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Relationship between pharmacokinetic parameters in patients and cytotoxicity *in vitro* of standard and investigational anticancer drugs

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The selection of the starting dose for initial clinical trials of anticancer agents is mostly determined by toxicological endpoints in mice (LD_{10}). So far, very few attempts have been made to evaluate the potential value of cytotoxicity assays for this purpose. The present study was undertaken as a first attempt to investigate the relationship between cytotoxicity of anticancer drugs *in vitro* and pharmacokinetic parameters *in vivo* in patients, at suggested maximum tolerated doses. Using the fluorometric microculture cytotoxicity assay (FMCA), we determined the concentration giving 50% cell survival (IC_{50}) *in vitro*, for 25 cytotoxic drugs in fresh preparations of normal peripheral blood mononuclear cells (PBMC) and of tumor cells from patients with acute or chronic lymphocytic leukemia (ALL or CLL). Using linear regression, we investigated the relationship between the IC_{50}s and clinically achievable peak plasma concentrations (C_{max}) or concentration–time products (C × T) in humans. The clinical data was obtained from the literature. Based on all drugs tested, good correlations were obtained between IC_{50}s for CLL cells, and both C_{max} and C × T ($R \approx 0.7$, $p < 0.0002$), and for ALL cells and normal PBMC between IC_{50} and C_{max}, while the two latter cell types showed somewhat weaker relationships to C × T. Using the IC_{50} data of CLL cells, predictions of C_{max} and C × T exceeded 1 log for only four drugs. No tendencies to under- or overprediction within different classes of drugs were noted. The results demonstrate a significant relationship between toxicity *in vitro* and achievable systemic exposure of anticancer drugs in humans, which suggests that non-clonogenic *in vitro* assays for drug sensitivity testing may provide pharmacokinetic information useful in the development of investigational cytotoxic drugs.

Key words: Anticancer agents, *in vitro* assay, pharmacokinetics.

Introduction

Cytotoxic drugs often exhibit an extremely narrow therapeutic index and phase I clinical trials of new anticancer agents therefore involve numerous dose escalation steps to determine the maximum tolerated dose (MTD). This is time consuming, financially costly and involves ethical problems since patients are enrolled at subtherapeutic doses. To circumvent these problems, several models have been proposed to predict target plasma concentrations for new cytotoxic drugs in humans.[1,2]

A phase I dose-escalation procedure based on plasma concentration–time products (C × T) in mice was suggested several years ago by Collins *et al.*[3] After initiating the human phase I study at 1/10 of the mouse lethal dose 10% (LD_{10}), plasma C × T data is generated and the dose escalated to approach a plasma C × T equal to that achieved in the mouse model at LD_{10}.

In 1988, Davis *et al.* described a statistical regression model, using the relationship between LD_{10} in mice and human plasma C × T.[2] Scheithauer *et al.* had earlier suggested a similar model, using mouse LD_{50} and clinical peak plasma concentrations (C_{max}), to find reasonable test concentrations for *in vitro* evaluation of new anticancer agents.[4] Good correlations were obtained in both of these studies confirming an overall relationship between toxicological endpoints in animal systems and pharmacokinetic parameters in humans for cytotoxic drugs.

Numerous methods exist for determination of drug sensitivity and resistance *in vitro* in tumor cells from patients. Most experience has been obtained with colony-forming assays, which have shown good correspondance with clinical response.[5,6] However, these assays have low evaluability rates, poor reproducibility and are technically difficult.[5] During recent years, several simple non-clonogenic cytotoxicity assays, based on detection of cell damage in the whole tumor cell population rather than in the proliferative or clonogenic cell fraction have evolved.[7,8] These short-term and rather easily performed assays have shown correlations with clinical outcome comparable to clonogenic assays.[5,7,8] Al-

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though efforts to predict myelosuppressive toxicity using clonogenic assays on bone marrow cells have been made for some investigational drugs,[9,10] little effort has been made to evaluate the potential value of non-clonogenic assays in preclinical toxicology testing and clinical trial planning.

In the present study we attempted to investigate the possible relationship between cytotoxicity *in vitro* in primary cultures of normal human peripheral blood mononuclear cells (PBMC) or human tumor cells from drug sensitive tumor types and pharmacokinetic estimates of systemic drug exposure at MTD in humans.

Materials and Methods

Cell preparation and drugs

The activity of 25 standard or experimental cytotoxic drugs was tested on fresh tumor cells from patients with acute lymphocytic leukemia (ALL) or chronic lymphocytic leukemia (CLL), and on fresh normal PBMC. For each cell type, usually five samples were analyzed, with the exceptions defined in Table 1. Tumor samples were obtained from routine blood/bone marrow sampling and normal PBMC from blood donors. Tumor cell and PBMC suspensions were prepared by Ficoll-Paque (Kabi-Pharmacia, Uppsala, Sweden) density gradient centrifugation, as described previously.[11] The cells were resuspended in culture medium RPMI 1640 (HyClone, Cramlington, UK), supplemented with 10% heat-inactivated fetal calf serum (HyClone), 2 mM glutamine, 50 μg/ml streptomycin and 60 μg/ml penicillin. 2-Chlorodeoxyadenoside (CdA) was a kind gift from Dr Jan Liliemark, Karolinska Hospital, Stockholm, Sweden. The other cytotoxic drugs were obtained from commercial sources. The drugs were tested at five different concentrations, obtained by 5-fold serial dilution. V-shaped 96-well microtiter plates (Nunc, Roskilde, Denmark) were prepared with 20 μl/well of the drug solutions at 10 times the desired final concentration and stored at −70°C until used, as described.[11]

Table 1. Clinically achievable C_{max}, C × T and IC_{50} *in vitro* of the indicated cytotoxic drugs at their proposed MTD

Drug	Dose	Route of administration	C_{max} (μg/ml)	C × T (μg × h/ml)	Reference	IC_{50}CLL[b] (μg/ml)	IC_{50} ALL (μg/ml)	IC_{50} PBMC (μg/ml)
5-Fluorouracil	15 mg/kg	i.v.[a]	60.0	16.33	12	120.0	140.0	230.0
6-Thioguanine	135 mg/m^2	i.v.	12.5	71.85	12	42.0	25.0	79.0
Amsacrine	120 mg/m^2	i.v.	6.90	22.22	12	0.20	0.14	1.6
Bleomycin	15 U/m^2	i.v.	3.0	4.99	12	27.0	23.0	35.0
Cytarabine	10 mg/kg	i.v.	250	15.23	12	0.32	0.42	1.0
Carboplatin	330 mg/m^2	30 min infusion	45.7	99.0	13	88.0	58.0	56.0
CdA	0.14 mg/kg	2 h infusion	0.056	0.168	14	0.75	0.30	0.016[c]
Chlorambucil	0.6 mg/kg	oral	1.10	2.38	12	2.8	0.88	3.5
Cisplatin	100 mg/m^2	i.v.	2.49	1.94	12	2.1	1.2	2.7
Daunorubicin	100 mg/m^2	i.v.	0.41	7.32	12	0.21	0.052	0.081
Doxorubicin	60 mg/m^2	i.v.	0.6	3.84	12	0.25	0.13	0.76
Dacarbazine	250 mg/m^2	i.v.	15.3	30.72	12	140.0	220.0	250.0
Epirubicin	70–100 mg/m^2	2 h and 5 min infusion	0.265	1.70	15,16	0.75	0.78	0.84
Etoposide	290 mg/m^2	i.v.	34.2	153.94	12	1.3	0.92	2.9
Fludarabine	18–25 mg/m^2	30 min infusion	0.509	2.98	17	7.0	1.9	2.1
Gemcitabine	790 mg/m^2	30 min infusion	15.0	8.75	18	6.3	31.0	0.80[c]
Idarubicin	10–15 mg/m^2	1–7 min infusion	0.189	0.195	19,20	0.16	0.10	0.085[c]
Melphalan	0.6 mg/kg	i.v.	3.38	2.47	12	2.5	2.5	4.0
Mitomycin	20 mg	i.v.	1.5	0.36	12	1.1	1.2	2.3
Mitoxantrone	12 mg/m^2	1 h infusion	0.31	0.52	21	0.050	0.020	0.10
Mitotane	5–15 g	oral	50.0	—	22	72.0[c]	65.0[c]	68.0[c]
Streptozocin	1.5 g/m^2	i.v.	381	72.1	23	420.0[c]	800.0[c]	490.0[c]
Taxol	175 mg/m^2	3 h infusion	3.65	14.3	24	3.9	5.6	3.0[c]
Vincristine	0.025 mg/kg	i.v.	0.372	0.064	12	0.015	2.7	9.4
Vinorelbine	30 mg/m^2	15 min infusion	0.78	0.80	25	0.49	0.86	3.6[c]

[a] i.v., intravenous bolus injection.
[b] The IC_{50} values are presented as the mean of five samples, with the exceptions indicated.
[c] Mean of three samples.

Cytotoxicity assay procedure

For each drug and cell type the concentration giving 50% cell survival *in vitro* (IC_{50}) was determined using the fluorometric microculture cytotoxicity assay (FMCA). The assay is based on measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA; Sigma, St Louis, MO) to fluorescein by living cells with intact plasma membranes. The principal steps of the FMCA procedure have been described previously.[11] Briefly, 180 µl/well of the cell suspension (50×10^3 and 100×10^3 cells/well for PBMC and ALL/CLL, respectively) were seeded into the prepared 96-well microtiter plates, using a pipetting robot (Pro/pette; Perkin Elmer, Norwalk, CT), and the plates were then incubated at 37°C and 5% CO_2 for 72 h without any change of medium. Each drug concentration was tested in triplicate wells. Six blank wells received only culture medium and six wells containing cells but no drug served as controls. At the end of the incubation period, the medium was removed, the cells washed once with phosphate buffered saline (PBS), and FDA at 10 µg/ml in PBS was added to blank, control and test wells. The plates were incubated for 1 h before measuring the generated fluorescence in a Fluoroscan II (Labsystems Oy, Helsinki, Finland).

Quantification of FMCA results

Cell survival was presented as survival index (SI), defined as fluorescence of experimental cultures in per cent of control cultures with blank values subtracted. A custom-made program in Excel (Microsoft Corporation) was used for computing the IC_{50}s, by extracting the concentration giving a SI of 50% from the obtained dose–response curves. Statistical correlations and fitted models were made by linear regression analysis using Statview (Abacus Concepts, Berkeley, CA). A logarithmic transformation of parameters was used in the regression analysis. For the IC_{50} data, the mean of the logarithms of the IC_{50}s of the individual samples was calculated.

Quality control

Quality criteria for a successful assay included >70% of tumor cells or >90% of PBMC in the cell preparation prior to incubation, a fluorescence signal in control cultures >5 times mean blank values and a coefficient of variation in control wells of <30%. Only successful assays were included in this study.

Clinical data

Clinical C_{max} and C × T were obtained from the literature, referenced in Table 1. For comparison, the same data as in the studies by Davis *et al.*[2] and Scheithauer *et al.*[4] were used where possible (Table 1). Otherwise, plasma concentrations at MTD measured after bolus administration or short-term infusions were used.

Results

The clinical C_{max} and C × T of each drug as well as the IC_{50}s (mean of five samples) for the three different cell types tested are presented in Table 1. When linear regression analysis was performed on the logarithms of the clinical C_{max} and C × T versus the mean of the logarithms of the IC_{50}s, significant correlations were observed between IC_{50} *in vitro* and clinical C_{max} for all three cell types ($R = 0.69$–0.72, $p < 0.0001$; Table 2). The relationship between IC_{50} *in vitro* and C × T was of the same magnitude for CLL ($R = 0.68$, $p = 0.0002$) but was somewhat weaker for the ALL and PBMC models ($R = 0.60$ and 0.57, $p = 0.002$ and 0.004, respectively). Figure 1 shows the regression lines with 95% confidence limits for CLL, and correlation coefficients and fitted models for all the three cell types are presented in Table 2.

To evaluate the reliability and variability of predicted drug concentrations, the deviations from observed C_{max} and C × T were calculated, on a log basis, for individual and classes of drugs, using the CLL models (Table 3). The overall mean of the absolute deviations between observed and predicted C_{max} and C × T was 0.51 and 0.54 logs, respectively. For C_{max}, cytarabine showed a deviation between the predicted and the observed values considerably greater than 1 log, and for C × T amsacrine and etoposide showed this magnitude of deviation. In all these cases, the predicted value underestimated the true value. Considered as different classes of drugs, deviations ranged from −0.39 to +0.24 for C_{max} and −0.47 to +0.15 for C × T.

Discussion

Previous studies have shown that toxicological endpoints like LD_{10} or LD_{50} in mice have a predictive value for plasma concentrations of anticancer agents achievable in man.[2,4] In the present study

Table 2. Correlation between log C_{max} or log C × T values, as indicated in Table 1 for all drugs tested, and mean log IC_{50} for normal PBMC, CLL and ALL cells *in vitro*

Cell type	Clinical variable	No. of drugs tested	R	R^2	p	Fitted model
CLL	C_{max}	25	0.70	0.50	<0.0001	log C_{max} = 0.44+0.58 × log IC_{50}
CLL	C × T	24	0.68	0.47	0.0002	log C × T = 0.59+0.52 × log IC_{50}
ALL	C_{max}	25	0.72	0.52	<0.0001	log C_{max} = 0.43+0.58 × log IC_{50}
ALL	C × T	24	0.60	0.36	0.0019	log C × T = 0.59+0.45 × log IC_{50}
PBMC	C_{max}	25	0.69	0.48	0.0001	log C_{max} = 0.26+0.60 × log IC_{50}
PBMC	C × T	24	0.57	0.33	0.0039	log C × T = 0.46+0.46 × log IC_{50}

we demonstrate a relationship also between pharmacodynamics *in vitro* of anticancer drugs in hematological cells and pharmacokinetic parameters in man. The predictive ability of the cellular model with respect to C_{max} is comparable to that of Scheithauer using LD_{50},[4] and for 15 of the 25 drugs the clinical pharmacokinetic data used was the same in the two models. The predictive ability of the C × T model was also good but the relationship was weaker than for the animal model of Davis *et al.*[2] It should be noted, however, that none of the animal models included the adenosine analogs fludarabine or CdA, which clearly would have reduced the magnitude of the correlations for these regression models (see below).

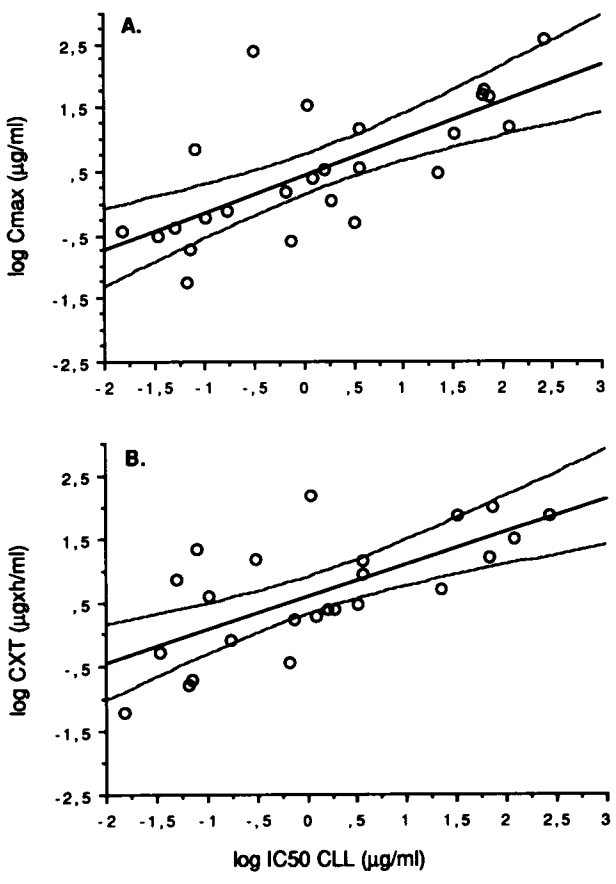

Figure 1. Linear regression lines with 95% confidence limits for IC_{50} of chronic lymphocytic leukemia cells versus C_{max} (A) and C × T(B) of cytotoxic drugs at the doses indicated in Table 1. Correlation coefficients were 0.70 and 0.68, respectively. Logarithmic transformation of data was used.

The observed relationship between pharmacokinetic parameters associated with MTD of cytotoxic drugs and pharmacodynamic data *in vitro* may provide information valuable in the development of investigational anticancer agents. The ability to predict target plasma concentrations could be of assistance in the selection of doses for human phase I trials. Furthermore, IC_{50} determinations *in vitro* could also provide an early prediction of clinical activity of a new agent. Thus, if a dose level associated with clinical toxicity yields plasma concentrations lower than those predicted by the *in vitro* model, clinical usefulness of the agent may be less likely and vice versa. The IC_{50} predictions may in this context preferably be used as complement to the animal toxicity models.

The combined use of animal toxicity and the IC_{50} *in vitro* models may be especially valuable for compounds like the deaminase-resistant adenosine nucleoside analogs fludarabine and CdA for which species differences at the target enzyme, deoxycytidine kinase, level exists.[26] For example, the LD_{50} model previously described[4] would considerably have overpredicted C_{max} for these drugs, approximately 100-fold. With the CLL *in vitro* models, on the other hand, C_{max} values were overpredicted only about 10-fold. This was true also when using models generated without fludarabine and CdA data (not shown). Indeed, both fludarabine and CdA showed significant clinical grade IV toxicity at the phase I starting dose based on toxicity data obtained from mice.[27,28]

Some limitations of the present approach should be considered. Some of the pharmacokinetic data was obtained several years ago with less than op-

Table 3. Deviation of predicted C_{max} and C × T from observed values (logarithmic values) using the IC_{50} CLL models for individual and classes of cytotoxic drugs

Agent	Deviation (logs) of predicted C_{max}	Deviation (logs) of predicted C × T
Alkylating agents		
chlorambucil	+0.56	+0.36
carboplatin	−0.14	−0.43
cisplatin	+0.09	+0.34
melphalan	+0.03	+0.30
Mean	**+0.14**	**+0.14**
Antibiotics		
bleomycin	+0.75	+0.59
daunorubicin	+0.08	−0.95
doxorubicin	+0.09	−0.50
epirubicin	+0.94	+0.29
idarubicin	+0.50	+0.70
mitomycin	+0.16	+0.94
mitoxantrone	+0.10	+0.11
streptozocin	−0.74	−0.01
Mean	**+0.24**	**+0.15**
Antimetabolites		
CdA	+1.01	+0.76
cytarabine	−2.25	−0.86
fludarabine	+1.03	+0.38
gemcitabine	−0.41	−0.06
5-fluorouracil	−0.28	+0.33
6-thioguanine	+0.22	−0.48
Mean	**−0.11**	**0.01**
Plant alkaloids		
etoposide	−1.07	−1.58
vincristine	−0.19	+0.84
vinorelbine	+0.10	+0.29
Mean	**−0.39**	**−0.15**
Miscellaneous		
amsacrine	−1.03	−1.32
dacarbazine	+0.46	+0.18
mitotane	−0.21	ND[a]
taxol	+0.20	−0.28
Mean	**−0.14**	**−0.47**

[a] Not done.

timal techniques and with MTDs not completely up to date. Also, protein binding was not considered, although the pharmacodynamic effect is probably better related to the free fraction of drug. The presence and influence of active metabolites was not accounted for since for many drugs the early pharmacokinetic data did not provide this information. The models were built using simple linear regression. More sophisticated techniques, capable of detecting non-linear relationships and using physicochemical characteristics like hydrophobicity and molecular weight in combination with animal toxicology data, may further improve the predictive ability of preclinical models. Finally, no attempt was made to account for differences in drug administration schedules in man and mice and all *in vitro* data was obtained using continuous drug exposure. Despite these obvious limitations, the correlations obtained and presented here are reasonably high to warrant further investigation.

Conclusion

A relationship between cytotoxicity *in vitro* and achievable plasma concentrations of anticancer agents at their MTD is described. The true predictive ability and potential usefulness of *in vitro* models in the development of new cytotoxic drugs will require prospective evaluation.

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References

1. Grieshaber CK, Marsoni S. Relation of preclinical toxicology to findings in early clinical trials. *Cancer Treat Rep* 1986; **70**: 65–72.
2. Davis LE, Alberts DS, Plezia PM, *et al.* Predictive model for plasma concentration-versus-time profiles of investigational anticancer drugs in patients. *J Natl Cancer Inst* 1988; **80**: 815–9.
3. Collins JM, Zaharko DS, Dedrick RL, *et al.* Potential roles for preclinical pharmacology in phase I clinical trials. *Cancer Treat Rep* 1986; **70**: 73–80.
4. Scheithauer W, Clark GM, Salmon SE, *et al.* Model for estimation of clinically achievable plasma concentrations for investigational anticancer drugs in man. *Cancer Treat Rep* 1986; **70**: 1379–82.
5. Weisenthal LM, Lippman ME. Clonogenic and nonclonogenic *in vitro* chemosensitivity assays. *Cancer Treat Rep* 1985; **69**: 615–32.
6. Von Hoff DD. Human tumor cloning assays: Applications in clinical oncology and new antineoplastic agent development. *Cancer Met Rev* 1988; **7**: 357–71.
7. Weisenthal LM. Predictive assays for drug and radiation resistance. In: Masters J, ed. *Human cancer in primary culture, a handbook.* Dordrecht: Kluwer 1991: 103–47.
8. Fruehauf JP, Bosanquet AG. *In vitro* determination of drug response: a discussion of clinical applications. *Principles & Practice of Oncology updates* 1993; **7**: 1–16.
9. Parchment RE, Volpe DA, LoRusso PM, *et al. In vivo–in vitro* correlation of myelotoxicity of 9-methoxypyrazoloacridine (NSC-366140, PD115934) to myeloid and erythroid hematopoietic progenitors from human, murine and canine marrow. *J Natl Cancer Inst* 1994; **86**: 273–80.
10. Schroyens WA, Meeker JB, Dodion P, *et al.* Comparative effect of cisplatin, spiroplatin, carboplatin, iproplatin and JM40 in a human myeloid clonogenic assay. *Eur J Cancer Clin Onocol* 1988; **24**: 1309–12.
11. Larsson R, Kristensen J, Sandberg C, *et al.* Laboratory determination of chemotherapeutic drug resistance in tumor cells from patients with leukemia, using a fluorometric microculture cytotoxicity assay (FMCA). *Int J Cancer* 1992; **50**: 177–85.
12. Alberts DS, Chen GHS. Tabular summary of pharmacokinetic parameters relevant to in vitro drug assay. In: Salmon S, ed. *Cloning of human tumor stem cells.* New York: Alan R Liss 1980: 351–359.
13. Elferink F, van der Vijgh WJF, Klein I, *et al.* Pharmacokinetics of carboplatin after iv administration. *Cancer Treat Rep* 1987; **71**: 1231–7.
14. Liliemark J, Juliusson G. On the pharmacokinetics of 2-chloro-2-deoxyadenosine in humans. *Cancer Res* 1991; **51**: 5570–2.
15. Eksborg S. Anthracycline pharmacokinetics. *Acta Oncol* 1990; **29**: 339–42.
16. Robert J, Vrignaud P, Nguyen-Ngoc T, *et al.* Comparative pharmacokinetics and metabolism of doxorubicin and epirubicin in patients with metastatic breast cancer. *Cancer Treat Rep* 1985; **69**: 633–40.
17. Hersh MR, Kuhn JG, Phillips JL, *et al.* Pharmacokinetic study of fludarabine phosphate (NSC 312887). *Cancer Chemother Pharmacol* 1986; **17**: 277–80.
18. Abbruzzese JL, Grunewald R, Weeks EA, *et al.* A phase I clinical, plasma and cellular pharmacology study of gemcitabine. *J Clin Oncol* 1991; **9**: 491–8.
19. Eksborg S, Söderberg M, Nilsson B, *et al.* Plasma pharmacokinetics of idarubicin and its 13-hydroxymetabolite after intravenous and oral administration under fasting and non-fasting conditions. *Acta Oncol* 1990; **29**: 921–5.
20. Gillies HC, Herriott D, Liang R, *et al.* Pharmacokinetics of idarubicin (4-demethoxydaunorubicin; IMI-30; NSC 256439) following intravenous and oral administration in patients with advanced cancer. *Br J Clin Pharmacol* 1987; **23**: 303–10.
21. Gruber A, Liliemark J, Tidefelt U, *et al.* Pharmacokinetics of mitoxantrone in plasma and leukemic cells during treatment of patients with acute non-lymphocytic leukemia. *Leukemia and Lymphoma* 1992; **6**: 493–6.
22. Calabresi P, Chabner BA. Antineoplastic agents. In: Goodman AG, Rall TW, Nies AS, *et al.* eds. *Goodman and Gilman's the pharmacological basis of therapeutics* **8**. New York: Pergamon Press, 1990: 1209–63.
23. Adolphe AB, Glashofer ED, Troetel WM, *et al.* Preliminary pharmacokinetics of streptozotocin, an antineoplastic antibiotic. *J Clin Pharmacol* 1977; **17**: 379–88.
24. Huizing MT, Keung ACF, Rosing H, *et al.* Pharmacokinetics of paclitaxel and metabolites in a randomized comparative study in platinum-pretreated ovarian cancer patients. *J Clin Oncol* 1993; **11**: 2127–35.
25. Marquet P, Lachatre G, Debord J, *et al.* Pharmacokinetics of vinorelbine in man. *Eur J Clin Pharmacol* 1992; **42**: 545– 7.
26. Habteyesus A, Nordenskjöld A, Bohman C, *et al.* Deoxynucleoside phosphorylating enzymes in monkey and human tissues show great similarities, while mouse deoxycytidine kinase has a different substrate specificity. *Biochem Pharmacol* 1991; **42**: 1829–36.
27. Grever M, Leiby J, Kraut E, *et al.* A comprehensive phase I and II clinical investigation of fludarabine phosphate. *Semin Oncol* 1990; **17**: 39–48.
28. Carson DA, Wasson DB, Beutler E. Antileukemic and immunosuppressive activity of 2-chloro-2-deoxyadenosine. *Proc Natl Acad Sci USA* 1984; **81**: 2232–6.

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