$100 to 200 mm^3 before the administration of irinotecan alone (25 or 50 mg/kg) or irinotecan (25 or 50 mg/kg) followed by two doses of AZD7762 (25 mg/kg; 2 and 14 h after irinotecan dose). Tumor volumes were measured with calipers. The data published in these studies was used in our mathematical model.

In vivo PK/PD studies in tumor-bearing mice with irinotecan and flavopiridol combination were reported by Motwani et al. [11]. According to the published study, athymic-NCr-nu male mice were inoculated subcutaneously in the flank with minced p21-intact Hct116 tumor cells mixed with Matrigel (Becton–Dickinson, San Jose, CA, USA). Treatment was initiated on the third day after the inoculation of tumors. Mice were treated with the maximum-tolerated dose of either a single agent or in combination. Mice received irinotecan (CPT-11) alone (100 mg/kg), flavopiridol alone (11 mg/kg), or CPT-11 (100 mg/kg) followed by flavopiridol (3 mg/kg) 4, 7, 16, or 24 h later. Mice in the control group were given vehicle (PBS) alone. All drugs were administered via the intraperitoneal (ip) route twice a week for a total of five injections. Tumor volume was measured every 3–4 days with calipers. The data published

Table 1 Bioanalytical conditions of plasma analysis of flavopiridol and AZD7762

Compound	Sample prep	Column	Mobile phase	LC–MS/MS		LOQ (ng/mL)
				Ionization mode	MRM transitions	
Flavopiridol	Protein precipitation	Phenomenex Synergi Hydro- RP 80A 4 μm 30 × 2.0 mm	A: water + 0.1% formic acid + 10 mM ammonium formate B: acetonitrile + 0.1% formic acid	ESI+	402.2/341.2	1
AZD7762	Protein precipitation	Waters Atlantis dC18 3um 50 × 3.0 mm	A: water + 0.1% formic acid B: acetonitrile + 0.1% formic acid	APCI+	363.2/220.3	3

in these studies of flavopiridol and irinotecan combination were used in our mathematical model.

Pharmacokinetic studies

The pharmacokinetics of AZD7762 and flavopiridol were determined in separate groups of mice ($n = 27$). All procedures were conducted in accordance within the guidelines approved by Astrazeneca's Institutional Animal Care and Use Committee. Flavopiridol was administered through the ip route at 3 mg/kg and AZD7762 by the intravenous (iv) route in mice at 3 mg/kg. Plasma samples for the flavopiridol and AZD7762 were assayed using LC–MS–MS technique. The bioanalytical conditions for the analysis are reported in Table 1. The PK of flavopiridol and AZD7762 were analyzed using standard extravascular and iv bolus two-compartmental models respectively using WinNonLin (Pharsight, CA, USA) without any weighting, and the PK parameters are summarized in Table 2. The PK parameters of irinotecan were taken from the literature [14]. Irinotecan was dosed via the iv route for the AZD7762–irinotecan combination, and it was dosed via the ip route for the flavopiridol–irinotecan combination. Since the iv and ip routes yield similar exposure profiles [7], the PK parameters of irinotecan for the ip route was assumed to be similar to the literature values and the absorption rate constant (k_{01}) was assumed to be similar to those estimates for flavopiridol.

PK/PD combination models

The mathematical model published by Simeoni et al. [14, 17] was modified to account for the combination therapy. The structures of these combination therapy models are summarized in Fig. 1.

Model I describes a synergistic effect on tumor growth between two agents delivered in combination such as that observed for combination therapy with irinotecan and AZD7762. The model is a system of differential equations that describe the volume of rapidly growing tumor cells (Z_1) or cells that have arrested growth (Z_2, Z_3, and Z_4) due to the effect of the antitumor agent(s) at concentration C_1 or C_2. Cells in the arrested state are then recruited into a cell death process following a delay. For the purpose of this model, it is assumed that dead cells are of zero volume:

$$\frac{dZ_1(t)}{dt} = \frac{\lambda_0 Z_1(t)}{\left[\left(1 + \frac{\lambda_0}{\lambda_1} W(t)\right)^{\phi}\right]^{\frac{1}{\phi}}} - k_2 C_1(t) Z_1(t) - k_3 C_2(t) Z_1(t) - k_2 C_1(t) k_3 C_2(t) Z_1(t) \quad (1)$$

$$\frac{dZ_2(t)}{dt} = k_2 C_1(t) Z_1(t) + k_3 C_2(t) Z_1(t) + k_2 C_1(t)_3 C_2(t) Z_1(t) - k_1 Z_2(t) \quad (2)$$

$$\frac{dZ_3(t)}{dt} = -k_1 Z_3(t) + k_1 Z_2(t) \quad (3)$$

$$\frac{dZ_4(t)}{dt} = -k_1 Z_4(t) + k_1 Z_3(t). \quad (4)$$

The variable C_1 is the concentration of drug 1 (irinotecan) and C_2 is the concentration of drug 2 (AZD7762), k_2 is the antitumor potency constant of drug 1 (irinotecan) and k_3 is the antitumor potency constant of drug 2 (AZD7762). In the model, the parameter $\psi = 20$ modulates the transition from exponential to linear growth, $W(t)$ represents the derivative of tumor weight in non-treated animals, λ_0 is rate of exponential growth and λ_1 is rate of linear growth as published by Simeoni et al. [14, 17]. The delay function, which is responsible for the death rate of damaged cells, is described by the constant k_1. Tumor volume is described as:

$$V_t = \sum_{n=1}^{4} Z_n. \quad (5)$$

Model II predicts the growth curve in a xenograft model assuming that drug 2 (flavopiridol) only works in the presence of drug 1 (irinotecan) and the maximal antitumor effect occurs at an optimal dosing interval between 1 and 2 administration.

The equations, modified from those above, that describe this combination effect are:

Table 2 Pharmacokinetic parameters along with % CV of flavopiridol and AZD7762 using two-compartment model

Compound	V_1 or V_1/F (L/kg)	k_{10} (hr^{-1})	k_{12} (hr^{-1})	k_{21} (hr^{-1})	k_{01} (hr^{-1})
Flavopiridol	1.6 (1,383%)	1 (922%)	1.7 (164%)	1.3 (700%)	11 (139%)
AZD7762	4.3 (11.2%)	0.59 (18.9%)	0.46 (77.5%)	0.57 (104%)	

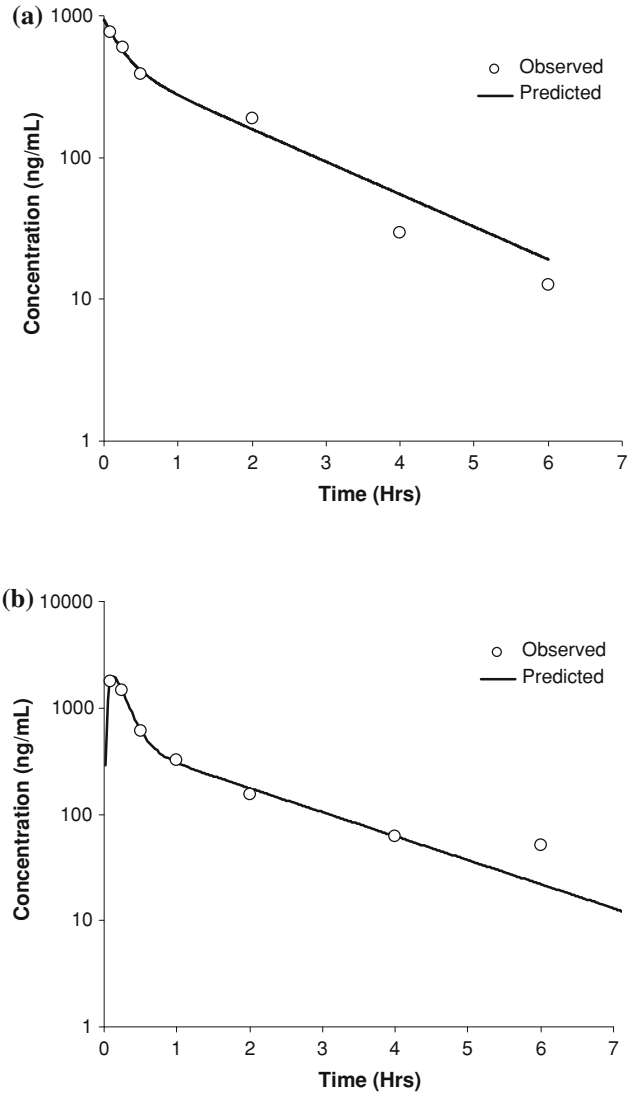

Fig. 1 **a** Pharmacokinetics and two-compartment model simulation of AZD7762 in CD-1 mice following 3 mg/kg iv bolus dose. **b** Pharmacokinetics and two-compartment model simulation of flavopiridol in CD-1 mice following 3 mg/kg ip dose

$$\frac{dZ_1(t)}{dt} = \frac{\lambda_0 Z_1(t)}{\left[\left(1+\frac{\lambda_0}{\lambda_1}W(t)\right)^{\phi}\right]^{\frac{1}{\phi}}} - k_2 C_1(t) Z_1(t) - k_2 C_1(t+lagt) k_3 C_2(t) Z_1(t) \quad (6)$$

$$\frac{dZ_2(t)}{dt} = k_2 C_1(t) Z_1(t) + k_2 C_1(t+\mathrm{lag}t) k_3 C_2(t) Z_1(t) - k_1 Z_2(t) \quad (7)$$

$$\frac{dZ_3(t)}{dt} = -k_1 Z_3(t) + k_1 Z_2(t) \quad (8)$$

$$\frac{dZ_4(t)}{dt} = -k_1 Z_4(t) + k_1 Z_3(t) \quad (9)$$

where, C_1 is the concentration of drug 1 (irinotecan) and C_2 is the concentration of drug 2 (flavopiridol), k_2 is the antitumor potency of drug 1 (irinotecan) and k_3 is the antitumor potency of drug 2 (flavopiridol). The variable lag$t$ is the lag time between doses of drug 1 and drug 2. Tumor volume is described in Eq. 5 and the rest of the parameters in Model II are similar to the one described in Model I.

Model simulations and data analysis

The dosing schedules reported by Zubladoff et al. [21] and Motwani et al. [11] used in the xenograft mice studies along with the PK parameters of the anticancer drugs generated or obtained from the literature were used in the PK/PD models to simulate plasma concentration time profiles. The vehicle control group predicts the rate of growth (λ_0) of the cancer cells [14]. The rate at which an anticancer compound suppresses these growing cells is given by the rate constant k_2 or k_3. The ratio of the growth rate to killing rate is called the threshold concentration (C_T) and determines the antitumor potency of the compound [14]. Maintaining steady-state levels of anticancer compound above this threshold concentration lead to tumor eradication [14].

The initial values for the PK/PD parameters of the combination therapy models were estimated manually using the Berkeley Madonna (Kagi, Berkeley, CA, USA) program to simulate the PK/PD model, which allowed us to interactively varying individual parameter values and evaluate the resulting fit in real-time, as described below for Model I and Model II.

Model I The initial values of λ_0, k_2 and k_3 were calculated by fitting the model parameters to the data for the vehicle, irinotecan, and AZD7762 data sets. The initial values of the growth rate parameters (λ_0 and λ_1) were estimated by fitting the model to the data for the vehicle group that received no treatment. The initial values of killing rates for irinotecan (k_2 and k_1) were estimated by fitting those parameters to the data from the 25 mg/kg irinotecan treatment group (Fig. 3), while using the previously determined growth rate parameter values. The initial

Table 3 PK/PD parameters of Model I and Model II

Parameter	Irinotecan + Flavopiridol combination (estimate) (low–high estimate reflecting 5% change in RMSE)	Irinotecan + AZD7762 combination (estimate) (low–high estimate reflecting 5% change in RMSE)	Note
k_1 (day^{-1})	0.312 (0.218 to >31.2)	0.168 (<0.168–0.252)	Transit rate constant
k_2 ($ng^{-1}ml\ day^{-1}$)	7.68×10^{-4} ($7.3–15.36 \times 10^{-4}$)	5.78×10^{-4} ($4.62–14.45 \times 10^{-4}$)	Irinotecan potency
k_3 ($ng^{-1}ml\ day^{-1}$)	23.04×10^{-4} (3.45 to $>23.04 \times 10^{-4}$)	8.64×10^{-4} ($2.59–1.3 \times 10^{-4}$)	Co-administered drug potency
λ_0 (day^{-1})	0.144 (0.13–0.216)	0.072 (0.065–0.144)	Control/vehicle tumor exponential growth phase
λ_1 ($g\ day^{-1}$)	0.019 (0.008 to >1.9)	0.019 (0.013–0.024)	Control/vehicle tumor linear growth phase
W_0 (g)	0.009 (0.007–0.0135)	0.054 (0.043–0.016)	Initial tumor weight
lag*t* (h)	9.8 (4.9–19.6)		Optimal interval time between co-administration drugs

values of killing rates for AZD7762 (k_3) were estimated from the AZD7762 25 mg/kg treatment group (Fig. 3), once again using the growth rates determined from the vehicle group data. Using these initial estimates of the model parameters, the growth and killing rate parameter values were optimized to minimize the error of the global fit of the model to all the treatment groups (results are listed in Table 3) using the curve fit minimization function in Berkeley Madonna (Kagi, Berkeley, CA, USA).

Model II The initial values of λ_0 and k_2 were estimated by fitting the model parameters to the data for the vehicle and irinotecan treatment groups. The initial values of the growth (λ_0 and λ_1) were estimated by fitting the model to the data for the vehicle group that received no treatment. The initial values of killing rates for irinotecan (k_2 and k_1) were estimated by fitting those parameters to the data from the irinotecan treatment group (Fig. 4), while using the previously determined growth rate parameter values. Because flavopiridol only acts in the presence of irinotecan, the simulation of the flavopiridol given alone was similar to the vehicle group (Fig. 4). The initial estimate of the killing rate for flavopiridol (k_3) was made by fitting the model to the data from the irinotecan–flavopiridol combination studies (4 h interval combination, Fig. 4), using the growth rates determined from the vehicle group. Using these initial estimates, a global fitting procedure was performed by simultaneously fitting all the treatment groups along with the vehicle groups to predict the growth and killing rate constants (shown in Table III) using the curve fit minimization function in Berkeley Madonna (Kagi, Berkeley, CA, USA) to minimize the global error.

Since Berkeley Madonna does not provide the capability to perform a sensitivity analysis for the model parameters, we conducted sensitivity analyses for each of the model parameters using a manual procedure previously reported in the literature [4] (Appendix). The difference in the predicted and observed values of the tumor growth rates for Model I and Model II was assessed by root mean square error (RMSE). A 5% change in RMSE was used as the cut off to calculate the low and high values of the model parameters (Table 3).

Results

The pharmacokinetics of AZD7762 determined following iv administration to mice was well described by a two-compartment model with elimination from the central compartment (Fig. 1a). The PK parameters used to describe the plasma PK of AZD7762 in the PK/PD Model I are summarized in Table 2. The PK of flavopiridol in plasma following ip administration was described by a similar two-compartmental model with first order absorption kinetics (Fig. 1b). The PK parameters used to describe the plasma profile of flavopiridol in the PK/PD Model II are summarized in Table 2. Although some of the model parameters have high % coefficient of variation values following ip administration for flavopiridol (Table 2), the fit was reasonably good (Fig. 1b) and this did not affect the overall PK/PD model output of tumor growth rates.

Model I (Fig. 2) was applied to simulate the growth curves of AZD7762 and irinotecan administered both as individual therapies and in combination to the xenograft-bearing mice (Fig. 3). The symbols in Fig. 3 represent the data published by Zabludoff et al. [21] and the lines represent the simulations from Model I at the respective doses. The growth curves of tumors treated only with the vehicle provided control experiments from which we derived the growth parameters λ_0 and λ_1 (Table 3). The growth inhibition constant for irinotecan (k_2), assessed from growth kinetic curves from the mice dosed at 25 mg/(kg day) (Table 3), allowed for accurate estimation

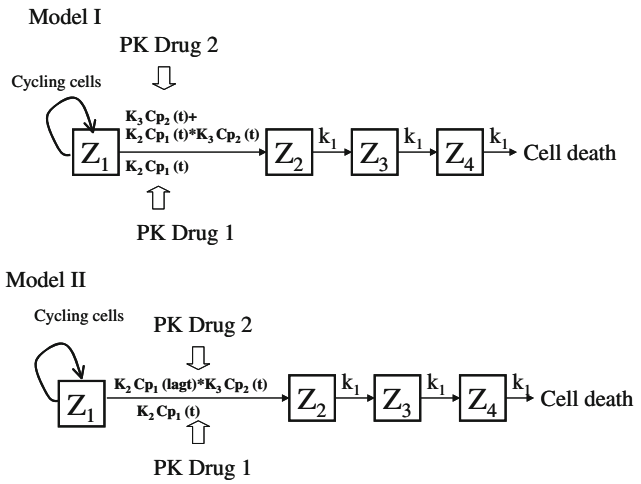

Fig. 2 Schematic representation of PK/PD models for combination antitumor therapy. Model I was used to describe the synergistic effects of the combination of AZD7762 (*Drug 2*) and irinotecan (*Drug 1*). Model II was used for time-dependent synergistic effect flavopiridol (*Drug 2*) and irinotecan (Drug 1) combination. The derivation of the differential equations is fully described in the "Materials and methods"

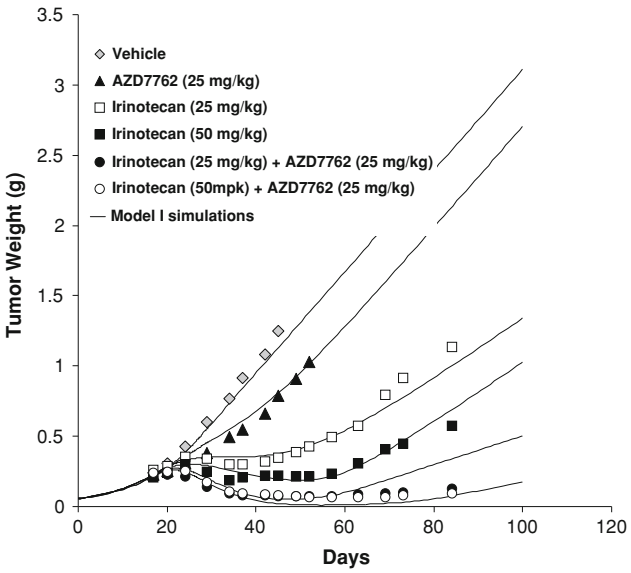

Fig. 3 Simulated (*lines*) and published (*symbols*) tumor growth kinetic data (Zabludoff et al.) following administration of irinotecan and the checkpoint abrogator AZD7762 either discretely or in combination to xenograft-bearing mice. PK of the individual compounds was simulated using compartmental models derived from PK studies in tumor-bearing mice. The PD Model I was used to simulate tumor growth kinetics

of tumor growth changes at the higher dose, 50 mg/(kg day). The growth inhibition constant values (antitumor potency) of irinotecan administered as a monotherapy in Model I was very similar to those previously reported in monotherapy studies by other groups [14]. The effect of AZD7762 on tumor growth when administered alone was modest but measurable (Fig. 3), and a constant (k_3) was derived from this data (Table 3). The effect of the combination of the two drugs was considered to be synergistic (i.e., a product) rather than additive given the marked change in growth curve following co-administration and tumor cures in the majority of animals (Fig. 3). Model I, when applied using the constants derived from monotherapy for each individual compound, was able to capture the synergistic effects of the AZD7762 and irinotecan combination therapy at two separate dose levels without further modification (Fig. 3) [14]. The simultaneous optimization of the Model I parameters to all of the tumor growth curves resulted in a global fit with a relatively low value of error (RMSE = 0.217).

Model II (Fig. 2) was developed to simulate the growth curves of tumors in xenograft-bearing mice treated with flavopiridol and irinotecan using both independent-administration and co-administration regimens (Fig. 4). The symbols in Fig. 4 represent the data that were published by Motwani et al. [11] and the lines represent the predictions from Model II at the respective doses. Because flavopiridol only acts in the presence of irinotecan, the prediction of the growth curves for the mice treated with flavopiridol monotherapy was similar to that for the vehicle-treated group (Fig. 4). The growth parameters λ_0 and λ_1 were derived from the vehicle control growth curves (Table 3). The constant for tumor growth inhibition by irinotecan (k_2), assessed from growth kinetic curves of mice dosed at 25 mg/(kg day) (Table 3), was similar to that estimated from the study described above and in the literature [14]. The effect of flavopiridol on tumor growth when administered alone was indistinguishable from that of the vehicle control (Fig. 4), and thus the constant (k_3) was obtained by fitting k_3 to the data for the group receiving the irinotecan and flavopiridol combination at 4 h interval (Table 3). The simultaneous optimization of the Model II parameters to all of the tumor growth curves resulted in a global fit with a relatively low value of error (RMSE = 0.011).

The timing of flavopiridol co-administration with irinotecan has been shown to have an impact on tumor suppression in preclinical xenograft-bearing tumor models [11]. The tumor growth rates were inhibited to a greater magnitude as the dose interval increased from 4 to 7 h. However, as the dose interval increased to 16 h, the tumor growth curves were less affected. Modeling of the combination therapy is made more complex by the variable period between irinotecan and flavopiridol doses. Incorporating a delay function in the synergistic effect term in Model II (Fig. 4) improved the quality of the model fit to the data. When the tumor size at maximal efficacy (circles) was plotted as a function of the dosing interval between irinotecan and flavopiridol from Motwani et al. published data [11], the simulations (lines) suggested that maximal

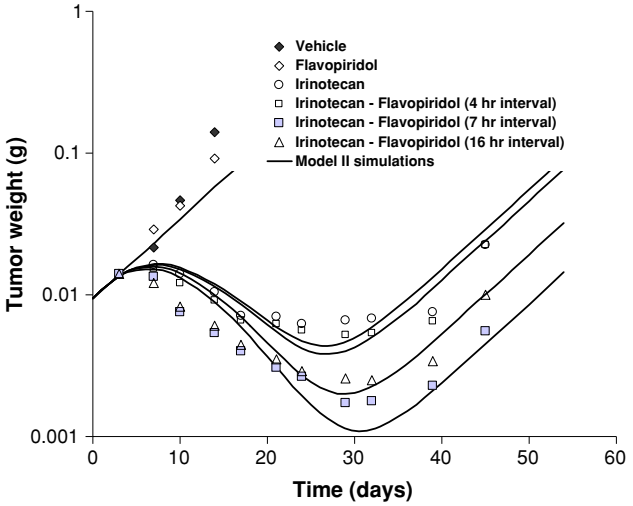

Fig. 4 Simulated (*lines*) and published (*symbols*) tumor growth kinetic data (Schwartz et al.) following administration of irinotecan and flavopiridol either discretely or in combination to xenograft-bearing mice. PK of the individual compounds was simulated using compartmental models derived from PK studies in tumor-bearing mice. The PD Model II was used to simulate tumor growth kinetics. Flavopiridol acts only in the presence of irinotecan so the simulations of flavopiridol given discretely overlap over the vehicle group

tumor suppression could be achieved at 9.8 h after the irinotecan dosing interval (Table 3).

Discussion

In recent years, there has been increased interest in the use of modeling and simulation in study design and interpretation/integration of data from preclinical and clinical antitumor studies [2, 19, 20]. These models, while of various design, all serve as a means of integrating information to facilitate dose/schedule selection and trial design. Importantly, the utility of these models as an aid to clinical decision-making has been accepted by regulatory agencies [3, 5, 12]. A recent FDA white paper emphasized the importance of integrating exposure–response information into the drug development process and recommended model-based drug development to aid decisions [1]. A dual transit compartment model of 5-fluorouracil and tegafur has accurately reproduced the additive response in a xenograft model [18]. This demonstrates the utility of combination PK/PD models to describe changes in tumor growth rates.

The present study provides an extension to the use of monotherapy PK/PD models [10, 13, 14, 17] to combination therapies of anticancer agents in preclinical tumor models. Our goal was to minimally elaborate these previous models to account for potential interactions when two compounds are co-administered. When the term $k_2 \times C_1 \times k_3 \times C_2 \times Z$ (1) in Eq. 2 is zero then Model I is equivalent to a previously reported additive model [18]. When the same term is greater than zero then Model I becomes a synergistic model. We included a new amplification parameter (γ) to investigate the possibility of superadditive synergy within the interaction term $k_2 \times C_1 \times k_3 \times C_2 \times Z \times \gamma$ (1) in Eq. 2. Since the value of this amplification parameter was close to unity (1.3), for the combination of AZD7762 and irinotecan, the additional parameter was not introduced to avoid over parameterization. In the case of AZD7762 and irinotecan, an additive model could not accurately reproduce the growth curves in the xenograft following co-administration, while Model I—which explicitly accounts for synergistic drug effects—accurately reproduced the effects of irinotecan and AZD7762 monotherapies to inhibit tumor growth as well as the synergistic effect due to their co-administration that leads to profound tumor suppression. This model may be used to identify the optimal timing and magnitude of the doses of the respective co-administered medications.

The preclinical studies with irinotecan and flavopiridol (Model II) were based on observations from in vitro studies which showed that SN-38, when followed by flavopiridol, gave the greatest enhancement of apoptosis when compared to a single agent or concomitant or the reverse sequence [11]. The published in vivo studies confirmed this observation but the intervals for this sequential therapy were in fact empirically selected. That is, the 4 to 7-h interval reported in preclinical studies, although effective was essentially an empirical finding. Our model rationally suggests (based on changes in growth kinetics), an optimal period of 8–10 h between doses (Table 3), which compares favourably with the 7-h interval currently used in clinical studies [16]. Due to the limitation of the data between 7 and 14 h only assumptions can be made based on the in vivo data as to the correct dosing interval. Especially in a drug discovery setting where only minimal data are often generated, these models can be significantly be helpful in designing future dosing intervals to be tested preclinically. Based on these findings with additional information about the effect of dosing intervals, it may be possible to guide clinical study design by the simulation of such co-administration studies.

We were also able to demonstrate that such PK/PD models may be useful to estimate the magnitude of the phase II biological effective dose of a potentiator drug when given in combination with cytotoxic agents. In recent communications, Rochetti et al. [14] demonstrated that for several antitumor monotherapeutics, the relationship between logarithm of cumulative effective clinical dose and the logarithm of the cross product of human systemic clearance,

Table 4 Predicted and clinical doses of flavopiridol and irinotecan in phase II studies

	Predicted weekly clinical dose (mg/m^2)	Weekly clinical dose (mg/m^2)
Irinotecan (Irinotecan + flavopiridol study)	110	83–116
Irinotecan (Irinotecan + AZD7762 study)	70	83-117
Flavopiridol	40	50

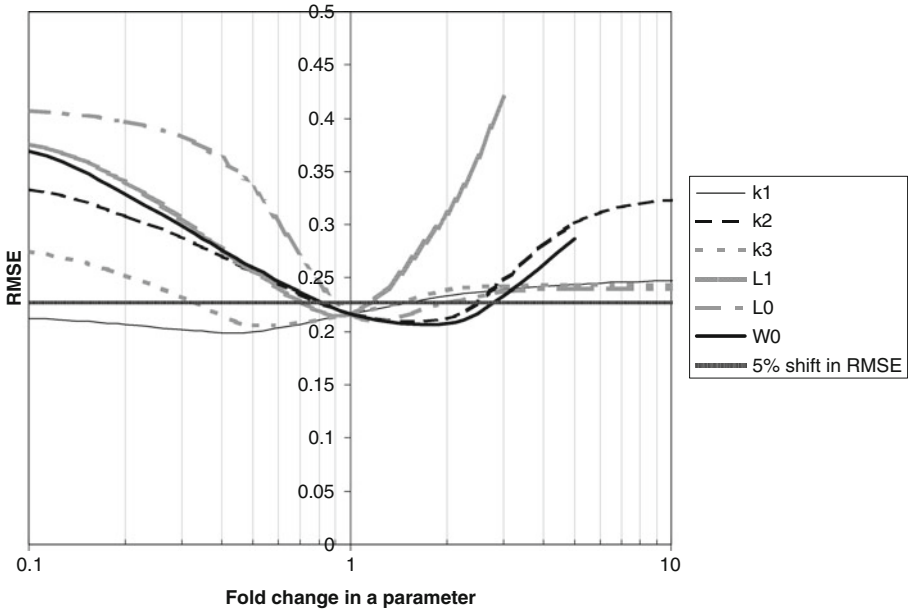

Fig. 5 Sensitivity analysis of various parameters for Model I

Cl_{sys} and C_T, ($C_T = \lambda_0 \backslash k_2$) was approximately linear. Thus, the measurement of this cross product $C_T \times Cl_{sys}$ may be used to estimate the magnitude of an effective clinical dose. These threshold values calculated for irinotecan for model I and model II were very similar to the ones observed in the literature [14]. Using the parameter $C_T \times Cl_{sy}$ derived from the current irinotecan studies, we estimated effective irinotecan doses of 70 and 110 mg/(m^2 week), which compare favorably with literature values of 80–120 mg/(m^2 week) (Table 4) [14]. By extension, Model II estimates an effective flavopiridol dose of 40 mg/(m^2 week), which is similar to the dose of 50 mg/(m^2 week), recently recommended for phase II studies (Table 4) [16]. If this relationship is demonstrated to hold across multiple therapeutics, it is conceivable that advanced models of human drug clearance [6] and preclinical tumor models may be combined to make early preclinical "go-no go" decisions.

Our objective in this work was to make incremental changes to an existing clinically relevant model to better describe combination therapy. Our priority was to preserve the simplicity of the initial model published by Simeoni et al. [14, 17], because incorporation of additional parameters would reduce the sensitivity of the model parameters to the data. The values of the parameters k_1 and λ_0 differed between models I and model II (Table 3). We ascribe this to intrinsic differences in the two cancer cell lines used in these studies, which we would expect to differ in their growth and death rates. Similarly, we would expect both cell lines to differ in their susceptibilities to chemotherapeutic drugs. Nevertheless, we used a single value of k_1 for both drugs in each of the models, as the resulting fits of the models were satisfactory. We also assumed that flavopiridol can only act in the presence of irinotecan, however, it is possible that flavopiridol may posses antitumor activity that this model would not represent. Finally, the form of the time-delay parameter *t*lag in model II is admittedly simplistic; while more complex models could be proposed, we wanted to determine the performance of the simplest possible model. As a result of these approximations, these models are likely not generally applicable to simulate the effects of all possible drug combination therapies, but rather they are specific to the drugs and dosing regimens described in this study.

Although these findings were described for these two specific combination therapies, we feel that such a PK/PD model will be useful not only to reproduce tumor growth rates in xenografts but also to simulate the dosing interval

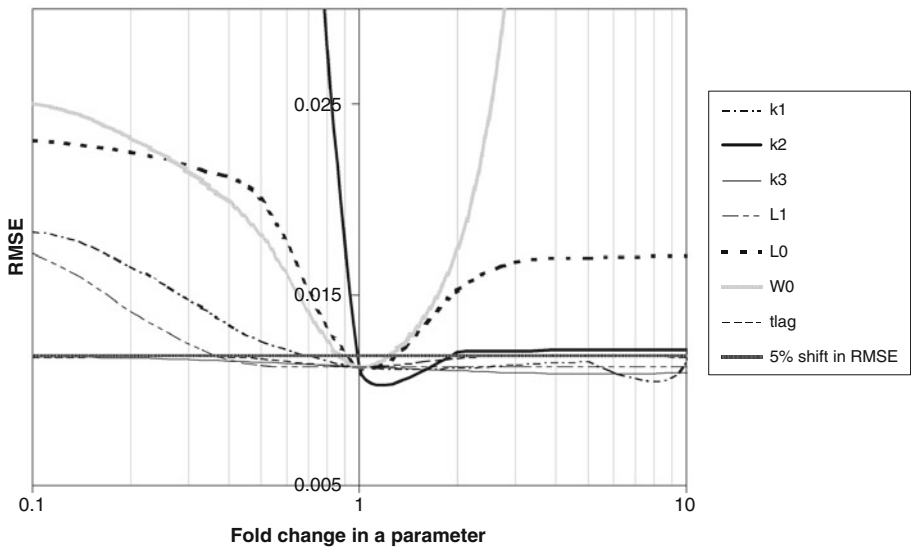

Fig. 6 Sensitivity analysis of various parameters for Model II

and schedules of combination therapies to maximize tumor suppression. These show that such mathematical PK/PD models can rapidly help to explain and optimize tumor growth dynamics following combination therapies in a rational manner compared with the traditional empirical approach which may aid in the estimation of the magnitude of phase II effective doses.

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Appendix

Both Model I and Model II were able to quantitatively describe several experimental data sets. Each parameter in these models was incrementally decreased and increased from the optimized fit and the effect on RMSE was evaluated. The sensitivity analysis of each of these parameters for Model I is given in Fig. 5. The sensitivity analysis of each of the parameters for Model II is given in Fig. 6. The sensitivity analysis shows which parameters are more sensitive to the RMSE. Based on this analysis a 5% shift in RMSE was considered to be the cut off to calculate the low and high value of the model parameter (Figs. 5, 6). These values low and high values for each model parameter estimates is given in Table 3. Parameters k_1 was least sensitive in model I, whereas parameters k_1, k_3 and λ_1 were less sensitive in model II. Although the low and high values were large for some parameters, the model does captures the tumor growth profiles when the drugs are given in combination. With limited data generated in drug discovery it is hard to get certainity around a parameter estimate, but the message of the utility of the models for such combinations remains.

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