A Mechanistic, Predictive Model of Dose-Response Curves for Cell Cycle Phase-specific and -nonspecific Drugs

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ABSTRACT

In vitro dose-response curves for anticancer agents are useful for predicting the clinical response to chemotherapy, and models to capture the time-dependency of dose-response curves are necessary for potential clinical extrapolation. Usually, the modified Hill model is used (see Levasseur et al., Cancer Res., 58: 5749–5761, 1998), although this model is neither mechanistic nor predictive for understanding how drug and tumor cell characteristics affect the shape of the dose-response curve. A new exponential kill (EK) model is proposed to predict the shape of dose-response curves based on the cell cycle phase specificity of a drug, the cell cycle time, the duration and concentration of drug exposure at the site of action, and a scaling factor for the level of drug resistance. Explicit analytical equations are presented for predicting the IC₅₀ (the concentration required to reduce cell growth by 50%), the maximum cell kill achievable at high doses after a given duration of drug exposure, and the slope of the survival fraction versus log (concentration) plot at the IC₅₀. Numerical solutions illustrate that there may be an optimal, finite duration of drug exposure that maximizes cell kill for a given area under the concentration versus time curve, and an analytical equation is given to calculate when such an optimal, finite duration exists.

The EK model generates sigmoidal dose-response curves, like those seen empirically and previously described by the Hill model, which eventually plateau with increasing drug concentration at levels that depend on the cycle specificity of the drug, the cell cycle time, and the duration of exposure to the drug. This study includes no original data. Instead, empirical results in the literature are used to test the model. Because data by Levasseur et al. (1998) was fit to the Hill model assuming the plateau in the effect versus concentration curve to be independent of exposure duration, a full test of the model is not possible using their published data. Some tests of the EK model were possible, however, showing that EK model predictions yield good fits to in vitro data published in that and in another study. In addition, combining the EK model with a pharmacokinetic model resulted in predictions that were consistent with results of clinical studies comparing etoposide given in different schedules. Further tests of the model are necessary.

INTRODUCTION

Skipper et al. (1, 2) and Bruce et al. (3) showed that the chemotherapy dose-response curve resulting from a single-dose spike of CS³ drugs levels off with increasing dose (reviewed in Ref. 4). This tapering dose-response curve contrasts with the exponential decrease in survival S with increasing dose D for CNS drugs, which has been described (1–4) by the log-linear relationship S = \exp[-(αD)], where α is an empirically measured parameter that depends on the level of susceptibility to the drug (α = 0 for completely resistant cells). Since then, a number of studies have shown that dose-response curves depend not only on the cycle specificity of a drug, but also the time of drug exposure, both in vitro (5–8) and in vivo (9, 10). In addition, recent in vitro experiments show that the cell kill resulting from CNS drugs also plateaus with increasing dose (7), in contrast to previous data indicating an exponential increase in cell kill. Usually, the modified Hill model (7) is used to describe chemotherapy dose-response curves based on statistical fits to a sigmoidal curve. However, the Hill model does not allow one to predict how features like the cycle specificity of the drug (i.e., what fraction of cells are in vulnerable phases of the cell cycle, and the cell cycle time) or the duration of exposure affect cell kill. A predictive, quantitative model of in vitro dose response is necessary because in vitro drug sensitivity correlates with the responses of individual patients to chemotherapy (6). Thus, it would be helpful in determining the best procedure for in vitro drug screening to have a mechanistic model that could predict the survival fraction at which the dose-response curve plateaus, the IC₅₀ (the dose required to inhibit colony growth by 50% relative to that of a control), and the slope of the curve at the IC₅₀, based on independently measured parameters such as the cycle specificity of the drug and the duration of exposure. This study proposes a simple, mechanistic equation to predict cell kill in vitro. Bearing in mind the limitations of extrapolating in vitro results to in vivo and clinical situations, the mechanistic approach presented here could be used to suggest levels of cell kill that might occur in vivo, given that one could determine the drug concentration at the site of action, the cell cycle time of the target cells, the percentage of cells in the drug-susceptible phase (e.g., S phase) at the start of drug exposure, and the level of resistance of the target cells. This study contains no original empirical data, but rather presents a new mathematical model and compares model predictions with results of empirical studies presented in the literature. In addition, results of such a model may shed light on potential reasons why some clinical trials have had better success than others (e.g., Refs. 9, 10).

The mathematical basis of dose-response curves for radiotherapy is well developed with log survival declining as a LQ function of dose S = \exp[-(αD + βD²)](11). It is thought that α has direct biophysical significance as the probability per unit dose that two critical sites within a cell are simultaneously damaged, leading to the death of the cell. In addition, β is another empirically determined parameter that describes the sublethal damage that may become lethal if compounded with a second hit (βD²). Data show a fundamental difference between radiotherapy and chemotherapy dose-response curves: in radiotherapy, the dose response of cell kill does not plateau with increasing radiation dose, whereas in chemotherapy, cell kill does level off with increasing drug dose. Moreover, experimental data indicate that the level of this plateau depends on the duration of drug exposure and the cycle specificity of the drug (7, 8). Thus, a mathematical model to predict in vitro cell kill for chemotherapy is called for which parallels the current theoretical understanding of radiation cell kill. It is possible that the qualitative difference between radiotherapy and chemotherapy dose-response curves results from a difference in the time scales of atomic and molecular reactions leading to cell death: ionizing radiation causes DNA strand breaks or creates oxidation products on a time scale of 10⁻⁹–10⁻¹² s (12), whereas the time scale of cellular uptake and chemical reactions of chemotherapeutics are orders of magnitude longer, pointing to the importance of exposure duration in addition to dose. However, further speculation as to the atomic and molecular bases of dose-response curves is beyond the scope of this study.
The dose-response curve is shaped like a roller coaster, with intervening plateaus before the final plateau $B$ is reached.

The Hill model generates sigmoidal dose-response curves, when dose is plotted on a log scale, like those measured empirically. The intuitive, mechanistic interpretations, or derivations, of the parameters $B$, $IC_{50}$, and $\gamma$, however, are unclear. In this study, an equation for effect versus concentration is presented, and is based on: (a) the cycle specificity of the drug; (b) for CS drugs, the fraction $f$ of cells in the vulnerable part of the cell cycle at the beginning of drug exposure ($f$ is a unitless fraction of cells); (c) the cell cycle time, $c$ (in hours); (d) the duration of drug exposure, $T$ (in hours); (e) the drug concentration $y(t)$ (in units of $\mu g$, mg, $\mu g$/m$^2$, and so on) at time $t$ at the site of action; and (f) the level of resistance, or concentration scaling factor, given by the parameter $a$ (in units of $\mu g^{-1}$, mg$^{-1}$, and so on). All of these parameters, except the last, may be estimated independently of dose-response data, in vitro or in vivo. Thus, a number of features about the dose-response curve may be predicted before any dose-response experiments are performed. Only $a$ must be determined from dose-response experiments because it is as a scaling factor depending on the units in which dose is measured. This EK model may enable one to predict the survival fraction at which the dose-response curve plateaus, the $IC_{50}$, and the slope of the dose-response curve as functions of cell cycle phase specificity of the drug, $f$, $c$, $T$, $y(t)$, and $a$.

### MATERIALS AND METHODS

#### The Model

Table 1 lists the independent parameters, which must be measured empirically, that the EK model requires. Table 2 summarizes the derived parameters that simplify the presentation of the key equations of the EK model, which are given in Table 3. The EK model assumes exponential cell kill over very short time intervals. But because the drug concentration may change over time, and for CS drugs only a fraction $f$ of cells are in the vulnerable part of the cell cycle at the start of treatment, with cells entering and leaving the vulnerable fraction at a rate inversely proportional to the cell cycle time $c$, that instantaneous kill fraction changes over time. Therefore, the survival fraction $S(T)$ after applying a drug for a duration of $T$, relative to the growth of a control colony not exposed to the drug, is not exactly exponential with drug concentration (Table 3 Eq. 2a, b, derived in Appendix A). For a CNS model is a subset of the and later applied to cancer chemotherapeutics (15), where the AUC was proposed as a modification of the AUC model of drug efficacy, originally developed to describe bacterial disinfectant action (14) and later applied to cancer chemotherapeutics (15), where the AUC model is a subset of the $IC_n \times T = k$ model with $n = 1$. Although the $IC_n \times T = k$ model describes empirical data well, the parameter $n$ is crucial for predicting the time-dependency of cell kill and must be estimated by fitting curves. The parameter $n$, however, has no intuitive basis and has been fit using a 4th order polynomial function of the logarithm of the surviving fraction $x$ of cells (15). The third model (7) combines both the $IC_n \times T = k$ relationship with the Hill model (16), which is used to describe pharmacodynamic effects, including in vitro drug-dose-response curves. This model, described by Levasseur et al. (7) to capture the time-dependency of in vitro drug cytotoxicity, will be referred to simply as the Hill model. It fits dose-response curves to the logistic function

$$E = B + (E_{con} - B) \left( \frac{C^n}{C^n + IC_{50}^n} \right)$$

where $E$ is the measured effect (e.g., cell survival, measured as a percentage of control colony growth), $B$ is the background effect observed at infinite drug concentration (i.e., the plateau of the dose response), $E_{con}$ is the control effect observed at zero drug concentration, $C$ is the drug concentration, $IC_{50}$ is the concentration of drug resulting in a 50% inhibition of the maximal effect ($E_{con} - B$), and $\gamma$ is the slope of the E versus C plot at the $IC_{50}$. For inhibitory drugs, $\gamma < 0$, and the larger the absolute value of $\gamma$, the steeper the dose-response curve. This redefinition of the $IC_{50}$, to be the drug concentration resulting in a % inhibition of the maximal effect ($1 - B$) differs from the traditional, literal definition as the concentration resulting in % inhibition of the effect relative to a control colony, and the alternate definitions will be referred to as the redefined and the literal, respectively. The distinction is important because if the plateau $B$ is >50% the literal $IC_{50}$ may not be achievable. Unless specified otherwise, this study will refer to the redefined $IC_{50}$.

Exposure time (7) is incorporated by expressing $IC_{50}$ as a function of drug exposure time, $IC_{50} = (k/T)^{1/n}$, or alternate, more complicated relationships based on fits to data. In addition, they present equations for double or triple Hill patterns, similar to the one above (Eq. A), except composed of sums of logistic equations, for situations in which

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Definition (units of measurement)</th>
<th>Method of determination</th>
</tr>
</thead>
<tbody>
<tr>
<td>$y(t)$</td>
<td>Drug concentration at time $t$ at the site of action (e.g., mg or mg/m$^2$)</td>
<td>In vitro, the amount of drug applied if there is no breakdown or removal of the drug; in vivo, measured empirically and modeled with established pharmacokinetic models.</td>
</tr>
<tr>
<td>$T$</td>
<td>Duration of drug exposure (hours)</td>
<td>In vitro, the time until the drug is washed away from the cells; in vivo, measured empirically in the tissue containing the tumor, or calculated based on drug half-life, the schedule of drug administration, and pharmacokinetic models.</td>
</tr>
<tr>
<td>$f$</td>
<td>Initial fraction of cells in the vulnerable part of the cell cycle for a CS drug (no units, expressed as a proportion of cells)</td>
<td>DNA/BrdUrd$^b$ distributions and flow cytometry, or thymidine labeling index, to determine the fraction of cells in S phase for an S phase-specific drug, G$_1$ phase for a G$_1$-specific drug, and so on.</td>
</tr>
<tr>
<td>$c$</td>
<td>Cell cycle time (hours), only needed for CS drugs</td>
<td>BrdUrd/DNA distributions, culture doubling times.</td>
</tr>
<tr>
<td>$a$</td>
<td>Level of drug resistance [mg$^{-1}$ or (mg/m$^2$)$^{-1}$]</td>
<td>Measured empirically by fitting the EK dose response curve for a given cell line/drug combination, assumed to be constant across values of $T$, $y(t)$, $f$, and $c$. This is the only parameter which is not measured independently from the dose response curve.</td>
</tr>
</tbody>
</table>

$^b$ BrdUrd, bromodeoxyuridine. 

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Table 2 Parameters derived from those presented in Table 1

<table>
<thead>
<tr>
<th>Parameter (Calculation)</th>
<th>Calculation</th>
</tr>
</thead>
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<tr>
<td>$\tau$</td>
<td>Minimum($T, f$)</td>
</tr>
<tr>
<td>$q$</td>
<td>$\begin{cases} \frac{T}{f} &amp; \text{for a CNS drug} \ \frac{T}{c} &amp; \text{for a CS drug} \end{cases}$</td>
</tr>
<tr>
<td>$z$</td>
<td>$1 - \frac{f}{100}$ for Literal $IC_{50}$</td>
</tr>
<tr>
<td></td>
<td>$1 - \frac{f}{100} (1 - B(T))$ for Redefined $IC_{50}$</td>
</tr>
</tbody>
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The LQ model may be extended to incorporate DNA repair of sublethal damage (11). Although, to date, for chemotherapy no model of dose-response curves has incorporated sublethal damage and DNA repair like has been done for radiotherapy. Such a model may be required to describe chemotherapeutics with a shoulder in the dose-response curve. This study, however, will focus only on lethal damage inflicted by chemotherapeutics.

Several relationships have been used to describe anticancer drug potency. First, it was suggested that the AUC ($C \times T$) may predict cytotoxicity (13). Second, the $IC_n \times T = k$ model describes empirical data well, the parameter $n$ is crucial for predicting the time-dependency of cell kill and must be estimated by fitting curves. The parameter $n$, however, has no intuitive basis and has been fit using a 4th order polynomial function of the logarithm of the surviving fraction $x$ of cells (15). The third model (7) combines both the $IC_n \times T = k$ relationship with the Hill model (16), which is used to describe pharmacodynamic effects, including in vitro drug-dose-response curves. This model, described by Levasseur et al. (7) to capture the time-dependency of in vitro drug cytotoxicity, will be referred to simply as the Hill model. It fits dose-response curves to the logistic function

$$E = B + (E_{con} - B) \left( \frac{C^n}{C^n + IC_{50}^n} \right)$$

where $E$ is the measured effect (e.g., cell survival, measured as a percentage of control colony growth), $B$ is the background effect observed at infinite drug concentration (i.e., the plateau of the dose response), $E_{con}$ is the control effect observed at zero drug concentration, $C$ is the drug concentration, $IC_{50}$ is the concentration of drug resulting in a 50% inhibition of the maximal effect ($E_{con} - B$), and $\gamma$ is the slope of the E versus C plot at the $IC_{50}$. For inhibitory drugs, $\gamma < 0$, and the larger the absolute value of $\gamma$, the steeper the dose-response curve. This redefinition of the $IC_{50}$, to be the drug concentration resulting in a % inhibition of the maximal effect ($1 - B$) differs from the traditional, literal definition as the concentration resulting in % inhibition of the effect relative to a control colony, and the alternate definitions will be referred to as the redefined and the literal, respectively. The distinction is important because if the plateau $B$ is >50% the literal $IC_{50}$ may not be achievable. Unless specified otherwise, this study will refer to the redefined $IC_{50}$.

Exposure time (7) is incorporated by expressing $IC_{50}$ as a function of drug exposure time, $IC_{50} = (k/T)^{1/n}$, or alternate, more complicated relationships based on fits to data. In addition, they present equations for double or triple Hill patterns, similar to the one above (Eq. A), except composed of sums of logistic equations, for situations in which the dose-response curve
In the results, it was assumed that a concentration curve at the IC longer durations of exposure (Fig. 1) drug concentration) when concentration is plotted on a logarithmic.

For higher values of T in vitro when comparing the model predictions with T otherwise, it will be assumed that T has a monotonically (become steeper) with the duration of exposure and also to be steeper (the absolute value of y(T,x) is larger) for drugs that exhibit less phase specificity (Fig. 1E).

Increasing the duration of drug exposure at a constant concentration y increases the AUC (yT mg h) as well as the cell kill. To hold the AUC constant across different exposure durations, one must set y = AUC/T. A plot of y versus y, in which the AUC remains constant across T, contrasts with Fig. 1C, in which y remains constant but the AUC rises in direct proportion to T. With a constant AUC, in the limit as T goes to infinity, log[y(T,x)] asymptotically approaches αAUC for CNS drugs, which is always a minimum, or αAUC/α for CS drugs, which may not be a minimum for low values of AUC combined with high values of αc (the exact formula is given in “Appendix B”), in which case there is a finite Topt that maximizes cell kill. Although it is not possible to find an analytical expression for the Topt, one may calculate numerically (Fig. 1G). For a CS drug with f > 0.1, Topt increases with c, AUC, and f. When f ≤ 0.1, Topt goes to zero for small c, but is finite for longer c.

**Testing the Model with Published Empirical Data.** Keefe et al. (8) measured in vitro dose-response curves of survival versus constant concentration of methotrexate after T = 3-, 6-, 18-, 24-, 36-, and 48-h exposures for murine leukemic lymphoblasts with a cycle time of c = 12 h. They observed clear plateaus in the survival fractions that were dependent on T. Because methotrexate is S phase-specific, the fraction f was assumed to be between 0.7 and 0.8, corresponding to the cells spending 8–9 h in S phase (8.5 h/12 h = 0.71). Estimating f using the fraction of time spent in the susceptible part of the cell cycle assumes that cells are not synchronized in the cycle, as is the case for malignant cells. The KE model with f = 0.8 closely predicts the observed values of the plateau B(T) (Fig. 2, paired t test of arc sine root transformations, mean difference of 1.0 × 10^4, P = 0.50).

Levasseur et al. (7) present parameters estimated from curve fits to the Hill model from an extensive set of experiments using seven drugs and two cell lines for each drug. However, in the Hill model it is assumed that the plateau of cell kill, B, is independent of T. The curve fits and parameters in the study by Levasseur et al. (7) are based on this assumption, and few data points at high doses are shown for short.
Fig. 1. Relationships predicted by the EK model. A, survival fraction \( S(T) \) versus concentration, from Eq. 3, after exposure to a CS drug with \( f = 0.4 \) or to a CNS drug, given for a duration of 1 or 24 h. Curves are sigmoidal and plateau at a level that depends on \( T \) and the cell cycle specificity. B, plateau in the survival fraction, \( B(T) \), versus the duration \( T \) of drug exposure, from Eq. 4, for CS drugs with different values of \( f \) and for a CNS drug. The plateau occurs at smaller survival fractions for longer \( T \). C, \( S(T) \) versus \( T \) at constant concentration. D, redefined IC\(_{50}\) and IC\(_{99}\) (in mg) versus \( T \), from Eq. 5. The IC\(_{x}\) is approximately constant across a short range of \( T \) for a CNS drug and \( T \), 10 h for a CS drug, and declines approximately linearly for longer \( T \). E, slope of the \( S(T) \) versus log (Concentration) plot, from Eq. 6. The slope is steeper for CNS than for CS drugs, and for longer \( T \). F, \( S(T) \) versus \( T \) for constant AUC (in units of mg/h), so that \( y \) for a given \( T \) is equal to \( AUC/T \). For CNS drugs or CS drugs with small \( f \) ≤ 0.1, the minimum survival fraction from a given AUC is approached asymptotically as \( T \) goes to \( \infty \), arguing for prolonged drug exposure. For most CS drugs, however, with 0.2 ≤ \( f \) ≤ 1 for the AUCs shown, there exists a \( T_{opt} \), ≤ \( \infty \) that maximizes kill, occurring at about 8–10 h for the case of \( f = 0.4 \). G, \( T_{opt} \) versus \( c \) for different combinations of \( f \) and the AUC (mg/h). The optimal exposure duration increases with \( c, f \), and the AUC across a range of values, but for combinations in which \( fc \) is small and the AUC is large, \( T_{opt} \) approaches \( \infty \). The parameter values were \( a = 1 \) mg\(^{-1}\) and \( c = 20 \) h.
Although there is no significant difference between the predicted and observed data for IC\textsubscript{50} upward rather than continuously increasing, and the slope at the model in Fig. 1 is not predicted to exist for exposure durations measured. On the log-log plots in Fig. 4, the value of IC\textsubscript{50} also stops increasing as \( t \) decreases, whereas the IC\textsubscript{50}s for the Hill model as \( T \) decreases, because the Hill equations use values of \( c \) given by Levasseur et al. (Ref. 7), and \( f \) is estimated based on knowledge of the phase specificity of the drug (Table 4). For example, for an S phase-specific drug assuming that S phase lasts 8 h, \( f \) is estimated to be 8/c. The value of \( a \) in the EK model for each drug/cell line combination is estimated using the Gauss-Newton method of nonlinear fitting to the IC\textsubscript{50} = \((k/T)^{1/n}\) relationship (Table 4). Note that \( a \) is a scaling factor that depends on the units in which dose is measured. On the log-log plots in Fig. 4, the value of \( a \) affects only the y-intercept but not the slope, which is predicted only from the parameters \( f \) and \( c \), estimated separately from the data used to generate the dose response or the IC\textsubscript{50} curves. The literal IC\textsubscript{50}s for CS drugs are not predicted to exist for exposure durations < 2 h. For the drugs cisplatin, doxorubicin, and paclitaxel, the EK model closely predicts the IC\textsubscript{50} captured in the IC\textsubscript{50} = \((k/T)^{1/n}\) relationship that Levasseur et al. (7) estimated from the data, at all exposure durations. Interestingly, the IC\textsubscript{50}s predicted by the EK model seem to make a better qualitative fit to the data plotted in (their Figs. 2 and 5) than do fits to the relationship IC\textsubscript{50} = \((k/T)^{1/n}\). In the latter function, plots of the log(IC\textsubscript{50}) versus log(T) are linear with slope \(-1/n\), for all \( T \). Such plots of their data, however, show a slope of 0 for \( T < 10 \) h for the CS drugs, a pattern predicted by the EK model. For CNS drugs, their data points match any of the three relationships IC\textsubscript{50} = \((k/T)^{1/n}\) or the literal or redefined IC\textsubscript{50}s predicted by the EK model, because all show a near-linear decline for \( T \geq 1 \) h.

Although application of the Hill model Levasseur et al. (7) estimates the survival fraction plateau to be constant across \( T \), there should, nevertheless, be a correlation between the observed \( BE\textsubscript{on} \) and \( B(T) \) predicted by the EK model across a range of \( T \), because the correlation was based on data collected across a range of \( T \). Using the ratio \( B/E\textsubscript{on} \) normalizes results so that the survival fraction at zero drug concentration is one. \( B(T) \) was predicted for each drug/cell line combination (Table 4). There was a significant correlation between observed and predicted values ranging from 0.65 (with \( T = 96 \) h, \( P = 0.02 \)) to 0.70 (with \( T = 1–24 \) h, \( P = 0.01 \), calculated on arc sine transformed values) if results for the drug paclitaxel were excluded from the analysis. The mean difference between the observed \( BE\textsubscript{on} \) and predicted \( B(10) \) was not significant (paired t test on arc sine square root transformations, \( P = 0.13 \)).

The empirical plateau in the survival fraction for paclitaxel was much lower than predicted by the model using Eq. 4, which ignores cell cycle delays. Because this drug promotes the assembly of microtubules and stabilizes them against depolymerization, thus inhibiting cell replication, it seems more appropriate to use Eq. A5 in the appendix, which includes a cell cycle delay effect in addition to the cell killing action of the drug. Using Eq. A5 and the ratio of the predicted to the observed value of the survival plateau, the delay in the cell cycle was estimated to be \( b = 85 \) h and 84 h for the cell lines A2780 and A2780/DX5B, respectively. Although extrapolation from theoretical predictions and in vitro data to clinical results demands a good measure of caution, it is interesting that EK model predictions regarding survival fractions are
Fig. 4. IC\textsubscript{50} (µM) versus \( T \) for the literal and the redefined IC\textsubscript{50}s as predicted by the EK model (see text) and the IC\textsubscript{50} = (k/T)\(^{1/n}\) relationship with \( n \) and \( k \) estimated from experiments in the study by Levasseur et al. (7). The drugs used were cisplatin (DDP; A), doxorubicin (DOX; B), paclitaxel (PTX; C), trimetrexate (TMQ; D), raltitrexed (RTX; E), methotrexate (MTX; F), and AG2034 (G). The three upper curves in each plot are for the more resistant cell line specified in Table 4, and the three lower curves are for the more susceptible cell line. For DDP, DOX, and PTX, all three relationships generate virtually identical IC\textsubscript{50}s. For the CS drugs TMQ, RTX, MTX, and AG2034, the EK model predicts lower values of the IC\textsubscript{50} for \( T < \) about 10 h and higher values for \( T > \) 10 h than those values predicted by the IC\textsubscript{50} = (k/T)\(^{1/n}\) relationship. Empirical curves and the few data points shown explicitly in the study by Levasseur et al. (7) show a qualitative pattern more like the redefined IC\textsubscript{50}s of the EK model than those of the IC\textsubscript{50} = (k/T)\(^{1/n}\) relationship, because the data show a constant IC\textsubscript{50} for \( T < \) 10 h.
consistent with clinical trials comparing different etoposide schedules for the treatment of small-cell lung cancer. In clinical trials, 500 mg/m² etoposide was administered by CI for 24 h, by CI of 2 h/day for 5 days, or by CI of 75 min/day for 8 days, once every 3 weeks (9, 10). These schedules resulted in response rates of 10%, 81%, and 87%, respectively, and survival durations of 6.3 months, 7.1 months, and 9.4 months, respectively. In a subsequent study in which etoposide was given by CI for 5 days, with individual monitoring resulting in variation in total dose among patients (median dose, 509 mg), the response rate was 70% and the survival duration was 8.9 months (19), between the 5- and 8-day schedules. To make predictions using the EK model, drug concentration y(t) was calculated using a single compartmental model with a drug half life of 6 h (corresponding to that of etoposide, 10) and a total dose of 500 mg/m² given by the four alternate schedules used in the clinical trials. Although a single compartmental model may not be the most appropriate for more detailed analyses, this simplification seemed adequate for the present purposes. f was assumed to be 0.4 for this CS drug (most active in late S phase and G₂)⁴. Eq. 2b was integrated numerically to predict the fraction of tumor cells surviving therapy for different drug schedules of administering a total dose of 500 mg/m². Predictions using a range of values of c were made, and qualitative patterns were approximately similar across the relevant ranges of the survival fraction, so only results using c = 48 h are shown (Fig. 5). Because the parameter a, which can be thought of either as the level of drug susceptibility or as a scaling factor that depends on the units in which drug dose is measured, is unknown, the predicted survival fractions are plotted for a range of values of a. To reduce the surviving fraction of tumor cells below 1%, Fig. 5 illustrates that a must be >0.01 (mg/m²)⁻¹. In fact, the survival fraction is probably orders of magnitude <1%, considering the success of clinical trials. The EK model leads to predictions that the 24-h continuous infusion allows a substantially greater fraction of tumor cells to survive than the other three schedules, and that the 75-min daily infusions for 8 days kills the highest fraction of cell. These predictions are consistent with the clinical results, both in terms of response rates and the duration of survival.

### Table 4: Observed plateaus in the survival fraction from Levasseur et al. (7) and predicted plateaus using the EK model for different drug/cell line combinations

<table>
<thead>
<tr>
<th>Drug</th>
<th>Cell line</th>
<th>c (h)</th>
<th>Phase specif.*</th>
<th>Est. of f (fraction of cells)</th>
<th>Est. of a (μM⁻¹)</th>
<th>B/E con</th>
<th>Obs. in Ref. 7</th>
<th>Pred.</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDP</td>
<td>A2780</td>
<td>18</td>
<td>CNS/C</td>
<td>1</td>
<td>0.044</td>
<td>0.001</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>DDP</td>
<td>A2780/CP3</td>
<td>29</td>
<td>CNS/C</td>
<td>1</td>
<td>0.004</td>
<td>0.024</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>DOX</td>
<td>A2780</td>
<td>18</td>
<td>S</td>
<td>1</td>
<td>1.063</td>
<td>0.016</td>
<td>0.007</td>
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<tr>
<td>DOX</td>
<td>A2780/DX5B</td>
<td>20</td>
<td>S</td>
<td>1</td>
<td>0.165</td>
<td>0.029</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>PTX</td>
<td>A2780</td>
<td>18</td>
<td>G₂-M</td>
<td>0.3</td>
<td>145.998</td>
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<td>0.341</td>
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*Phase specificity is based on pharmacological information about the drugs available in a number of sources (e.g., web sites such as www.bccancer.bc.ca/cdm/monographs and www.vet.purdue.edu/depts/bms/courses/bms445/chmrx/anticah2.htm#top). f and a were estimated as described in the text, B/E con is from Levasseur et al. (7), and B(T) was calculated using Eq. 4.

### DISCUSSION

The EK model generates dose-response curves like those measured empirically (5–8): sigmoidal curves (with dose on a log scale) that plateau at high doses, and in which survival declines as an exponential function of exposure time (8, 20, 21). Although the Hill model also generates sigmoidal curves, the EK equation allows one to go beyond a statistical description of data because it predicts the shape of the curve based on the following parameters determined independently from measures of dose response: the cell cycle phase specificity of the drug, the fraction of cells initially in a vulnerable part of the cell cycle, the cell cycle time, and the duration of drug exposure. The only parameter that must be estimated from a dose-response curve is a, the level of drug resistance, and once it is measured for a particular drug/cell line combination, a can be used to predict dose-response curves at different exposure durations. The EK model generates a quantitative, as well as a qualitative, fit to published empirical data, both in vitro (7, 8) and in vivo (9, 10, 19). However, additional tests of the model are needed. It is hoped that application of the model like in the example comparing different schedules of etoposide could assist in designing efficient clinical trials by identifying drug sched-
ules likely to yield the most substantial improvements in survival, and minimizing the number of treatments to be compared.

The EK model indicates that for CS drugs there may be an optimal duration of exposure to maximize cell kill with a given cumulative drug dose or AUC (Fig. 1, F and G). For some reasonable combinations of f, c, and AUC, that optimal duration is predicted to lie between 5 and 15 h. For situations in which the product fc is very small, however, even longer exposures may inflict greater cell kill. Many researchers would agree that, for CS drugs, prolonged exposure kills more cells with a given cumulative dose or AUC than does bolus administration (1, 2, 8, 9, 22, 23).

Results of the EK model suggest that prolonged exposure may augment cell kill relative to bolus treatment for a CNS drug, as well. Experiments using bleomycin and doxorubicin (CNS drugs) suggest that sustained drug delivery may improve survival and decrease toxicity (24, 25).

The duration of drug exposure within the cell is a crucial factor determining the shape of the dose-response curve. However, it may not be exactly what the experimenter intends or may differ across experiments: intracellular drug binding, breakdown, or excretion (in vitro) may differ across drugs, cells, and between in vitro and in vivo studies. In addition, washing procedures in vitro may also differ between studies and may be less effective than desired, resulting in persistent kill even after removal of extracellular sources of the drug. This makes it all of the more important to have a model that takes into account drug persistence when (cautiously) extrapolating from in vitro results to in vivo predictions. Rupniak et al. (6) suggested that more accurate representations of plasma half-lives should be considered when designing in vitro drug sensitivity tests. Ozawa et al. (13) found that taking the drug half-life into account improved predictions about dose-response curves. The full form of the EK model (Eq. 2a, b) with y(t) variable over time could enable researchers to use in vivo pharmacokinetic data to predict doses response, as well as the exposure duration to maximize cell kill with a given AUC.

There are several possible explanations for drugs with double or triple Hill curves like those documented by Levasseur et al. (7): (a) multiple molecular drug targets or modes of action that are affected across different ranges of drug concentration, and for which the parameter f differs; (b) effects on cell cycle time that differ across concentrations; or (c) differences in intracellular drug concentration or protein binding across different applied, extracellular concentrations, affecting T and/or f. When the parameters T, f, or a vary across different ranges of drug concentration, the EK model could describe roller coaster patterns similar to those of the double and triple Hill curves. For example, if a drug were to bind within a cell only at high concentrations, then washing procedures might be ineffective. Then the actual T at high concentrations would be longer than the T at low concentrations, and two plateaus would be observed in the dose-response curve. Because most drugs have multiple modes of action, roller coaster curves may be common. Fluorouracil, for example, seems to kill cells by different modes of action at low versus high concentrations. Thus, both the cycle specificity of the drug (the parameter f) and the mean level of resistance (the parameter a) probably change above a threshold drug concentration. This causes chemotherapeutic outcomes to diverge as a result of applying this drug by continuous infusion versus bolus, in terms of efficacy, types of toxicity, and mechanisms of resistance (26). Although EK model equations for such curves are not presented here, their derivation is a straightforward extension of the equations presented in Table 3 if one expresses f(y), T(y), and/or c(y) to be functions of drug concentration y.

In addition, tumors are composed of heterogeneous populations of cells that differ in their levels of resistance and, thus, have contrasting values of a. Thus, it might be better to use a frequency distribution for a rather than a single value. As cells with low levels of drug resistance are eliminated, the distribution of a changes with selection imposed by the drug (27). Although the EK model concludes that prolonged drug exposure to a CNS drug can kill more cells with a given dose than can short exposure or bolus treatment, an extension of the model that includes resistance evolution (27) concludes that it is possible to infuse a drug too slowly, facilitating the evolution of resistance through processes such as gene amplification, sequential modifications of a gene, or polygenic mechanisms of resistance. Indeed, for some drugs, low-concentration, continuous exposure facilitates the evolution of resistance (28), and some theoretical models have argued for dose-intense pulsed intermittent therapy from the beginning of treatment (29, 30).

One can envision more complicated models of cell kill in which the instantaneous kill fraction is exponential with dose, as assumed here (compared with a linear or a power function as assumed in previous models), but in which the cell population is subdivided into different phases of the cell cycle. One could characterize the survival fraction using a system of differential equations, requiring a number of parameters to describe transition rates between each phase of the cell cycle. A number of researchers have done this well (13, 17, 32). Such approaches describe cell kinetics more accurately than either the EK or the Hill model. However, they also demand more complicated analyses than the relatively simple EK model using Eqs. 2 or 3, and require that many more parameters be estimated. Thus, the advantage of the EK model is that it is not complex, yet provides an intuitive and mechanistic description of dose response.

In conclusion, a new EK model is proposed to describe dose-response curves. The model is mechanistic, allowing one to make quantitative predictions about how the cell cycle phase specificity of a drug, the cell cycle time, and the duration of drug exposure affect the dose response of cell kill by a cancer chemotherapeutic drug. Model predictions achieve a good fit to results of published empirical studies, both in vitro and clinical. Additional tests are needed.

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APPENDICES

Appendix A: Derivation of the EK Model. To include time-dependency in the formulation of cell kill, it is assumed that cell kill over a short interval of time ∆t is the exponential of drug concentration y(t) at time t. Thus, the fraction of cells killed in t + ∆t by a CNS drug of concentration y(t) is

\[ k_{cs}(t) \Delta t = \left[ 1 - \exp(-ay(t)) \right] \Delta t + o(\Delta t) \]  

where \( \lim_{\Delta t \to 0} o(\Delta t) = 0 \). Considering a CS drug, the instantaneous kill fraction \( k(t) \) is conditional on the probability that cells are in a vulnerable part of the cell cycle. Initially a fraction f of cells are in the vulnerable part of the cycle, and cells leave and enter this fraction at a rate \( c \). Thus, the instantaneous kill fraction is exponential with dose, as assumed here (27). Assuming exponential growth as in previous analyses (13, 32) at a rate \( 1/c \), the number of cells changes according to

\[ k_{cs}(t, c) \Delta t = \left[ 1 - \exp(-ay(t)) \right] \left[ f - \tau / c + 1 / c \right] \Delta t + o(\Delta t) \]  

where \( \tau = \min(t, f c) \).
with the solution
\[ \frac{dN}{dt} = N(t) \left( \frac{1}{c} - k(t, f) \right) \]  
(A3)

where \( b \) is the length of a cell cycle delay. In a control colony of cells that is not exposed to the drug, \( b = 0 \) and \( k(t, f) = 0 \). Thus, the ratio of the number of cells \( N_{\text{untreated}}(T) \) in a colony exposed to a drug relative to the number \( N_{\text{control}}(T) \) in an untreated colony, following exposure of duration \( T \), assuming both start at the same \( N(0) \), is
\[ S(T) = \frac{N_{\text{untreated}}(T)}{N_{\text{control}}(T)} = \exp(-bc) \exp(-\int k(t, f) dt) \]  
(A5)

This ratio is referred to as the survival fraction. In most of these analyses, it is assumed that \( b = 0 \), so any drug-inflicted delays in the cell cycle are ignored.

In the LQ model of radiotherapy and the AUC model of chemotherapy, damage per unit time is assumed to be directly proportional to the concentration \( y(t) \) at that time, and in the IC\(_{\alpha} \times T = k \) model cell kill is assumed to be proportional to some power of the concentration. In contrast, the assumption of the model proposed here, based on empirical results (1–3), is that kill per unit time increases exponentially with drug concentration, rather than as a linear or power function. Thus, the EK results (1–3), is that kill per unit time increases exponentially with drug concentration, rather than as a linear or power function. Thus, the EK

\[ \left( \frac{a_{\text{AUC}}}{1 - \exp(-a_{\text{AUC}}/c)} \right) \leq c \]  
(1)

Rearranging gives
\[ fc \geq \frac{a_{\text{AUC}}}{\pi} \left[ 1 - \exp(-a_{\text{AUC}}/c) \right] \]  
(2)
A Mechanistic, Predictive Model of Dose-Response Curves for Cell Cycle Phase-specific and -nonspecific Drugs

Shea N. Gardner


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