Predictive Pharmacokinetic-Pharmacodynamic Modeling of Tumor Growth Kinetics in Xenograft Models after Administration of Anticancer Agents

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ABSTRACT

The available mathematical models describing tumor growth and the effect of anticancer treatments on tumors in animals are of limited use within the drug industry. A simple and effective model would allow applying quantitative thinking to the preclinical development of oncology drugs. In this article, a minimal pharmacokinetic-pharmacodynamic model is presented, based on a system of ordinary differential equations that link the dosing regimen of a compound to the tumor growth in animal models. The growth of tumors in nontreated animals is described by an exponential growth followed by a linear growth. In treated animals, the tumor growth rate is decreased by a factor proportional to both drug concentration and number of proliferating tumor cells. A transit compartmental system is used to model the process of cell death, which occurs at later times. The parameters of the pharmacodynamic model are related to the growth characteristics of the tumor, to the drug potency, and to the kinetics of the tumor cell death. Therefore, such parameters can be used for ranking compounds based on their potency and for evaluating potential differences in the tumor cell death process. The model was extensively tested on discovery candidates and known anticancer drugs. It fitted well the experimental data, providing reliable parameter estimates. On the basis of the parameters estimated in a first experiment, the model successfully predicted the response of tumors exposed to drugs given at different dose levels and/or schedules. It is, thus, possible to use the model prospectively, optimizing the design of new experiments.

INTRODUCTION

A fundamental step of the preclinical development of oncology drugs is the in vivo evaluation of the antitumor effect. For this purpose, a series of experiments are performed, in which tumor cells from immortalized cell lines are inoculated into athymic mice. Tumor volumes are measured at different times throughout the experiment in all of the animals, treated either with a vehicle (control) or with an active drug. The effect of the active molecule is then measured by comparing the average tumor weights in treated and control animals at the end of the experiment, or by recording the animals surviving their disease (1–4). This approach can be used to select the most potent candidate within a series using the same dosing regimen, or the most appropriate dosing regimen among those tested for a specific compound. However, in this way, the time course of the tumor growth is often neglected, so that only partial use of the information available from the experiment is made.

A pharmacokinetic-pharmacodynamic (PK-PD) model, linking the administration regimen of a candidate to tumor growth dynamics, would greatly improve the preclinical development of oncology drugs. For developing this kind of model, the first step is the definition of a mathematical model describing the progression of the disease (5). A number of tumor growth models are reported in the literature, reflecting different paradigms. Empirical models use mathematical equations (e.g., sigmoid functions, such as logistic, Verhulst, Gompertz, and von Bertalanffy; Refs. 6, 7) to describe the tumor growth curve, without an in-depth mechanistic description of the underlying physiological processes. In this context, the effect of a drug can be evaluated only in terms of changes of the parameter values describing the tumor growth. Such changes depend on the dose level and the administration schedule, so that these approaches can be applied only retrospectively and not as predictive tools when used outside the tested regimens.

FUNCTIONAL MODELS, conversely, are based on mechanistic, physiology-based hypotheses. They make a set of assumptions about the tumor growth, involving cell-cycle kinetics and biochemical processes, such as those related to antiangiogenic and/or immunological responses (7, 8). Such models usually represent the cell population in its heterogeneity, splitting it into at least two subpopulations: the proliferating and the quiescent cells. More complex models describe the cell population as age-structured and take into account subpopulations related to specific phases of the cell cycle. These models have a much larger number of parameters compared with the empirical ones. Their development is time consuming and a number of quantitative observations (e.g., flow cytometry analyses, biochemical and immunological marker measurements, and so forth) are required to avoid the identifiability problems due to the overparameterization. The situation becomes even more complex when the effect of the treatment with an anticancer drug is considered (9–12), also because of the incomplete knowledge of the mode of action in vivo. As a consequence, these models are rarely used in industrial drug research.

In conclusion, despite the existence of several tumor growth models, a practical tool that supports oncology drug development is still missing. In this respect, the only metrics of success are its application to the experimental data and the savings of experiments, time, costs, resources, and animal requirements. In this article, we describe a model that is an effective compromise between empirical and mechanistic-based approaches. This model is currently used with success in the preclinical development of a number of oncology drug candidates. It relies on a few identifiable and biologically relevant parameters, the estimation of which requires only the data typically available in the preclinical setting: the pharmacokinetics of the anticancer agents and the tumor growth curves in vivo.

MATERIALS AND METHODS

Experimental Methods

Compounds

Paclitaxel and 5-fluorouracil (5-FU), which are commercially available; irinotecan (CPT-11), Drug A and Drug B, which have been synthesized by Pharmacia (purity, >95%), were used.

Animals

Female Hsd, athymic nude-nu mice, 5–6 weeks of age (20–22 g), were obtained from Harlan, S. Pietro al Natisone, Italy. Animals were maintained in cages with paper filter covers, sterilized food and bedding, and acidified water.
All of the animal experiments were conducted in accordance with the current best practices and ethic principles.

**In Vivo Tumor Growth Experiments**

A2780 human ovarian carcinoma and HCT116 colon carcinoma cell lines (from American Type Culture Collection) were maintained by s.c. transplantation in athymic mice using 20–30 mg of tumor brei. For the experiments, tumors were excised and fragments were implanted s.c. into the left flank. One week after tumor inoculation, mice bearing a palpable tumor (~100–200 mm³) were selected and randomized into control and treated groups; tumor weight mean for all of the groups was ~0.15 g. One to 6 days after randomization (i.e., from Day 8 to 13 of the experiment), the treatment with the anticancer compounds started.

Mice were clinically evaluated daily and were weighed two times weekly. Dimensions of the tumors were measured regularly by caliper during the experiments (typically from once daily to once every 4 days), and tumor masses were calculated as follows:

\[
\text{Tumor weight (mg)} = \frac{\text{length (mm)} \cdot \text{width}^2 \text{(mm)}^2}{2} \rho \quad \text{(A)}
\]

assuming density \(\rho = 1 \text{ mg/mm}^3\) for tumor tissue.

**Drug Treatments**

All of the drugs were prepared immediately before use, and treatments were given i.v. at a dose volume of 10 ml/kg.

- CPT-11 (i.v. bolus) was given as a water solution to two groups of five HCT116 tumor-bearing mice; the drug was given as a single dose at the dose levels of 45 and 60 mg/kg on Day 13.
- Paclitaxel (i.v. bolus) was given in two different experiments as an alcoholic solution of Cremophor (Cremophor dose, 1.2 ml/kg) starting either one day (Day 8 of experiment 1) or 6 days (Day 13 of experiment 2) after randomization. In both cases, the drug was given once every 4 days for 3 days (4d×3) at the dose level of 30 mg/kg to groups of eight animals bearing A2780 tumors.
- 5-FU was given as an i.v. bolus to two groups of eight HCT116 tumor-bearing mice. The drug was given in two different experiments as a water solution at a dose level of 50 mg/kg once weekly from Day 8 either for 2 (qw×2, experiment 1) or for 4 weeks (qw×4, experiment 2).

Drug A and B are two novel anticancer candidates, part of a Pharmacia research program. Drug A was given i.v. as bolus administration starting from Day 9 of the experiment at the dose level of 60 mg/kg using three different schedules: three times daily for 1 day (tid×1), two times daily for 4 days (bid×4), and once daily for 11 days (qd×11). For each schedule, eight animals were treated. In a preliminary experiment, Drug B was given i.v. at the dose level of 15 and 30 mg/kg as bolus administration. Doses were given twice daily for 5 days (bid×5) starting from Day 13 of the experiment. Eight animals were used for each group. In a second experiment Drug B was given as a 7-day infusion (83 mg/kg/day) to 10 mice starting on Day 9 of the experiment. Experiments for Drug A and B were performed in A2780 tumor-bearing mice.

**Pharmacokinetic Studies**

The pharmacokinetics of CPT-11, paclitaxel, 5-FU, Drug A, and Drug B were investigated in separate groups of tumor-bearing mice (historical data from previous experiments or data obtained in small ancillary groups). Three to five animals were used for these assessments. Blood samples (~80 μl) were collected using an appropriate sampling schedule either after a single dose or before and after dosing on the last day of treatment. Blood samples were collected in heparinized tubes; the samples were immediately centrifuged at 4°C (1200 × g for 10 min), and plasma samples were kept at ~ −20°C. The plasma concentrations of the drug were determined using liquid chromatographic methods with mass spectrometry detection. The lower limits of quantification were 0.25 ng/ml for CPT-11, 1 ng/ml for paclitaxel, 5.4 ng/ml for 5-FU, and 10 ng/ml for Drugs A and B.

**Model Development**

**Pharmacokinetic Model**

Plasma pharmacokinetics were described using the built-in compartmental models of Winnolín (version 3.1, Pharsight, Mountain View, CA). Nonlinear least squares were applied using nlminb as weighting function. For CPT-11, 5-FU, and Drugs A and B, pharmacokinetic parameters were estimated from mean drug levels. Because plasma concentration data for paclitaxel were obtained in different experiments (with the same dosing schedule but different sampling times), the pharmacokinetic analysis was performed using a naive pooled approach.

**Pharmacodynamic Model**

**Unperturbed Growth Model (Control Group).** In vivo tumor growth in xenograft models is known to follow exponential growth, at least in its early phases of development (13). Subsequently, the tumor growth follows a linear growth, reaching eventually a plateau. This behavior can be described using a Gompertz model (14). Because a plateau was never observed in the experimental datasets, we preferred adopting a more flexible model focused on the exponential and linear phases. In our approach, we assumed that there is a threshold tumor mass \((w_{th})\) at which the tumor growth switches from exponential to linear (i.e., from a first-order to a zero-order process; Ref. 15).

In terms of differential equations, we have the following:

\[
\frac{dw(t)}{dt} = \lambda_0 \cdot w(t), \quad w(t) \leq w_{th}
\]

\[
\frac{dw(t)}{dt} = \lambda_1, \quad w(t) > w_{th}
\]

\[
w(0) = w_0
\]

where \(w_0\) represents the tumor weight at the inoculation time \((t = 0)\), and \(\lambda_0\) and \(\lambda_1\) are parameters characterizing the rate of exponential and linear growth, respectively. The value \(w_{th}\) can be expressed as a function of \(\lambda_0\) and \(\lambda_1\), imposing the continuity of the derivatives of the model (Eq. B) at \(w = w_{th}\): \(\lambda_0 \cdot w_{th} = \lambda_1\) (C).

From a mechanism-based perspective, \(\lambda_0\) and \(\lambda_1\) may be indicative of the aggressiveness of the cell line in the in vivo experiment and of the response of the animal (immunological, antiangiogenic, and so forth) to the tumor progression, respectively.

A model such as that described by Eq. B and Eq. C adequately describes the tumor growth in control animals (15). However, for computational reasons, it is convenient to use a single differentiable function, especially in view of the subsequent introduction of the effect of an anticancer agent (see "Perturbed Growth Model"): \(\frac{dw(t)}{dt} = \lambda_0 \cdot w(t) \left[1 + \frac{w(t)}{w_{th}}\right]^{\alpha} \)

\(w(0) = w_0\)

For values of \(\Psi\) large enough, Eq. D is a good approximation of the original switching system. In fact, as long as the tumor weight \(w(t)\) is smaller than \(w_{th}\), the term \((\lambda_0/\lambda_1) \cdot w(t)\)^\(\Psi\) in the denominator is negligible compared with 1; thus, the growth rate is approximated by \(\lambda_0\cdot w(t)\) (exponential growth). On the contrary, when the tumor weight \(w(t)\) becomes larger than \(w_{th}\), 1 can be neglected, so that the growth rate becomes equal to \(\lambda_1\) (linear growth). In practice, in our experience, the value \(\Psi = 20\) allows the system to pass from the first-order to the zero-order growth sharply enough, as in the original switching model.

**Perturbed Growth Model (Treated Groups).** Whereas in the unperturbed model, all tumor cells are assumed to be proliferating, the perturbed growth model assumes that the anticancer treatment makes some cells nonproliferating (see Fig. 1), eventually bringing them to death. For a given time \(t\), let \(x(t)\) indicate the portion of proliferating cells within the total tumor weight \(w(t)\) and let \(c(t)\) indicate the plasma concentration of the anticancer agent. The growth
rate of proliferating cells is still given by an equation similar to Eq. D, but with $x_1(t)$ replacing $w(t)$ in the numerator, because $x_1(t)$ represents the portion of $w(t)$ that is actually proliferating. The overall tumor weight $w(t)$ in the denominator of Eq. D is maintained instead; in some sense, it acts as a penalty on the growth rate, reflecting the fact that a large tumor mass hampers the nutrient supply. The latter choice was also justified by better fitting results.

The model assumes that the drug elicits its effect decreasing the tumor growth rate by a factor proportional to $c(t)$, through

$$k_2 \cdot c(t) \cdot x_1(t)$$

(E)

Because the death of tumor cells is delayed with respect to the drug treatment, a transit compartment model is used for describing this feature, as derived from the model: a time efficacy index (TEI) and a threshold concentration ($C_{th}$) may be determined from the model. For anticancer treatments applied to tumors in exponential growth, such a TEI can be derived with good approximation from Eq. E as follows:

$$TEI = \frac{k_2 \cdot AUC}{C_{th}}$$

(F)

where $AUC$ is the area under the plasma concentration-time curve of the drug. Notably, in these conditions, the TEI is directly proportional to the total drug exposure to the drug and does not depend on the specific shape of the plasma concentration-time curve of the anticancer agent. TEI may also be determined experimentally as the tumor growth delay reported by Bissey (13).

Threshold Concentration for Tumor Eradication. For animals exposed to a steady-state drug concentration $c(t) = C_{ss}$, a threshold concentration $C_{th} = \frac{x_1}{k_1}$ can be derived such that, if $C_{ss} < C_{th}$, the tumor will asymptotically converge to the steady-state weight $w_{ss} = \frac{x_0}{k_1} \cdot (k_2 \cdot C_{th})$, which may or may not be reached.

To appreciate the effect of $k_2$ on the response curves, one may refer to Fig. 3, in which the simulated curves relative to different values of $k_2$ are plotted.

Secondary Parameters. Two important secondary parameters can be derived from the model: a time efficacy index (TEI) and a threshold concentration for tumor eradication.

TEI. If the exposure to the drug has a finite duration, when the therapeutic effect becomes negligible, the model predicts that the time course of the tumor weight will eventually follow a linear growth with slope $\lambda_2$. Thus, different treatments give rise, after a transient, to parallel straight lines, the most leftward being the one relative to the controls. In this case, the efficacy of a treatment may be measured using the delay of the tumor growth. This delay can be defined as the difference in time required to achieve a predefined tumor weight in treated animals compared with control animals during the linear growth (Fig. 4). For anticancer treatments applied to tumors in exponential growth, such a TEI can be derived with good approximation from Eq. E as follows:

$$TEI = \frac{k_2 \cdot AUC}{C_{th}}$$

(F)
be compatible with the survival of the experimental unit. Conversely, if $C_e > C_p$, the model eventually predicts the tumor eradication independently from the weight of the tumor at the start of the treatment. This can be demonstrated based on the annihilation of the differential equations (Eq. E). It is interesting to note that $C_p$ may be also calculated based on the experimental evaluation of TEI as $AUC/TEI$.

Data Analysis

The PK-PD model was implemented using Winnonlin (version 3.1, Pharsight). Parameters were estimated by using weighted nonlinear least squares. Different weighting strategies (uniform, $1/y_{observed}$, $1/y_{observed}^2$) were applied and chosen based on the analysis of residuals and on the coefficient of variation of the estimated parameters. Drug plasma concentrations in input to the pharmacodynamic model were derived from the pharmacokinetic parameters, using the appropriate dosing regimen. Pharmacokinetic data were obtained in separate experiments or ancillary groups, so that average pharmacokinetics were used. Also the unperturbed and perturbed tumor growth curves were obtained in different groups of animals. Therefore, model parameters were estimated by simultaneous fitting of average data of control and treated groups, which allows sharing the same tumor-related parameters $w_0$, $\lambda_0$, and $\lambda_1$ between the two groups.

Simulations of tumor growth curves at different doses and/or regimens were performed fixing the PK-PD parameters to their previously estimated values.

RESULTS

Unperturbed Growth Curves

The unperturbed model, with only three parameters, fitted well the average growth curves of control animals observed for different cell lines. In Fig. 5, as an example, the fitted curves are plotted against the data collected in experiments using two different cell tumor lines (A2780 and HCT116).

Perturbed Growth Curves

CPT-11. The pharmacokinetics of CPT-11 in nude mice were described using a two-compartment model; pharmacokinetic parameters after a single i.v. bolus at 45 mg/kg dose level were $V_1 = 4.85$ liter $\cdot$ kg$^{-1}$, $k_{10} = 0.553$ h$^{-1}$, $k_{01} = 0.0115$ h$^{-1}$, $k_{23} = 0.0616$ h$^{-1}$ (model 7 of Winnonlin). Maximal concentration and plasma clearance were $\sim 10 \mu g/ml$ and $2.7$ liter $\cdot$ h$^{-1}$ kg$^{-1}$, respectively, consistent with the data reported in the literature (19). In Fig. 6 the fitting of the average plasma concentrations (inset) and the average observed and model-fitted tumor weights are shown. The PK-PD model was able to correctly describe the growth of tumors in both untreated and treated mice given a single i.v. dose of CPT-11 at 45 and 60 mg/kg dose levels ($r^2 > 0.99$). It is important to notice that the fitting of the control and treatment arms was simultaneous, which is indicative of the consistency of the parameters across the two dose levels; results are reported in Table I.

Paclitaxel. In Fig. 7, the observed and the model-fitted average tumor growth curves are shown in control and i.v.-treated animals in two different experiments. The results of the pharmacokinetic fittings, obtained in previous experiments, are shown in the same figure. The

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline Parameter & Estimate & CV\(^a\) (%) \\
\hline $k_1$ (day$^{-1}$) & 0.469 & 22.30 \\
$k_2$ (ng $\cdot$ ml$^{-1}$day$^{-1}$) & $8.42 \times 10^{-4}$ & 9.43 \\
$\lambda_0$ (day$^{-1}$) & 0.146 & 4.05 \\
$\lambda_1$ (g/day) & 0.334 & 6.67 \\
$w_0$ (g) & 0.085 & 10.01 \\
\hline
\end{tabular}
\caption{Pharmacodynamic parameters obtained fitting the model to the tumor weight data of control and treated animals on Day 13.}
\end{table}

\(^{a}$CV, coefficient of variation; $k_1$, first-order rate constant of transit; $k_2$, measure of drug potency; $\lambda_0$, first-order rate constant of tumor growth; $\lambda_1$, zero-order rate constant of tumor growth; $w_0$, tumor weight at the inoculation time.
Table 2. Pharmacodynamic parameters obtained fitting simultaneously the model to the tumor weight data of two different experiments in control and treated animals

Nude mice received i.v. either the vehicle or paclitaxel [experiment 1 (exp 1), 30 mg/kg every 4 days for 3 Days from day 8; experiment 2 (exp 2), 30 mg/kg every 4 days for 3 days from Day 13].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV(^\circ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 ) (day(^{-1}))</td>
<td>0.968</td>
<td>14.53</td>
</tr>
<tr>
<td>( k_2 ) (ng (^{-1})ml/day(^{-1}))</td>
<td>6.29 \times 10(^{-4})</td>
<td>8.03</td>
</tr>
<tr>
<td>( a_{00} ) (day(^{-1}))</td>
<td>0.273</td>
<td>8.08</td>
</tr>
<tr>
<td>( a_1 ) exp 1 (day(^{-1}))</td>
<td>0.814</td>
<td>2.34</td>
</tr>
<tr>
<td>( w_0 ) exp 1 (g)</td>
<td>0.055</td>
<td>31.56</td>
</tr>
<tr>
<td>( a_0 ) exp 2 (day(^{-1}))</td>
<td>0.311</td>
<td>7.54</td>
</tr>
<tr>
<td>( a_1 ) exp 2 (g/day(^{-1}))</td>
<td>0.656</td>
<td>4.19</td>
</tr>
<tr>
<td>( w_0 ) exp 2 (g)</td>
<td>0.033</td>
<td>28.17</td>
</tr>
</tbody>
</table>

\(^\circ\) CV, coefficient of variation; \( k_1 \), first-order rate constant of transit; \( k_2 \), measure of drug potency; \( a_{00} \), first-order rate constant of tumor growth; \( a_1 \), zero-order rate constant of tumor growth; \( w_0 \), tumor weight at the inoculation time.

Fig. 7. Observed and model-fitted tumor growth curves obtained in two different experiments in nude mice given i.v. either the vehicle or paclitaxel [experiment 1 (exp 1), 30 mg/kg every 4 days for 3 days from Day 8; experiment 2 (exp 2), 30 mg/kg every 4 days from Day 13]. Inset, the fitting of the pharmacokinetic data of paclitaxel given as repeated i.v. bolus doses at 30 mg/kg dose level. Conc., concentration.

pharmacokinetics were described using a two-compartment open model (model 7 of Winnolin). Pharmacokinetic parameters obtained after i.v. paclitaxel (30 mg/kg, every 4 days for 3 days) were \( V_1 = 0.81 \) liter \( \cdot \) kg\(^{-1}\), \( k_{10} = 0.868 \) h\(^{-1}\), \( k_{12} = 0.0060 \) h\(^{-1}\), and \( k_{21} = 0.0838 \) h\(^{-1}\). Plasma clearance was approximately 0.7 liter \( \cdot \) h\(^{-1}\) \( \cdot \) kg\(^{-1}\). The comparison of these pharmacokinetics with those reported in the literature is difficult because of the known effect of Cremophor on the pharmacokinetics of paclitaxel (20); however, the shape of the curve and the maximal concentrations (\( \approx 110 \) \( \mu \)g/ml) were in reasonable agreement with the values reported in the literature (20). The two experiments were performed using the same administration schedule for paclitaxel. However, the anticancer regimen were started either on study Day 8 or on study Day 13, at which times the tumor weights were \(-0.2\) and 2 g, respectively. The data from the two experiments were fitted simultaneously, assuming the same \( k_1 \) and \( k_2 \) across experiments. Conversely, because some differences were observed between the tumor growth in controls across experiments, different values of \( a_{00} \), \( a_1 \), and \( w_0 \) (parameters related to the growth of control tumors) were estimated for each experiment. In Table 2, the eight pharmacodynamic parameters are reported. The simultaneous modeling captured well the features of the tumor growth and the effect of the anticancer treatment. The goodness of the simultaneous fitting (\( r^2 > 0.99 \)) confirms the consistency of the drug-related parameters across experiments, supporting the use of the model for prediction purposes.

5-FU. An analogous evaluation, as performed with paclitaxel, was done with 5-FU, for evaluating the applicability of the model to a drug with a different mode of action. Again, two experiments were performed using the same dose levels, but, in this case, different durations of the treatments (2 and 4 weeks) were used. Observed and predicted plasma concentrations are shown in the inset of Fig. 8. Pharmacokinetic parameters (model 7 of Winnolin) were \( V_1 = 0.71 \) liter \( \cdot \) kg\(^{-1}\), \( k_{10} = 6.30 \) h\(^{-1}\), \( k_{12} = 0.234 \) h\(^{-1}\), \( k_{21} = 0.0964 \) h\(^{-1}\). Plasma clearance was 4.5 liter \( \cdot \) h\(^{-1}\) \( \cdot \) kg\(^{-1}\), consistent with the values obtained from the literature (21). Again, the data from the two experiments were fitted simultaneously, assuming the same \( k_1 \) and \( k_2 \) across-experiment, and different \( a_{00} \), \( a_1 \), and \( w_0 \) for each experiment. The model captured well the features of the tumor growth and the effect of the anticancer treatment (\( r^2 > 0.98 \); Fig. 8). In Table 3 the pharmacodynamic parameters are reported. In this case, the large coefficient of variation of \( k_1 \) is possibly due to the nonoptimal experimental design. Parameter \( k_2 \), which is the measure of drug potency, however, was well estimated using the simultaneous fitting of all data.

Drug A. An i.v. experiment was performed with a control arm and three different dosing regimens with the active compound. To validate the within-experiment predictivity of the model, two treatment arms were intentionally omitted and the model was fitted simultaneously to the tumor weight data of two different experiments in control and treated animals.

Table 3. Pharmacodynamic parameters obtained fitting simultaneously the model to the tumor weight data of two different experiments in control and treated animals

Nude mice received i.v. either the vehicle or 5-FU [experiment 1 (exp 1), 50 mg/kg every week for 2 weeks from Day 8; experiment 2 (exp 2), 50 mg/kg every week for 4 weeks from Day 8].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Estimate</th>
<th>CV(^\circ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_1 ) (day(^{-1}))</td>
<td>0.056</td>
<td>212.04</td>
</tr>
<tr>
<td>( k_2 ) (ng (^{-1})ml/day(^{-1}))</td>
<td>20.2 \times 10^{-4}</td>
<td>10.98</td>
</tr>
<tr>
<td>( a_{00} ) exp 1 (day(^{-1}))</td>
<td>0.270</td>
<td>10.00</td>
</tr>
<tr>
<td>( a_1 ) exp 1 (g/day(^{-1}))</td>
<td>1.11</td>
<td>5.60</td>
</tr>
<tr>
<td>( w_0 ) exp 1 (g)</td>
<td>0.037</td>
<td>44.08</td>
</tr>
<tr>
<td>( a_0 ) exp 2 (day(^{-1}))</td>
<td>0.215</td>
<td>13.98</td>
</tr>
<tr>
<td>( a_1 ) exp 2 (g/day(^{-1}))</td>
<td>0.412</td>
<td>5.20</td>
</tr>
<tr>
<td>( w_0 ) exp 2 (g)</td>
<td>0.065</td>
<td>48.98</td>
</tr>
</tbody>
</table>

\(^\circ\) CV, coefficient of variation; \( k_1 \), first-order rate constant of transit; \( k_2 \), measure of drug potency; \( a_{00} \), first-order rate constant of tumor growth; \( a_1 \), zero-order rate constant of tumor growth; \( w_0 \), tumor weight at the inoculation time.

Fig. 8. Observed and model-fitted tumor growth curves obtained in two different experiments in nude mice given i.v. either the vehicle or 5-fluorouracil [5-FU; experiment 1 (exp 1), 50 mg/kg every week for 2 weeks from Day 8; experiment 2 (exp 2), 50 mg/kg every week for 4 weeks from Day 8]. Inset, the fitting of the pharmacokinetic data of 5-FU given as a single i.v. bolus at 50 mg/kg dose level. Conc., concentration.
the data of the control arm and only one of the active treatments (the one in which 60 mg/kg was given every day for 11 days). The outcome of the pharmacokinetic fitting of the ancillary group is shown in the inset of Fig. 9. Plasma pharmacokinetic parameters (model 7 of Winnonlin) were $V_1 = 1.96$ liter $\cdot$ kg$^{-1}$, $k_{10} = 1.56$ h$^{-1}$, $k_{12} = 0.552$ h$^{-1}$, $k_{21} = 0.233$ h$^{-1}$. The results of the PK-PD modeling were excellent ($r^2 > 0.99$); the time course of the tumor weights was well described (Fig. 9), and the model parameters were estimated with good precision (Table 4). Using these parameter values and the appropriate pharmacokinetic profiles (see inset Fig. 10), the tumor weight curves of the two remaining treatment arms were simulated. Predicted tumor growth rates were in excellent agreement with the observations (Fig. 10). As expected, when the model was fitted to the whole dataset the results were again satisfactory ($r^2 > 0.98$) and the pharmacodynamic parameters were essentially identical to those obtained using one treatment arm only (Table 4).

The data obtained with this drug were used to validate Eq. F. In Fig. 11, the average TEI values (calculated in this case for achieving a tumor mass of 4 g) are plotted against the AUC; the slope of the straight line (0.0012 ml/ng) was in excellent agreement with the theoretical one ($k_f\lambda_0 = 0.0011$ ml/ng).

**Drug B.** In a first experiment, Drug B was given i.v. twice a day for 5 days at the dose level of 15 and 30 mg/kg as bolus administrations. The pharmacokinetic profile obtained in a previous experiment is shown in the inset of Fig. 12 ($V_1 = 1.42$ ml $\cdot$ kg$^{-1}$, $k_{10} = 1.17$ h$^{-1}$, $k_{12} = 0.260$ h$^{-1}$).

Table 4 Pharmacodynamic parameters obtained fitting the model to tumor weight data in nude mice given i.v. either the vehicle or Drug A at a dose level of 60 mg/kg.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Controls, qd $\times$ 11</th>
<th>Control, qd $\times$ 11, bid $\times$ 4, tid $\times$ 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_1$ (day$^{-1}$)</td>
<td>0.405 (29.14)</td>
<td>0.355 (20.57)</td>
</tr>
<tr>
<td>$k_2$ (ng$^{-1}$ ml$^{-1}$day$^{-1}$)</td>
<td>3.45 $\times$ 10$^{-4}$ (5.02)</td>
<td>3.76 $\times$ 10$^{-4}$ (5.27)</td>
</tr>
<tr>
<td>$\lambda_0$ (day$^{-1}$)</td>
<td>0.349 (3.89)</td>
<td>0.355 (3.52)</td>
</tr>
<tr>
<td>$\lambda_1$ (ng day$^{-1}$)</td>
<td>0.363 (3.60)</td>
<td>0.366 (3.47)</td>
</tr>
<tr>
<td>$w_{01}$ (g)</td>
<td>0.010 (14.77)</td>
<td>0.009 (13.08)</td>
</tr>
</tbody>
</table>

* CV, coefficient of variation; $k_f$, first-order rate constant of transit; $k_e$, measure of drug potency; $\lambda_0$, first-order rate constant of tumor growth; $\lambda_1$, zero-order rate constant of tumor growth; $w_{01}$, tumor weight at the inoculation time.

In the left panel, the parameters refer to the fitting of controls and animals treated every day for 11 days (qd $\times$ 11) from Day 9. In the right panel, the parameters refer to the data of the whole experiment (controls, and dosages given every day for 11 days (qd $\times$ 11), twice a day for 4 days (bid $\times$ 4), three times a day for one day (tid $\times$ 1); all treatments started from Day 9.

On the basis of the $\lambda_0$ and $k_2$ values previously estimated, a threshold concentration for tumor eradication ($C_T$) of $\sim$1100 ng/ml was calculated. An infusion experiment was designed to target a steady-state concentration of Drug B of $\sim$2000 ng/ml (1.8-fold higher than $C_T$) to observe tumor shrinkage within the time frame of the experiment. On the basis of the plasma clearance obtained in the previously described experiment (1.7 liter $\cdot$ h$^{-1}$ kg$^{-1}$) and on basic pharmacokinetic principles (22), an infusion rate of 80 mg/kg/day was calculated: the corresponding profile is shown in the inset of Fig. 13. The experiment was performed at an effective infusion rate of 83 mg/kg/day: the average observed steady-state concentration was 2085 ng/ml. In Fig. 13A, observed and predicted tumor weights are shown, showing an excellent agreement. In Fig. 13B observed and model-fitted data are reported ($r^2 > 0.99$). Pharmacodynamic parameters of the infusion are also reported in Table 5. As predicted, the observed tumor weights in treated animals indeed showed the start of tumor eradication, confirming the utility of the secondary parameter $C_T$. This example demonstrates the cross-experiments predictive power of the model using different administration modes.

**DISCUSSION**

In this article, a novel PK-PD model was presented for predicting and describing the tumor growth and the effect of anticancer agents in animal models. The pharmacodynamic part of the model was based on five physiologically relevant parameters. These parameters are identifiable without additional efforts, using the typical experiments performed in nude mice as part of the drug research and development process. Three parameters ($w_{01}$, $\lambda_0$, and $\lambda_1$) describe the features of the tumor kinetics in control animals, characterized by an exponential growth followed by a linear growth. This approach was flexible enough for describing accurately the growth patterns of different cell lines in untreated nude mice (Fig. 5). It is also interesting to notice that, in its first implementation (15), the model of unperturbed growth was successfully used for modeling the tumor growth curves in rats. This flexibility is particularly important for accommodating the var-
stochastic description of the events, in which the passage from one
passing through the origin. The slope (0.0012 ml/ng) was in excellent agreement with that
calculated from Eq. F (0.0011 ml/ng).

Table 5 Pharmacodynamic parameters obtained fitting the model to the tumor weight
data in nude mice given i.v. either the vehicle or Drug B

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimate</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>k1 (day^-1)</td>
<td>0.517</td>
<td>16.72</td>
</tr>
<tr>
<td>k2 (ng^-1ml^-1day^-1)</td>
<td>2.89 x 10^-4</td>
<td>9.15</td>
</tr>
<tr>
<td>k3 (day^-1)</td>
<td>0.309</td>
<td>22.06</td>
</tr>
<tr>
<td>k4 (gday^-1)</td>
<td>0.796</td>
<td>4.82</td>
</tr>
<tr>
<td>w0 (g)</td>
<td>0.034</td>
<td>91.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.016</td>
</tr>
</tbody>
</table>

CV, coefficient of variation; k1, first-order rate constant of transit; k2, measure of
drug potency; k3, first-order rate constant of tumor growth; k4, zero-order rate constant of
tumor growth; w0, tumor weight at the inoculation time.

The effect of an anticancer drug on the tumor weight is typically
delayed and smoothed with respect to the drug exposure. For address-
ing this behavior, we used a transit compartmental model, a typical
way to include a delay in a PK-PD model. This corresponds to a
stochastic description of the events, in which the passage from one
compartment to the other is assumed to be independent from what is
occurring in the other compartments, and each passage is assumed to
occur individually and at a uniform rate (16). A system with three
compartments with a unique rate coefficient ki was able to generate a
probability distribution of cell-death times flexible enough for accom-
modating all of the analyzed datasets.

The secondary parameters obtained from the model have an imme-
diate biological meaning. The threshold concentration for tumor erad-
ication, CT, may be regarded as a reference concentration to be
realized in vivo for achieving a significant activity. Only schedules
able to give, at least for a certain period, concentrations higher than
CT may have some chance to give tumor reduction. The TEI value,
just as the survival time, the relapse-free survival, and so forth, is a
measure of efficacy expressed in time metrics. Both CT and TEI may
be useful for extrapolating the preclinical results to humans.

The examples reported in this article referred to the modeling of
average curves, which is the main goal of the exploratory studies
performed in early drug discovery and development. Because of the
typical features of these experiments, with separate control and treated
groups and pharmacokinetics evaluated in ancillary studies, the si-
multaneous fitting of average data is the most efficient way for using
the all of the available information. Because the perturbed growth
collapses into the unperturbed one in the absence of treatment, this
kind of analysis can be easily implemented with our model, validating
the approach and increasing the robustness of the estimates.
The examples reported on discovery candidates illustrate how this approach could expedite oncology drug discovery and development. The example concerning Drug A illustrated that, given the control and one treatment arm, the model could simulate the other two treatment arms with good accuracy. This shows that, exploiting the predictive capability of the model, a substantial simplification of the experiment can be used for the design of subsequent experiments, identifying the appropriate dosing regimens and schedules.

In this article, we presented predictions of different dosing regimens using the same administration route. In case of changes of route, many factors can affect the response of the system (first-pass metabolism, formation of active metabolites, effect of formulations, differences in the manipulation of animals, and so forth). In such cases, caution must be exercised when the model is applied prospectively.

In conclusion, the model presented in this article correctly describes the inhibition caused by anticancer drugs with different modes of action and in different cell lines. The model has proven to be reliable enough for predicting the effect of different dosing regimens. We are currently using \( k_1 \) estimates for ranking candidates based on their potency and for giving hints on the dynamic of cell death. The model is also being used prospectively for an educated design of the \textit{in vitro} experiments; in this way, a number of unnecessary or less informative studies can be avoided, priority can be given to the most discriminating dosing regimens, and a more realistic evaluation of the safety margins can be obtained.

The availability of tumor growth models, achieving the right compromise between empirical and mechanism-based approaches, is also a real need in clinical practice (23). Indeed, appropriate mathematical approaches (24) may have a big impact both for driving the design of oncology trials (25–26) and for devising optimal strategies for the application of diagnostic tools in the clinics (27). The approach presented in this article may also be useful for additional considerations in this context.

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Predictive Pharmacokinetic-Pharmacodynamic Modeling of Tumor Growth Kinetics in Xenograft Models after Administration of Anticancer Agents

Monica Simeoni, Paolo Magni, Cristiano Cammia, et al.


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