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Predicting the active doses in humans from animal studies: A novel approach in oncology

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ABSTRACT

The success rate of clinical drug development is significantly lower in oncology than in other therapeutic areas. Predicting the activity of new compounds in humans from preclinical data could substantially reduce the number of failures. A novel approach for predicting the expected active doses in humans from the first animal studies is presented here. The method relies upon a PK/PD model of tumour growth inhibition in xenografts, which provides parameters describing the potency of the tested compounds. Anticancer drugs, currently used in the clinic, were evaluated in xenograft models and their potency parameters were estimated. A good correlation was obtained between these parameters and the exposures sustained at the therapeutically relevant dosing regimens. Based on the corresponding regression equation and the potency parameters estimated in the first preclinical studies, the therapeutically active concentrations of new compounds can be estimated. An early knowledge of level of exposure or doses to be reached in humans will improve the risk evaluation and decision making processes in anticancer drug development.

1. Introduction

Drug development in oncology is a process that requires on average more than 10 years and investments of the order of 1 billion dollars for a drug to reach marketing approval and successful use in clinical practice.[1,2] This figure includes the cost of developing other drugs that fail, in most cases due to the insufficient efficacy observed in the clinical phases, possibly because the appropriate dose levels for eliciting significant activity have not been reached. Also considering the new target-based drugs, the major causes of losses are due to lack of drug efficacy and/or problems associated with toxicology and clinical safety.[3,4] The number of failures in the clinic could be substantially reduced if in the first phases of drug development it were possible to combine the toxicological, pharmacokinetic and pharmacodynamic properties with reliable estimates of active doses in patients.[5]

Although several vitro and vivo preclinical models have been developed for screening new anticancer agents, their relevance in terms of ability to predict the clinical outcomes is still under debate.[6–11] In addition, even if different statistical and mathematical approaches have been attempted,[12–15] no generally accepted methodologies for extrapolating the *in vivo* evaluation of the anti-tumour effect measured in animal models to the human being are currently available. A possible cause of this deficiency may be the absence of

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appropriate metrics of efficacy from these preclinical studies. In particular, considering the xenograft mice, the most common animal model used for this purpose, the evaluation of the efficacy is usually based on the ratio of the tumour volumes in treated and control animals (%T/C) measured at specified time points. The decision of advancing or discarding compounds from development is then simply based on the achievement of some reference values of %T/C (e.g. <30% or < 42%).[4,16] Some qualitative rules for anticipating the activity of new candidate drugs in cancer patients by combining these %T/C thresholds with the available pharmacokinetic information[16,17] have been proposed, but methods for providing quantitative predictions of activity in humans are still lacking. Indeed, the evaluation of %T/C, as metric of efficacy, is dose, schedule and time dependent and cannot be used for extrapolating the anticancer activity in case the dosing regimen and/or the experiment schedule are changed. This limitation is likely to be the most important cause of the poor reliability of extrapolations to humans.

These problems have been overcome by a recent pharmacokinetic-pharmacodynamic model linking the plasma concentrations of the anticancer compounds to the effect on the tumour growth curves observed in the treated animals.[18–20] As a result, in addition to the other properties, the model also provides quantitative estimates of two parameters, k_2 and C_T, that can be regarded as drug-specific measurements of the potency of the tested compounds.[18–20] The present paper explores the use of these parameters for predicting the response in humans. For this purpose, known anticancer drugs were tested in the xenograft model and the experimental data were used to evaluate the corresponding potency parameters. Based on the correlation observed between these parameters and the exposures commonly achieved in patients with these drugs, a novel methodology for anticipating the expected active doses in humans for anticancer compounds in preclinical development is proposed.

2. Materials and methods

2.1. General

The following anticancer drugs, typically included in the pharmacological studies for reference purposes, were considered in this paper: 5-fluorouracil, cisplatin, docetaxel, doxorubicin, etoposide, gemcytabine, irinotecan, paclitaxel, vinblastine, vincristine. The experiments and data analysis processes related to 5-fluorouracil, irinotecan, paclitaxel have already been presented.[18] Herein, we briefly summarise the experimental procedures for the remaining drugs and the major characteristics of the PK/PD model.

2.2. In vivo experiments

Female Hsd athymic nude-*nu* mice of 20–22 g, 5–6 weeks of age (Harlan, S. Pietro al Natisone, Italy), were used. Human A2780 ovarian carcinoma fragments (cell lines obtained from American Type Culture Collection) were implanted s.c. into the left flank of mice on day 1. One week after inoculation, mice bearing a palpable tumour (approximately 100–300 mm^3) were randomised into control and treatment groups. After treatment (typically on day 8), mice were clinically evaluated daily and tumours were measured, usually every 2 or 3 days, using calipers. Tumour masses (mg) were calculated as length (mm) × $width^2$ (mm^2)/2, assuming unit density.

Cisplatin, gemcytabine, docetaxel, vinblastine, and vincristine were given to two groups of eight animals at dose levels of 4, 120, 10, 3, and 0.5 mg/kg, respectively, either as a single administration on day 8 or as two administrations on

Table 1 – Major bioanalytical conditions of the plasma concentration assays for the evaluated anticancer drugs

Compound	Sample preparation	Column	Mobile phase	LC-MS/MS		Limit of quantitation (ng/mL)
				Ionisation mode	MRM transition	
5-fluorouracil	Protein Precipitation	Phenomenex Ashaipak NH2	Acetonitrile 10 mM ammonium formate pH 3.5	APCI negative	129–42 m/z	5
Cisplatin	Total Pt in plasma was measured by Flameless Atomic Absorption Spectrophotometry[21]					
Docetaxel	Protein Precipitation	Zorbax SB C8	Acetonitrile 2 mM ammonium acetate pH 5	ESI positive	809–527 m/z	5
Doxorubicin	Protein Precipitation	Zorbax SB C8	Acetonitrile 10 mM ammonium formate pH 3.5	ESI positive	544–397 m/z	5
Etoposide	Liquid–liquid extraction	Lichrosorb RP-8	Methanol Water	HPLC-UV (254 nm)[22]		100
Gemcytabine	Protein Precipitation	Phenomenex Ashaipak NH2	Acetonitrile 10 mM ammonium acetate	APCI negative	262–110 m/z	5
Irinotecan	Protein Precipitation	Chromolith SpeedROD RP-18e	Acetonitrile 2 mM ammonium formate pH 3	ESI positve	587–245 m/z	0.25
Paclitaxel	Liquid–liquid extraction	Zorbax SB C18	Acetonitrile 2 mM ammonium acetate pH 5	ESI positve	855–286 m/z	1
Vinblastine	Protein Precipitation	Zorbax SB C8	Acetonitrile 10 mM ammonium formate pH 3.5	ESI positive	812–224 m/z	5
Vincristine	Protein Precipitation	Zorbax SB C8	Acetonitrile 10 mM ammonium formate pH 3.5	ESI positive	825–224 m/z	5

day 8 and day 13 (q4d × 2). Doxorubicin was given to eight animals as a single dose of 12 mg/kg on day 8. Etoposide was given to two groups of eight animals at a dose level of 20 mg/kg either as a single administration on day 8 or as two administrations on day 8 and day 15 (q7d × 2).

Except for cisplatin and etoposide, for which data from the literature were considered,[21,22] the pharmacokinetics of the anticancer drugs were investigated in separate groups of tumour-bearing mice. Blood samples for the pharmacokinetic assessment were collected and the drugs were assayed in plasma using LC-MS-MS techniques based on a generic procedure adopted for compounds in discovery phases.[23] A list of the major bioanalytical conditions is reported in Table 1.

2.3. *Model structure*

The tumour growth curves were analysed using a recently published mathematical model,[18] whose main features are summarised in Fig. 1. The tumour growth in non-treated animals is described by an exponential phase followed by a linear growth phase. For facilitating the fitting procedure and for reducing the complexity of the system, a single differential equation was used for modelling the derivative of the tumour weight ($w(t)$), see Fig. 1, left panel. In this equation, w_0 represents the tumour weight at the inoculation time ($t = 0$), and λ_0 and λ_1 are parameters characterising the rate of exponential and linear growth, respectively. Ψ is a constant modulating the transition from the exponential to the linear growth model; according to Simeoni and colleagues,[18] a Ψ value of 20 was used.

In the treated animals it is assumed that the anticancer treatment makes some cells non-proliferating eventually bringing them to death through a mortality chain as summarised in Fig. 1, right panel. In the same figure, the system of differential equations is reported: for a given time t, $x_1(t)$ indicates the portion of proliferating cells within the total tumour weight $w(t)$ and $c(t)$ indicates the plasma concentrations of the anticancer agent. In these equations, $w(t)$ is the total tumour

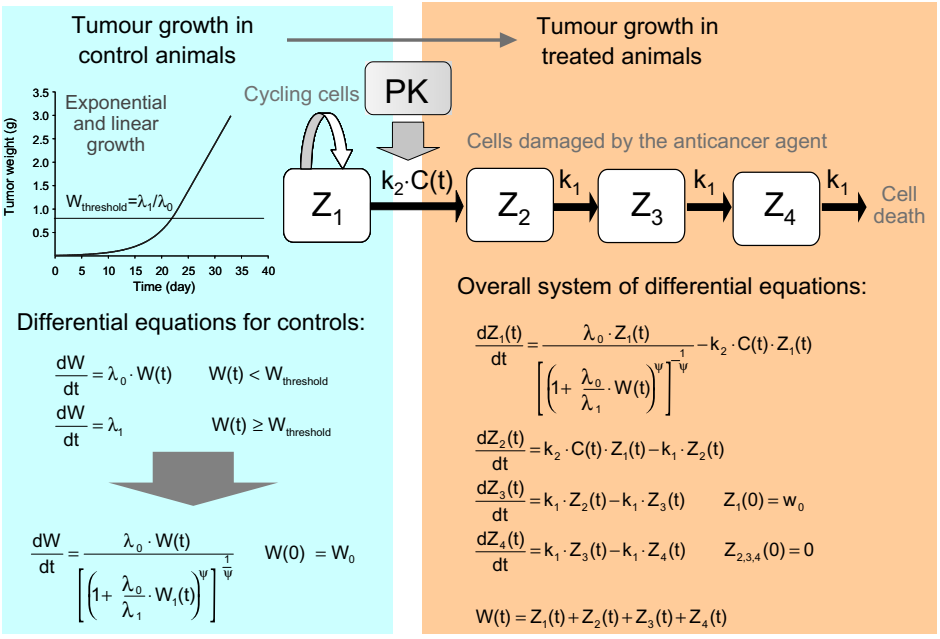

Fig. 1 – Scheme and equations of the PK/PD tumour growth inhibition model.

Table 2 – Pharmacokinetic parameters of the evaluated anticancer drugs used for generating plasma profiles as input to the PK/PD analysis

Drug	V_1 mL/kg	k_{10} 1/h	k_{12} 1/h	k_{21} 1/h	k_{13} 1/h	k_{31} 1/h
5-fluorouracil	714.1	6.301	0.234	0.096		
Cisplatin	394.9	4.417	4.523	1.971		
Docetaxel	824.9	1.960	2.048	2.405	0.320	0.114
Doxorubicin	2040.1	1.377	4.264	0.508	3.432	0.069
Etoposide	124.2	4.372	7.945	4.310		
Gemcytabine	887.2	4.053	0.849	1.883		
Irinotecan	4852.9	0.553	0.011	0.062		
Paclitaxel	813.2	0.868	0.006	0.084		
Vinblastine	4175.2	1.254	1.838	0.642		
Vincristine	2235.1	0.897	4.180	1.458		

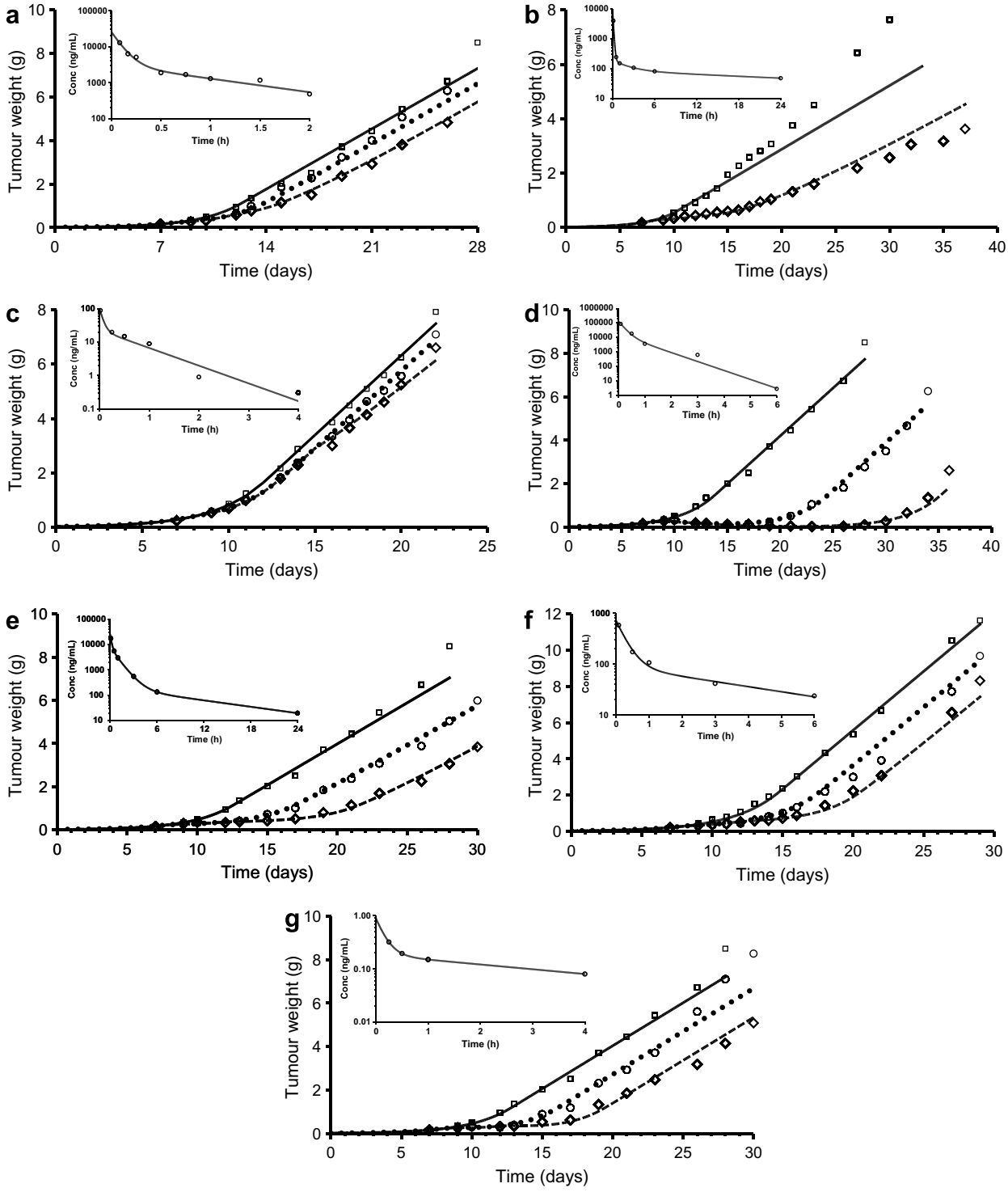

Fig. 2 – Observed and model-fitted tumour growth curves obtained in nude mice given i.v. established anticancer drugs. In the insets of each panel, the fittings of the pharmacokinetic data are shown. Panel (a) Cisplatin: vehicle (□), 4 mg/kg as single (○) or q4dx2 treatment (◇). Pharmacokinetics obtained from literature.[21] Panel (b) Doxorubicin: vehicle (□), 12 mg/kg as single dose (○). Pharmacokinetics obtained in an ancillary group of animals. Panel (c) Etoposide: vehicle (□), 20 mg/kg as single dose (○) or q7d × 2 treatment (◇). Pharmacokinetics from the literature.[22] Panel (d) Gemcytabine: vehicle (□), 120 mg/kg as single dose (○) or q4d × 2 treatment (◇). Pharmacokinetics obtained in an ancillary group of animals.Panel (e) Docetaxel: vehicle (□), 10 mg/kg as single dose (○) or q4d × 2 treatment (◇). Pharmacokinetics after a single bolus dose of 20 mg/kg. Panel (f) Vinblastine: vehicle (□), 3 mg/kg as single dose (○) or q4d × 2 treatment (◇). Pharmacokinetics obtained in an ancillary group of animals. Panel (g) Vincristine: vehicle (□), 0.5 mg/kg as single dose (○) or q4d × 2 treatment (◇). Pharmacokinetics after a single bolus dose of 2 mg/kg.

weight, represented by the sum of the weights of the cells in the various states 1, 2, 3 and 4, and w_0, λ_0 and $\lambda_1$are the parameters describing the growth of the proliferating cells as in the control animals. The action of the drug on the tumour growth is determined uniquely by the two parameters k_1, the micro-rate constant describing the kinetics of cell death, and k_2, the proportionality factor linking the plasma concentration to the effect. In this context, k_2 is the parameter describing the antitumour potency of the compound. Based on the annihilation of the differential equations, a threshold concentration $C_T = \lambda_0/k_2$ can be derived, such that, if animals are exposed to a steady state drug concentration $Css > C_T$, the model eventually predicts the tumour eradication.

2.4. Data analysis

For each drug, the pharmacokinetics were analysed using standard compartmental models; a list of the corresponding pharmacokinetic parameters is presented in Table 2. The plasma profiles corresponding to the different schedules adopted in the pharmacological studies were thus generated and used in the PK/PD model. The corresponding values of the potency parameter k_2 and $C_T$were then estimated using a simultaneous fitting of the average tumour growth curves observed in control and treated animals. Both PK and PK/PD analysis were performed using Winnonlin (version 3.1, Pharsight, CA, USA).

2.5. Definition of 'active clinical doses'

According to the different situations encountered in the clinical practice, anticancer drugs are given to patients at different doses and administration schedules. As a consequence, it is difficult to identify a unique clinically active dose for each drug. Due to this, the lowest and highest dose levels commonly encountered in the clinical literature,[24,25] expressed in terms of cumulative doses given over a 3-week period, were considered 'active clinical doses' for the purpose of this study. The 3-week period was chosen because it is the most frequently adopted cycle duration for the majority of these compounds.

3. Results

The PK/PD model of tumour growth inhibition was applied to the panel of anticancer drugs considered in this paper. The fittings of the average plasma concentrations (*inset*) and tumour weights are shown in Fig. 2. The values of the potency related parameters k_2 and C_T of the complete set of drugs are reported in Table 3. In the same table, for each drug, the corresponding range of clinical active doses[24,25] and plasma clearance values in humans (CL_h) taken from the literature are reported.[26,27]

In Fig. 3, a scatter plot on a log-log scale of the systemic exposures, simply derived from the clinical doses as AUC = Dose/CL_h (where AUC = area under the plasma concentration-time curve and Dose = midpoint of the range of active clinical doses), *versus* the k_2 values estimated in animals is shown. The relationship is evident and a strong correlation between the two variables was obtained ($r = -0.927$). Similar results were also obtained using C_T. In this case, assuming a direct proportionality between C_T and the active exposure in patients, multiplying both terms by the corresponding clearance in humans (CL_h), the $C_T \times CL_h$ values can be directly plotted versus the corresponding clinical dose levels (see Fig. 4). A strong correlation was observed ($r = 0.939$) and the slope of the regression line on a log-log scale was not significantly different from one ($p > 0.05$, *t*-test), in agreement with the presence of a direct proportionality between the two variables.

Table 3 – Relevant pharmacokinetic and pharmacodynamic parameters and doses used in the clinical practice for the evaluated anticancer agents

Drug	k_2 mL/μg/h (CV %)	λ_0 1/h (CV%)	C_T ng/mL	Plasma clearance in humans[27] L/h/m^2	Range of clinical doses mg/m^2 in 3-week periods	
5-fluorouracil	0.0842[18] (11.0)	0.0101 (8.3)	120	39.5 ± 17.3	1000[25]	5250[25]
Cisplatin	0.265 (11.2)	0.0152 (3.6)	57.5	15.6 ± 3.0	60[25]	100[25]
Docetaxel	0.277 (8.4)	0.0143 (3.6)	51.5	22.6 ± 7.7	75[24,25]	100[24,25]
Doxorubicin	0.686 (33.7)	0.0158 (12.6)	23.0	40.0 ± 20.3	50[25]	90[26]
Etoposide	0.00892 (15.8)	0.0147 (4.2)	1650	1.68 ± 0.57	300[25]	360[25]
Gemcytabine	0.0970 (6.3)	0.0126 (6.0)	130	91.8 ± 47.9	2000[25]	3750[24]
Irinotecan	0.0351[18] (9.4)	0.0061 (4.0)	174	14.8 ± 4.0	250[24,25]	350[24,25]
Paclitaxel	0.0262[18] (8.0)	0.0122 (5.5)	465	13.6 ± 8.6	175[24,25]	250[25]
Vinblastine	1.579 (7.9)	0.0125 (4.4)	7.91	29 (range 24–35)[28]	8[25]	12[25]
Vincristine	4.595 (9.1)	0.0142 (5.3)	3.10	4.92 ± 3.01	1[25]	2[25]

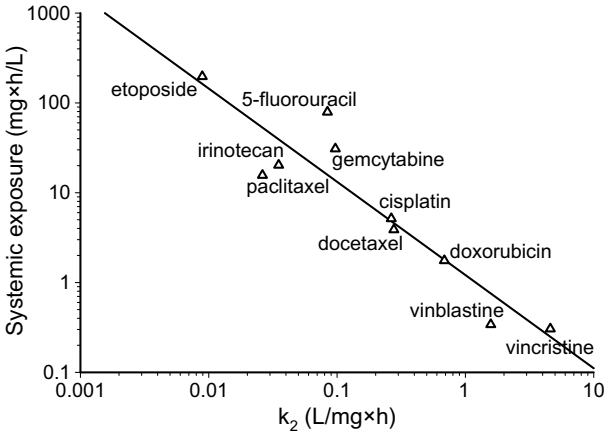

Fig. 3 – Relationship between the systemic exposure obtained at the midpoint of the dose range used in the clinics (cumulative doses given in 3-week cycles) and k_2 (potency parameter) estimated in animals. Regression performed on log-log scale: intercept = 0.0835, slope = –1.03, r = – 0.927.

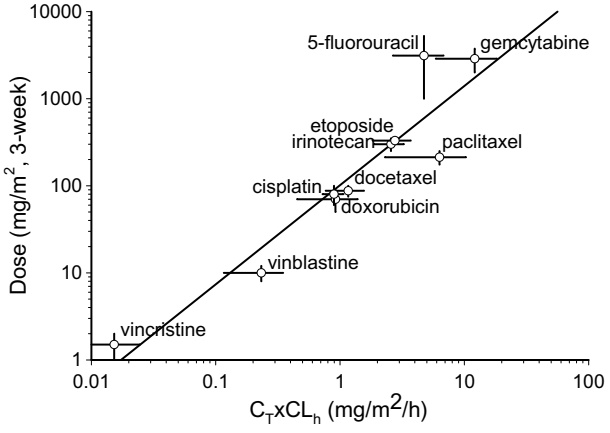

Fig. 4 – Relationship between the doses used in the clinics (cumulative doses given in 3-week cycles) and $C_T \times CL_h$. Regression (based on the midpoint of the dose range) performed on log-log scale: intercept = 2.01, slope = 1.14, r = 0.939. Vertical error bars represent the dose range reported in the literature. Horizontal error bars based on ± standard deviation of CL_h.

4. Discussion

A recent PK-PD model provides drug-specific measurements of the potency of anticancer compounds from preclinical studies. On these bases, we investigated whether doses and exposures required to achieve a clinical response in humans can be predicted from the potency parameters k_2 and C_T estimated in animals. A strong correlation was observed between the parameter k_2 and the systemic exposure in the clinical use. Analogous results were obtained when C_T estimates were correlated with the clinical doses. The latter correlation is consistent with the former one: in fact, $C_T = \lambda_0/k_2$, and the parameter λ_0, characterising the growth rate of the tumour, is relatively constant across the experiments of the present study (see Table 3).

Even allowing for the limited number of drugs and the degree of variability and uncertainty associated with the observations in the plots, the large interval of clinical doses covered by the tested drugs, the appropriate ranking of the compounds and the closeness of the data to the regression lines provide a strong support to the use of this approach for an early estimation of the active doses in humans of candidate compounds. This can be easily obtained introducing the k_2 and C_T values, estimated from the first animal studies, into the regression equations (plasma clearance in humans may be derived using the common interspecies scaling procedures) and making a simple extrapolation to the vertical axis.

We derived the approach using known anticancer drugs with different mechanisms of action, including topoisomerase inhibitors, antimicrotubule assembly inhibitors, antimetabolites and alkylating agents. This finding is consistent with the concept that the PK-PD model used for estimating the potency of the compounds is not intended to describe the specific molecular mechanism of action, but merely relates the degree of cell damage and cell death with the plasma concentrations of the drugs,[18,20] so that the model was also successfully employed for modelling the PK-PD of compounds with novel mechanism of action. Although no molecular targeted agents are present in the dataset used for building the correlations, preliminary analyses are also supporting the use of this methodology for this kind of compound.

If further confirmed and extended, this approach might significantly improve the drug discovery and development process, which for the oncology therapeutic area is particularly expensive and time-consuming. For example, the active dose of a new compound, estimated as described here, can be compared with the expected maximal tolerated dose based on toxicological findings or determined in phase I studies. This would provide a further support to the risk evaluation process required to take decisions on the progression to the subsequent phase II or III. As a consequence, ineffective drugs can be deprioritised and/or discarded earlier, the whole clinical development program shortened and, in turn, the number of patients undergoing ineffective treatments sensibly reduced.

Conflict of interest statement

None declared.

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