Pergamon

*European Journal of Cancer*, Vol. 35, No. 3, pp. 424–432, 1999

Printed in Great Britain
0959-8049/99/$ - see front matter

PII: S0959-8049(98)00286-X

## Original Paper

# Relationship Between Diagnosis-specific Activity of Cytotoxic Drugs in Fresh Human Tumour Cells *Ex Vivo* and in the Clinic

H. Fridborg,[1] E. Jonsson,[1] P. Nygren[2] and R. Larsson[1]

[1]Division of Clinical Pharmacology; and [2]Department of Oncology, University Hospital, SE-751 85 Uppsala, Sweden

**The aim of this investigation was to evaluate the relationship between the disease-specific activity of cytotoxic drugs in the clinic and in fresh human tumour cells from patients, as detected by a non-clonogenic cytotoxicity assay. The activity of 18 different cytotoxic drugs in fresh human tumour cells *ex vivo* was analysed in up to 15 samples from each of 13 different diagnoses using the fluorometric microculture cytotoxicity assay (FMCA). For each drug and diagnosis, relative activity indices (RAIs) were calculated, defined as the fraction of samples within the diagnosis having a survival index (SI) less than the median SI for the drug in all tested samples. Clinical response rates for the drug in the same diagnoses were collected from published phase II trials and were compared with the RAIs using Spearman's rank correlation (Rho) and Pearson's correlation coefficient (*R*). Correlation coefficients could be calculated for 12 drugs. The coefficients varied between 0.02 and 0.92 (Rho) and between 0.07 and 0.91 (*R*), but for most drugs the correlation between RAI and clinical response rates was good, with Rho > 0.6 and *R* > 0.7. Weak correlations were observed for cyclophosphamide and vincristine (Rho = 0.32 and 0.16, respectively), which might be due to old clinical data, and for paclitaxel (Rho = 0.02), which perhaps could be explained by *in vitro* activity of the solvent, Cremophor EL. The 18 drugs were also categorised according to their suggested clinical use in solid or haematological tumours, and were then compared regarding the activity in solid compared with haematological tumours *ex vivo*, expressed as the ratio between the number of responders among solid and haematological tumours (S/H ratio). The mean *ex vivo* S/H ratios in the group of drugs registered for use in haematological tumours was only 0.09 and was significantly different (*P* = 0.05) from the mean S/H ratios for the drugs used in both haematological and solid tumours (0.31) and in solid tumours only (0.47). Furthermore, the FMCA could identify the 50% most and least sensitive diagnoses with respect to clinical phase II activity with 74% (78/106) correct classifications. The results indicate that the relative activity of cytotoxic drugs in different diagnoses may be detected by the FMCA. Thus, 'phase II trials *ex vivo*' using non-clonogenic cytotoxicity assays might be used to make clinical trials more effective by targeting trials to diagnoses in which a new agent is most likely to be active.** 

**Key words: drug development, anticancer drugs, disease specificity, cytotoxicity assay, *ex vivo*, FMCA**
*Eur J Cancer*, Vol. 35, No. 3, pp. 424–432, 1999

## INTRODUCTION

THE MEANS to determine the activity of new cytotoxic drugs in different tumour types has thus far been the empirical phase II clinical trial. This approach involves financial as well as ethical problems, since there is a risk of giving a new agent to a large number of patients with diagnoses in which the drug turns out to be ineffective [1]. A valid method for preclinical evaluation of disease-specific activity of new anticancer agents could, therefore, be of value in the planning of

Correspondence to H. Fridborg.
Received 6 Mar. 1998; revised 29 Jun. 1998; accepted 15 Jul. 1998.

clinical trials, by directing the phase II trials into the specific tumour types in which the new agent is most likely to be effective.

During the few last decades, a number of laboratory assays for the determination of drug resistance in fresh tumour cells from patients *ex vivo* have evolved [2]. The primary objective of the development of these assays was to tailor the individual patient's treatment. We have developed a non-clonogenic fluorometric microculture cytotoxicity assay (FMCA) which is a rapid and efficient method, applicable to most tumour types [3, 4]. The feasibility of the FMCA for prediction of individual cytotoxic drug sensitivity is well documented. The assay has so far been applied to more than 2500 patient tumour samples, and the specificity and sensitivity of the test for prediction of individual tumour response are in the same range as for other non-clonogenic cytotoxicity assays, such as the MTT and differential staining cytotoxicity (DiSC) assays [5–7]. Much less is known about the ability of the FMCA and other assays to detect disease-specific activity. Although some studies have indicated this potential [8–18], no quantitative estimates of this relationship have been reported.

The aim of the present study was to evaluate the feasibility of the FMCA for prediction of cytotoxic drug activity in different diagnoses, by performing statistical analyses of the relationship between the activity pattern of cytotoxic drugs as determined in primary cultures of human tumour cells using the FMCA and as observed in the clinic. To obtain this, we used a defined number of patient samples for the determination of an activity index *ex vivo* and phase II clinical response rates as a numerical measure of clinical activity.

## MATERIALS AND METHODS

*Patient samples and assay procedure*

Tumour samples from patients were obtained by routine surgery, diagnostic biopsy or bone marrow/peripheral blood sampling, and this sampling was approved by the ethical committee at the University of Uppsala. Up to 15 consecutive successfully analysed samples of each of 13 different diagnoses were included in the study (Table 1).

Leukaemic cells were isolated from bone marrow or peripheral blood by Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) density gradient centrifugation [3]. Tissue from solid tumour samples was minced with scissors and tumour cells were then isolated by collagenase dispersion followed by Percoll (Pharmacia Biotech) density gradient centrifugation [4]. Viability was determined by trypan blue exclusion test and the proportion of tumour cells in each cell preparation was judged by inspection of May–Grünwald–Giemsa–stained cytocentrifuge preparations by a trained cytopathologist.

The FMCA is based on the measurement of fluorescence generated from hydrolysis of fluorescein diacetate (FDA; Sigma Chemicals, St. Louis, Missouri, U.S.A.) to fluorescein by cells with intact plasma membranes. The assay has been described in detail previously [3, 14] and only the major steps will be outlined here. Tumour cells, resuspended in culture medium RPMI 1640 (HyClone, Cramlington, U.K.), were seeded into 96 well microtitre plates prepared with drugs. The drugs were tested in triplicate wells, six untreated wells served as controls and six wells containing culture medium without cells as blanks. The cell densities varied from 10 000 to 100 000 cells/well depending on the tumour type. The plates were incubated for 72 h at 37°C and then the medium and drugs were removed before adding FDA to each well. The plates were incubated for 30–45 min and the generated fluorescence in each well, proportional to the number of living cells in the well, was measured in a Fluoroscan II (Labsystems Oy, Helsinki, Finland). Cytocentrifuge preparations were made from the untreated control wells to check cell identity.

The quality criteria for a successful assay included $\geq 70\%$ tumour cells in the cell preparation prior to and/or in the control wells after incubation, a fluorescence signal in control cultures of more than five times mean blank values and a coefficient of variation (CV) in the control cultures of $< 30\%$. Only successfully analysed samples were included in the study. The overall success rates of the assay are approximately 85% and 60% for haematological and solid tumours, respectively, with a low yield of cells after separation or a too low proportion of tumour cells in the preparation being the most common causes of assay failure.

*Drugs*

Eighteen different cytotoxic agents, clinically well-established as well as recently introduced, were analysed at their empirically derived cut-off concentrations (EDCC), defined as the concentration of a drug which produces the largest scatter of cell survival (highest standard deviation) among patient samples [3]. The drugs and the concentrations tested are listed in Table 1.

For most drugs, commercially available clinical formulations, either powder or solutions for injection/infusion, were used. Thus, the paclitaxel used *in vitro* (Taxol®) contained the vehicle Cremophor EL. The drugs or drug solutions were dissolved or further diluted in sterile water or phosphate buffered saline (PBS) to the desired test concentrations. Since cyclophosphamide is metabolically activated *in vivo*, its active metabolite, 4-hydroxycyclophosphamide (4-HC; Asta Medica, Frankfurt, Germany) was used for the *ex vivo* experiments. Thioguanine was obtained from Sigma and was dissolved in 0.04 M NaOH and diluted in sterile water. The final NaOH concentration showed no effect on cell survival. Topotecan (SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania, U.S.A.) and vinorelbine tartrate (Pierre Fabre Medicament, Muret, France) were dissolved in sterile water.

*Definitions and statistical analyses*

The FMCA results are presented as survival index (SI), calculated as the fluorescence in drug treated wells as a percentage of the fluorescence in control wells with blank values subtracted.

For each drug, the disease-specific activity in patient tumour cells *ex vivo* is presented as relative activity indices (RAIs) for the different diagnoses, defined as the fraction of samples within a diagnosis having a SI less than the median SI for the drug in all analysed patient samples. The RAI was not calculated for diagnoses from which less than eight samples were analysed, although data for all successfully analysed samples were included in the calculation of the median SI for the drug. The higher the RAI, the more effective the drug *ex-vivo* in the particular diagnosis compared with other diagnoses, and vice versa.

Clinical response rates were collected from published single agent phase II trials. Where possible, comprehensive tabular summaries were used [19–21]. For drugs and diagnoses not included in the summaries, a Medline search for single-agent phase II studies was performed and all retrieved studies were included. For the recently introduced drugs, a letter was

*Table* 1. *Investigated drugs and diagnoses. The table shows the number of samples analysed* ex vivo *(from untreated patient/previously treated patient/patient with unknown treatment status). The drug concentrations used (μg/ml) and the empirically derived cut-off concentrations, are indicated*

| Diagnosis | Amsacrine 1.0 | Cytarabine 0.5 | Cisplatin 2.0 | Doxorubicin 0.5 | Cyclophosphamide 2.0* | Mitomycin 0.5 | Mitoxantrone 0.5 | Prednisolone 50 | 6-thioguanine 10 |
|---|---|---|---|---|---|---|---|---|---|
| AML | 15 (6/8/1) | 15 (5/9/1) | 15 (3/11/1) | 15 (5/9/1) | 15 (5/9/1) | 15 (6/8/1) | 15 (4/10/1) | 15 (5/9/1) | 15 (6/8/1) |
| CLL | 15 (3/12/0) | 15 (3/12/0) | 15 (3/12/0) | 15 (3/12/0) | 15 (3/12/0) | 15 (5/10/0) | 15 (3/12/0) | 15 (3/12/2) | 15 (3/12/0) |
| CML | 15 (7/8/0) | 15 (7/8/0) | 15 (7/8/0) | 15 (7/8/0) | 15 (7/7/1) | 7 (1/5/1) | 15 (7/8/0) | 15 (7/8/0) | 15 (8/7/0) |
| NHL | 15 (6/9/0) | 15 (5/10/0) | 15 (6/9/0) | 15 (6/9/0) | 15 (7/8/0) | 15 (4/11/0) | 15 (7/8/0) | 15 (7/8/0) | 15 (6/9/0) |
| Bladder cancer | 5 (3/2/0) | 6 (4/2/0) | 15 (11/4/0) | 15 (11/4/0) | 14 (10/4/0) | 10 (6/4/0) | 12 (9/3/0) | 4 (3/1/0) | 3 (2/1/0) |
| Breast cancer | 12 (2/3/7) | 14 (2/4/8) | 15 (0/14/1) | 15 (0/14/1) | 15 (2/12/1) | 15 (1/13/1) | 15 (2/12/1) | 4 (0/3/1) | 12 (3/2/6) |
| Colorectal cancer | 8 (8/0/0) | 8 (8/0/0) | 15 (14/1/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 6 (6/0/0) | 6 (6/0/0) |
| NSCLC | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) | 15 (15/0/0) |
| Ovarian cancer | 15 (10/4/1) | 15 (11/3/2) | 15 (6/6/3) | 15 (7/5/3) | 15 (8/4/3) | 15 (9/5/1) | 15 (9/5/1) | 15 (10/4/1) | 15 (9/5/1) |
| Renal cancer | 11 (11/0/0) | 11 (11/0/0) | 12 (12/0/0) | 12 (12/0/0) | 12 (12/0/0) | 11 (11/0/0) | 12 (12/0/0) | 9 (9/0/0) | 11 (11/0/0) |
| Sarcoma | 15 (11/4/0) | 15 (12/3/0) | 15 (9/6/0) | 15 (10/5/0) | 15 (11/4/0) | 15 (9/6/0) | 15 (9/6/0) | 12 (9/2/1) | 15 (12/3/0) |
| SCLC | 7 (1/2/4) | 8 (2/2/4) | 11 (3/3/5) | 11 (3/3/5) | 8 (3/1/4) | 6 (2/2/2) | 10 (3/2/5) | 5 (2/1/2) | 8 (2/2/4) |
| Solid paediatric tumours† | 9 (2/7/0) | 11 (2/9/0) | 15 (6/9/0) | 15 (5/10/0) | 15 (6/9/0) | 15 (5/10/0) | 15 (5/10/0) | 8 (2/6/0) | 7 (0/7/0) |

| Diagnosis | Vincrinstine 0.5 | Etoposide 5.0 | Cladribine 0.2 | Fludarabine 2.5 | Gemcitabine 62.5 | Idarubicin 0.1 | Paclitaxel 5.0 | Topotecan 0.5 | Vinorelbine 2.5 |
|---|---|---|---|---|---|---|---|---|---|
| AML | 15 (6/8/1) | 15 (5/9/1) | 15 (6/9/0) | 15 (4/11/0) | 15 (3/12/0) | 15 (4/11/0) | 15 (6/9/0) | 15 (8/7/0) | 15 (4/11/0) |
| CLL | 15 (3/12/0) | 15 (3/12/0) | 15 (3/12/0) | 15 (3/12/0) | 15 (3/9/3) | 15 (2/13/0) | 15 (5/10/0) | 13 (4/7/2) | 15 (2/13/0) |
| CML | 15 (7/8/0) | 15 (7/8/0) | 15 (7/8/0) | 11 (6/5/0) | 9 (4/4/1) | 15 (6/9/0) | 15 (6/9/0) | 5 (2/2/1) | 11 (7/4/0) |
| NHL | 15 (7/8/0) | 15 (6/9/0) | 15 (5/10/0) | 15 (5/10/0) | 15 (3/12/0) | 15 (5/10/0) | 15 (3/12/0) | 15 (3/12/0) | 15 (7/8/0) |
| Bladder cancer | 9 (5/4/0) | 15 (12/3/0) | 5 (3/2/0) | 4 (3/1/0) | 11 (9/2/0) | 3 (2/1/0) | 15 (12/3/0) | 4 (3/1/0) | 11 (8/3/0) |
| Breast cancer | 15 (2/11/2) | 15 (1/13/1) | 6 (1/2/3) | 2 (0/2/0) | 15 (2/13/0) | 9 (2/3/4) | 15 (1/13/1) | 8 (1/7/0) | 15 (1/13/1) |
| Colorectal cancer | 15 (15/0/0) | 15 (15/0/0) | 8 (8/0/0) | 6 (6/0/0) | 15 (13/2/0) | 3 (3/0/0) | 15 (13/2/0) | 11 (11/0/0) | 15 (14/1/0) |
| NSCLC | 15 (15/0/0) | 15 (13/0/2) | 15 (15/0/0) | 15 (15/0/0) | 15 (13/0/2) | 15 (11/2/2) | 15 (13/0/2) | 15 (15/0/0) | 15 (13/0/2) |
| Ovarian cancer | 15 (9/3/3) | 15 (6/6/3) | 15 (10/4/1) | 15 (10/4/1) | 15 (9/4/2) | 15 (10/4/1) | 15 (7/5/3) | 15 (9/6/0) | 15 (10/4/1) |
| Renal cancer | 12 (12/0/0) | 12 (12/0/0) | 9 (9/0/0) | 1 (1/0/0) | 9 (9/0/0) | 10 (10/0/0) | 11 (11/0/0) | 1 (1/0/0) | 10 (10/0/0) |
| Sarcoma | 15 (10/5/0) | 15 (9/5/1) | 15 (11/3/1) | 4 (3/1/0) | 14 (9/4/1) | 15 (12/3/0) | 15 (10/5/0) | 7 (5/2/0) | 13 (9/3/1) |
| SCLC | 11 (3/3/5) | 11 (3/3/5) | 7 (2/2/3) | 1 (0/1/0) | 5 (2/1/2) | 6 (2/2/2) | 8 (3/2/3) | 2 (1/1/0) | 4 (1/1/2) |
| Solid paediatric tumours† | 15 (6/9/0) | 15 (5/10/0) | 11 (2/9/0) | 10 (2/8/0) | 7 (2/5/0) | 7 (2/5/0) | 15 (5/10/0) | 5 (1/4/0) | 7 (2/5/0) |

**In vitro* as the active metabolite 4-hydroxycyclophosphamide. †The solid paediatric tumour group contained Ewing's sarcomas, neuroblastomas and Wilms' tumours. AML, acute myelocytic leukaemia; CLL, chronic lymphocytic leukaemia; CML, chronic myelocytic leukaemia; NHL, non-Hodgkin's lymphoma; NSCLC, non-small cell lung cancer; SCLC, small cell lung cancer.

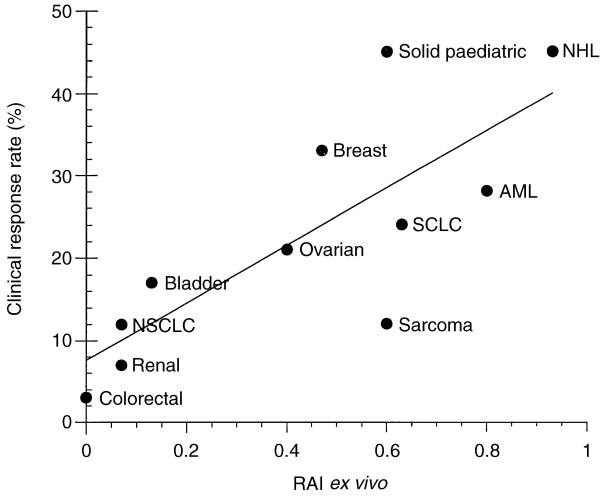


**Figure 1. Illustration of the linear relationship between clinical response rates and relative activity indices (RAIs) *ex vivo* for doxorubicin in different diagnoses ($R = 0.78$). Abbreviations as in Table 1.**

sent to the respective pharmaceutical companies requesting results of not yet published clinical trials. However, the latter approach did not add any new information. Response rates were calculated as the pooled number of partial and complete responses as a percentage of the total number of evaluable patients for a particular drug. A minimum of 14 evaluable patients was required [1].

The agreement between the disease-specific activity detected by the FMCA and the clinical response pattern of the investigated drugs was evaluated by correlating the RAI and the clinical response rate using two different correlation analyses: Spearman's rank correlation (Rho), a non-parametric test where the diagnoses are ranked according to RAI and response rate and the rank orders are compared, and Pearson's correlation coefficient ($R$) measuring the degree of linear relationship between the variables. As an example, the clinical response rates for doxorubicin are plotted versus the RAIs in Figure 1. The statistical analyses were performed using Statview (Abacus Concepts, Berkeley, California, U.S.A.) for Macintosh. Correlation coefficients were only calculated for drugs for which clinical response rates and RAIs were available in six or more diagnoses.

To evaluate whether FMCA results could predict for solid or haematological tumour specificity a solid/haematological (S/H) activity ratio was calculated for each drug, defined as the fraction of the solid tumour samples showing a SI < 50% to the drug divided by the fraction of haematological tumour samples showing a SI < 50%. Thus, high and low ratios indicate a relatively high activity in solid and haematological tumours, respectively. The leukaemias and non-Hodgkin's lymphomas (NHL) were included in the haematological tumour group and the remaining diagnoses in the solid tumour group. The drugs were categorised according to their suggested clinical use in haematological or solid tumours, and the mean S/H ratios in the different groups were compared using Student's $t$ test.

The ability of the FMCA to predict the most drug sensitive diagnoses was evaluated using a two-way table. For each drug, the diagnoses were ranked according to RAI and clinical response rate and positioned in the table, with the median diagnosis as a cut-off for 'sensitive' and 'resistant' diagnoses. Sensitivity was calculated as true 'sensitives'/(true 'sensitives' + false 'resistant') and specificity as true 'resistant'/(true 'resistant' + false 'sensitives'). The agreement between RAI and clinical activity was evaluated by the chi-square test.

## RESULTS

Table 2 shows the RAIs, as detected by the FMCA for the investigated drugs in different diagnoses, and in Table 3 the

*Table 2. Relative activity indices (RAI)* ex vivo *for the investigated drugs in different diagnoses. RAI was defined as the fraction of samples within a diagnosis having a survival index (SI) less than the median SI of all tested samples for the drug. The higher the RAI the more effective the drug* ex vivo *in the particular diagnosis compared with other diagnoses and* vice versa

| Diagnosis | Amsacrine | Cytarabine | Cisplatin | Doxorubicin | Cyclophosphamide* | Mitomycin | Mitoxantrone | Prednisolone | 6-thioguanine | Vincristine | Etoposide | Cladribine | Fludarabine | Gemcitabine | Idarubicin | Paclitaxel | Topotecan | Vinorelbine |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AML | 0.73 | 0.80 | 0.60 | 0.80 | 0.73 | 0.87 | 0.80 | 0.80 | 0.80 | 0.67 | 0.67 | 1.0 | 1.0 | 0.87 | 0.93 | 0.60 | 0.53 | 0.73 |
| CLL | 0.87 | 0.93 | 0.20 | 0.87 | 1.0 | 0.80 | 0.93 | 0.93 | 0.67 | 0.93 | 0.60 | 1.0 | 1.0 | 0.93 | 0.80 | 0.80 | 0.79 | 0.87 |
| CML | 0.87 | 0.73 | 0.67 | 0.80 | 0.60 | nd | 1.0 | 0.93 | 0.67 | 0.73 | 0.27 | 1.0 | 0.64 | 1.0 | 0.87 | 0.47 | nd | 0.73 |
| NHL | 0.87 | 0.80 | 0.33 | 0.93 | 0.87 | 0.60 | 0.87 | 0.87 | 0.73 | 0.87 | 0.67 | 0.53 | 0.60 | 0.87 | 0.87 | 0.80 | 0.93 | 0.87 |
| Bladder cancer | nd | nd | 0.60 | 0.13 | 0.29 | 0.40 | 0.33 | nd | nd | 0.67 | 0.40 | nd | nd | 0.09 | nd | 0.33 | nd | 0.27 |
| Breast cancer | 0.58 | 0.43 | 0.53 | 0.47 | 0.60 | 0.53 | 0.40 | nd | 0.58 | 0.33 | 0.27 | nd | nd | 0.20 | 0.33 | 0.53 | 0.38 | 0.53 |
| Colorectal cancer | 0.0 | 0.38 | 0.13 | 0.0 | 0.0 | 0.07 | 0.0 | nd | nd | 0.0 | 0.0 | 0.12 | nd | 0.20 | nd | 0.13 | 0.0 | 0.13 |
| NSCLC | 0.40 | 0.40 | 0.67 | 0.08 | 0.27 | 0.67 | 0.33 | 0.27 | 0.33 | 0.47 | 0.33 | 0.27 | 0.27 | 0.40 | 0.20 | 0.33 | 0.20 | 0.33 |
| Ovarian cancer | 0.27 | 0.13 | 0.73 | 0.40 | 0.20 | 0.47 | 0.33 | 0.20 | 0.27 | 0.33 | 0.53 | 0.40 | 0.40 | 0.40 | 0.20 | 0.27 | 0.40 | 0.40 |
| Renal cancer | 0.0 | 0.18 | 0.25 | 0.07 | 0.08 | 0.07 | 0.17 | 0.0 | 0.09 | 0.0 | 0.50 | 0.11 | nd | 0.22 | 0.10 | 0.18 | nd | 0.20 |
| Sarcoma | 0.07 | 0.40 | 0.60 | 0.60 | 0.33 | 0.27 | 0.33 | 0.17 | 0.33 | 0.47 | 0.60 | 0.13 | nd | 0.36 | 0.20 | 0.60 | nd | 0.23 |
| SCLC | nd | 0.38 | 0.64 | 0.64 | 0.88 | nd | 0.50 | nd | 0.62 | 0.64 | 0.73 | nd | nd | nd | nd | 0.50 | nd | nd |
| Solid paediatric tumours | 0.67 | 0.18 | 0.53 | 0.60 | 0.60 | 0.33 | 0.40 | 0.25 | nd | 0.33 | 0.73 | 0.18 | 0.10 | nd | nd | 0.73 | nd | nd |

**In vitro* as the active metabolite 4-hydroxycyclophosphamide. Abbreviations as in Table 1. nd, not determined. Less than eight samples available for calculation of the RAI.

*Table 3. Clinical response rates (%) in different diagnoses for the drugs included in the correlation analyses. The response rates were collected from single agent phase II studies (references are indicated in the table)*

| Diagnosis | Amsacrine | [ref.] | Cytarabine | [ref.] | Cisplatin | [ref.] | Doxorubicin | [ref.] | Cyclophosphamide | [ref.] | Mitomycin | [ref.] | Mitoxantrone | [ref.] | Vincristine | [ref.] | Etoposide | [ref.] | Idarubicin | [ref.] | Paclitaxel | [ref.] | Vinorelbine | [ref.] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AML | 26 | [22] | 27 | [20] | nf | | 28 | [19] | 10 | [20] | nf | | 21 | [19] | 14 | [30] | 23 | [33] | nf | | nf | | nf | |
| CLL | nf | | nf | | nf | | nf | | 42 | [20] | nf | | nf | | nf | | nf | | nf | | nf | | nf | |
| CML | 15 | [22] | nf | | nf | | nf | | 44 | [20] | nf | | 14 | [19] | nf | | nf | | 1 | [35] | nf | | nf | |
| NHL | 21 | [19] | 44 | [24, 25] | 18 | [19] | 45 | [19] | 54 | [20] | nf | | 32 | [19] | nf | | 24 | [19] | 8 | [36, 37] | nf | | 35 | [48] |
| Bladder cancer | 10 | [1] | nf | | 33 | [1] | 17 | [1] | nf | | 25 | [21] | 7 | [1] | 8 | [31] | 11 | [1] | nf | | 42 | [44] | nf | |
| Breast cancer | 5 | [1] | 9 | [21] | 17 | [1] | 33 | [1] | 34 | [21] | 37 | [21] | 18 | [1] | 20 | [21] | 8 | [1] | 14 | [35] | 37 | [45] | 40 | [48] |
| Colorectal cancer | 1 | [1] | 10 | [21] | 4 | [1] | 3 | [1] | 21 | [21] | 16 | [21] | 1 | [1] | 0 | [21] | 4 | [1] | 3 | [38] | nf | | 11* | [49] |
| NSCLC | 2 | [1] | 0 | [20] | 18 | [1] | 12 | [1] | 20 | [21] | 23 | [21] | 4 | [1] | 14 | [21] | 11 | [1] | 0 | [39] | 22 | [45] | 22 | [48] |
| Ovarian cancer | 4 | [1] | nf | | 47 | [1] | 21 | [1] | 44 | [21] | 19 | [27, 28] | 1 | [19] | 0 | [21] | 12 | [34] | 0 | [40] | 23 | [45] | 14 | [48] |
| Renal cancer | 1 | [1] | nf | | 1 | [1] | 7 | [1] | 19 | [20] | 11 | [21] | 0 | [1] | nf | | 3 | [1] | 0 | [41] | 0 | [45] | 3 | [50, 51] |
| Sarcoma | 4 | [19] | 11 | [26] | 11 | [19] | 12 | [1] | 52 | [21] | 15 | [21, 29] | 1 | [1] | 47 | [21] | 6 | [19] | nf | | 13 | [46] | nf | |
| SCLC | 0 | [1] | nf | | 17 | [1] | 24 | [1] | nf | | nf | | 1 | [1] | 42 | [32] | 25 | [1, 33] | 9 | [42, 43] | 31 | [45] | 16 | [48] |
| Solid paediatric tumours | 6 | [23] | nf | | 22 | [19] | 45 | [19] | 53 | [20] | nf | | nf | | 51 | [20] | 25 | [19] | nf | | 13 | [47] | nf | |

Abbreviations as in Table 1. *Response rate in 29 gastrointestinal tumours. nf, not found.

clinical response rates used in the correlation analyses are listed. The correlation between the two parameters for each drug is presented as Rho and $R$ in Table 4. The two coefficients paralleled each other and for most drugs the correlation was good, with Rho and/or $R > 0.7$. For cyclophosphamide, vincristine and paclitaxel, however, the correlations were much weaker ($R = 0.37$, 0.25 and 0.07, respectively). For prednisolone, 6-thioguanine and some of the recently introduced drugs, clinical data were missing or too few samples for *ex vivo* evaluation were obtained in certain diagnoses, prohibiting correlation analysis.

The activity of the tested agents in solid compared with haematological tumours *ex vivo* is shown as S/H ratios in Figure 2, where the drugs are grouped according to their approved clinical use in Sweden according to the Swedish Drug Compendium [52]. The mean S/H ratios in each group are listed in Table 5. The mean ratios in the S and HS groups of drugs were significantly higher than that in the H group (Table 5). However, the S drugs gemcitabine, mitomycin and vinorelbine showed S/H ratios comparable to those of the H drugs. Gemcitabine together with the other nucleoside

*Table 4. Spearman's rank correlation coefficient (Rho) and Pearson's correlation coefficient (*R*) for clinical response rate versus relative activity index (RAI)* ex vivo *for some cytotoxic drugs*

| | Rho | *R* | *n* |
|---|---|---|---|
| Amsacrine | 0.92* | 0.77* | 10 |
| Cytarabine† | 0.63 | 0.91* | 6 |
| Cisplatin | 0.62 | 0.71* | 10 |
| Doxorubicin | 0.78* | 0.77* | 11 |
| Etoposide | 0.79* | 0.74* | 11 |
| Cyclophosphamide† | 0.32 | 0.37 | 11 |
| Idarubicin | 0.77 | 0.25 | 6 |
| Mitomycin | 0.76 | 0.69 | 7 |
| Mitoxantrone | 0.76* | 0.75* | 11 |
| Paclitaxel | 0.02 | 0.07 | 8 |
| Vincristine† | 0.16 | 0.25 | 9 |
| Vinorelbine | 0.83 | 0.80* | 6 |
| **Mean** | **0.61** | **0.59** | |

*$P$ values < 0.05. †Clinical data based on studies from 1975 or earlier.

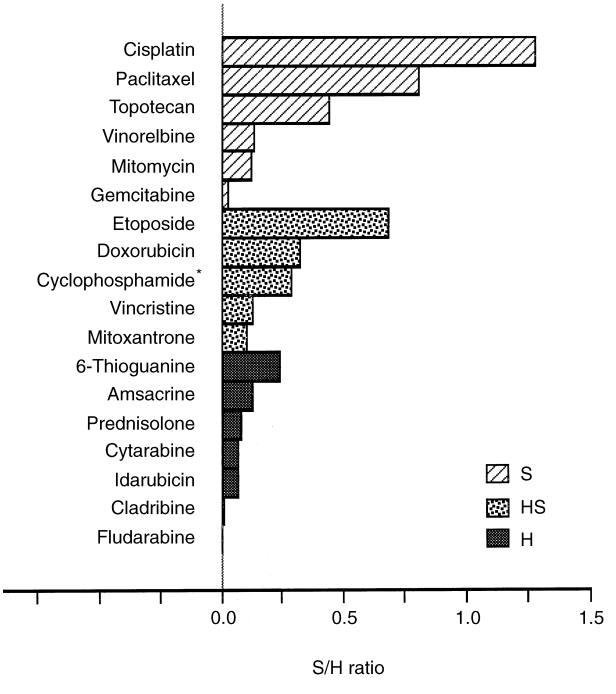


**Figure 2. Solid/haematological drug activity ratios *ex vivo* for different cytotoxic drugs. The S/H ratio is defined as the ratio between the fractions of solid and haematological tumour samples with a survival index (SI) < 50%. The drugs are grouped according to their indications in the Swedish drug compendium. Drugs registered for use in haematological malignancies only are denoted H, drugs registered for haematological as well as solid tumours HS and drugs registered for use in solid tumours only S. **In vitro* as the active metabolite 4-hydroxycyclophosphamide.**

*Table 5. Mean S/H ratios in different groups of drugs, defined in Figure 2. The mean value for the H group was significantly different from the values for the HS and S groups*

| Drug group | Mean S/H ratio | *n* |
|---|---|---|
| H | 0.09 | 7 |
| HS | 0.31 | 5 |
| S | 0.47 | 6 |

analogues cytarabine, fludarabine and cladribine showed the lowest ratios of all tested drugs, thus having the highest relative activity in haematological compared with solid tumours *ex vivo*. Vinorelbine showed a S/H ratio comparable to that of the other vinca alkaloid tested, vincristine. The highest ratios were observed for the S drugs cisplatin and paclitaxel and the HS drug etoposide.

Figure 3 shows the ability of the RAI, as detected with the FMCA, to predict the 50% most and least sensitive diagnoses with respect to clinical phase II activity. The sensitivity was 0.75 and the specificity was 0.72, indicating the ability of the test to detect the most and least sensitive diagnoses, respectively. The agreement between RAI and clinical response rate was statistically significant by the chi-square test ($P < 0.0001$). The number of correct classifications was 78/106 (74%).

## DISCUSSION

The accuracy of clonogenic and non-clonogenic cytotoxicity assays for the prediction of individual tumour response has been well studied and documented, for example by clinical correlations. Lately, a growing interest in using *ex vivo* assays in the development of new drugs has emerged. There are indications that activity patterns in fresh patient tumour cells *ex vivo* as measured by non-clonogenic assays may mirror clinical disease-specific activity of cytotoxic drugs [9, 10]. Previous studies have, however, been limited to a smaller number of diagnoses tested *ex vivo*, or, in the case of new drugs, in the clinic. Thus far no quantification of the predictive ability of these assays regarding diagnosis-specific activity has been made, and the present study was performed as an attempt to accomplish this.

RAI *ex vivo*

| Clinical response rate | Above median | Below median | |
|---|---|---|---|
| Above median | 42 | 14 | **56** |
| Below median | 14 | 36 | **50** |
| | **56** | **50** | |

**Figure 3. The ability of relative activity indices (RAI) *ex vivo* to identify the 50% most (including the median) and least drug sensitive diagnoses with respect to phase II clinical activity. For each of the 12 drugs, the diagnoses were ranked according to RAI and clinical response rates and positioned in a two-way table with the median used as a cut-off. Sensitivity and specificity were 0.75 and 0.72, respectively. The agreement between RAI and clinical response rates was statistically significant ($P < 0.0001$) by the chi-square test.**

The study was designed to compare the activity of a certain drug in different diagnoses in the clinic and *ex vivo*. The drug concentrations used in the *ex vivo* experiments were chosen to give the greatest separation between sensitive and resistant samples, and are not necessarily comparable to clinically active concentrations. Thus, it has to be kept in mind that comparisons between drugs may not be quite accurate, and that the fraction of responders *ex vivo* is not a prediction of the absolute clinical response rate. Use of the median SI as a cut-off for activity *ex vivo* may, however, give a measure of the relative activity of the drug in different diagnoses. Furthermore, the use of the median to define statistically *ex vivo* sensitive and resistant patients is a recognised method for prediction of individual patient drug sensitivity. It has been shown that a patient with a tumour cell survival for a drug less than the median of a large patient material in an *ex vivo* assay has a higher probability of responding clinically to the drug than the average patient, whilst patients with tumour cell survival higher than the median have a lower probability of response [5, 53, 54].

Overall, a good agreement between clinical response rates and *ex vivo* activity of the tested drugs was observed, indicating that predictions of clinical disease-specific activity could be made using the FMCA. However, this was not the case for all individual drugs. The weak correlation observed for cyclophosphamide and vincristine may, to some extent, be due to the fact that the clinical response rates for these drugs were obtained from studies from the 1960s or early 1970s [20, 21], when the concept of phase II dosage was not yet clearly defined. For example, for cyclophosphamide a comparably low clinical response rate (10%) was reported in acute myelocytic leukaemia (AML) from studies using low intensity schedules [20]. If the AML data were removed from the correlation analyses for cyclophosphamide, Rho increased from 0.32 to 0.60 and $R$ from 0.37 to 0.64. The weak correlation observed for vincristine could not be explained by a single value, but appears not to be valid for the vinca alkaloids as a class, since for vinorelbine the correlation between *ex vivo* and clinical data was good. In the case of paclitaxel, there are indications that cremophor EL, the solvent in the Taxol® formulation of the drug, which was the formulation used in the *ex vivo* experiments in the present study, contributes to the activity *ex vivo* [13, 55], but whether these findings are of clinical importance needs to be elucidated. Findings of a low distribution volume and tissue accumulation of cremophor EL in mice [56] indicate that the solvent does not contribute significantly to the clinical activity of paclitaxel, which might be an explanation of the weak correlation between RAI and clinical response rates observed for paclitaxel in the present study.

For some of the investigated drugs there were too few clinical data to, on a diagnosis level, conclude to what extent the clinical activity pattern correlates with the FMCA results. As a more rough measurement of tumour-type specificity, the activity in solid compared with haematological tumours was investigated. As a clinical estimate of solid or haematological tumour-specific activity, we used the indications listed for each drug in the Swedish Drug Compendium [52], a classification system which, although not perfect, is reasonably objective and unbiased.

There was a clear tendency to higher solid tumour activity *ex vivo* for the drugs considered as clinically active in solid tumours, but there were some exceptions. Gemcitabine, although regarded a solid tumour-specific drug, showed high activity against haematological malignancies *ex vivo*. The S/H ratio of gemcitabine was close to that of the related drugs cytarabine, fludarabine and cladribine, agents that are all dependent on deoxycytidine kinase activity in the cell to be effective [57]. There are very few or no studies on the clinical activity of gemcitabine in haematological tumours, and thus the proposed solid tumour-specificity of the drug needs further evaluation. This is also the case for vinorelbine, another drug registered for use in solid tumours only, but showing a S/H ratio close to that of the other vinca alkaloid tested, vincristine, a drug commonly used in haematological malignancies.

The type of phase II trial *ex vivo* described here may not be applicable to all types of anticancer drugs. Since the FMCA measures cell damage in the whole tumour cell population, consisting of largely non-dividing cells, purely antiproliferative drugs may not be accurately detected by this assay [9]. Furthermore, the mechanisms of cell death may be different *in vivo* and *ex vivo*. For example, *in vivo* a drug may cause cell death by producing DNA strand lesions, inhibiting or disturbing cell division, whereas *ex vivo*, damage of non-dividing cells may be produced only after induction of more extensive DNA damage. However, if the drug resistance mechanisms protecting the cells are similar *ex vivo* and *in vivo*, a cytotoxicity assay may still be valid. Alternatively, for some drugs *ex vivo* resistance may not parallel clinical resistance [9, 58]. Another problem may arise if a drug works by an indirect mechanism, for example biological response modification, interference with tumour vasculation or activation to cytotoxic metabolites [59]. Thus, an inactive drug *ex vivo* may be potentially active *in vivo*.

It should also be emphasised that *ex vivo* indication of high drug activity in a specific tumour type is not the same as prediction of clinical utility, which also depends on the achievable plasma concentrations of the particular drug. *In vivo*, it may not be possible to achieve cytotoxic concentrations of the drug either in plasma or in the tumour, due to dose-limiting toxicity, pharmacokinetic factors or factors associated with the biology of the tumour itself [59]. Cytotoxicity assays may, to some extent, be used for preclinical evaluation of dose-limiting toxicity by comparing tumour cell responses with those of normal cells, for example normal peripheral blood mononuclear cells [60].

The FMCA is rapid, objective, comparatively easy to perform and applicable on most tumour types, which makes it suitable for the performance of phase II trials *ex vivo*. However, for some diagnoses, a sufficiently high tumour cell content in the cell preparation may be difficult to achieve, for example multiple myeloma, where complementary morphological evaluation is needed, for example by the DiSC assay. Another obvious practical problem of this type of investigation is the supply of patient samples. A drug should preferably be tested in a broad spectrum of diagnoses and in a sufficient number of samples from each tumour type. In the present investigation, we used eight samples as a minimum for the determination of the RAI and treatment status of the patients was not considered in the calculations. However, we have observed that the difference in drug sensitivity *ex vivo* between samples from untreated and treated patients within a diagnosis is generally small compared with the difference between sensitive and resistant diagnoses. In the present study, most of the samples from the clinically more drug resistant solid tumours were obtained from primary operations, whilst the haematological and the more drug sensitive solid tumour groups contained samples from treated patients. Nevertheless, the haematological diagnoses were more sensitive *ex vivo* than the solid tumour groups, indicating that treatment status is less important in a study where the purpose is to detect differences between diagnoses. However, optimally, *ex vivo* studies should be performed using a larger number of samples, and samples from previously treated and untreated patients should be evaluated separately. Since the FMCA is also applicable on cryopreserved cells [61], it is possible to establish a 'tumour cell bank', which would facilitate the performance of such studies.

To summarise, the results in the present investigation show a good agreement between the cytotoxic drug activity patterns observed in fresh human tumour cells *ex vivo* and in the clinic, indicating that the FMCA and probably similar assays are well suited for preclinical evaluation of disease specificity of new drugs with a direct cytotoxic mode of action.


1. Marsoni S, Hoth D, Simon R, Leyland-Jones B, De Rosa M, Wittes RE. Clinical drug development: an analysis of phase II trials, 1970–1985. *Cancer Treat Rep* 1987, **71**, 71–80.
2. Fruehauf JP, Bosanquet AG. In vitro determination of drug response: a discussion of clinical applications. *Principles & Practice of Oncology Updates* 1993, **7**, 1–16.
3. Larsson R, Kristensen J, Sandberg C, Nygren P. 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**, 177–185.
4. Csoka K, Larsson R, Tholander B, Gerdin E, de la Torre M, Nygren P. Cytotoxic drug sensitivity testing of tumor cells from patients with ovarian carcinoma using the fluorometric microculture cytotoxicity assay (FMCA). *Gynecol Oncol* 1994, **54**, 163–170.
5. Larsson R, Nygren P. Prediction of individual patient response to chemotherapy using drug specific cut-off limits and a Bayesian model. *Anticancer Res* 1993, **13**, 1825–1830.
6. Nygren P, Hagberg H, Glimelius B, Sundström C, Christiansen I, Larsson R. In vitro drug sensitivity testing of tumor cells from patients with non-Hodgkin's lymphoma using the fluorometric microculture cytotoxicity assay. *Ann Oncol* 1994, **5**(Suppl. 1), S127–S131.
7. Csoka K, Tholander B, Gerdin B, de la Torre M, Larsson R, Nygren P. In vitro determination of cytotoxic drug response in ovarian carcinoma using the fluorometric microculture cytotoxicity assay (FMCA). *Int J Cancer* 1997, **72**, 1008–1012.
8. Weisenthal LM, Dill P, Birkhofer M. Accurate identification of disease-specific activity of antineoplastic agents with an in vitro fresh tumor assay measuring killing of largely non-dividing cells. *Proc Am Assoc Cancer Res* 1991, **32**, 2280.
9. Weisenthal LM. Predictive assays for drug and radiation resistance. In Masters J, ed. *Human Cancer in Primary Culture, a Handbook*. Dordrecht, Kluwer, 1991, 103–147.
10. Nygren P, Fridborg H, Csoka K, *et al.* Detection of tumor-specific cytotoxic drug activity *in vitro* using the fluorometric microculture cytotoxicity assay and primary cultures of tumor cells from patients. *Int J Cancer* 1994, **56**, 715–720.
11. Larsson R, Fridborg H, Csoka K, *et al.* In vitro activity of 2-chlorodeoxyadenosine (CdA) in primary cultures of hematological and solid tumors. *Eur J Cancer* 1994, **30A**, 1022–1026.
12. Fridborg H, Nygren P, Csoka K, *et al.* Activity of cyclosporins as resistance modifiers in primary cultures of human hematological and solid tumors. *Br J Cancer* 1994, **70**, 11–17.
13. Nygren P, Csoka K, Jonsson B, *et al.* The cytotoxic activity of Taxol in primary cultures of tumor cells from patients is partly mediated by Cremophor EL. *Br J Cancer* 1995, **71**, 478–481.

14. Csoka K, Nygren P, Graf W, Påhlman L, Glimelius B, Larsson R. Selective sensitivity of solid tumors to suramin in primary cultures of tumor cells from patients. *Int J Cancer* 1995, **63**, 356–360.
15. Csoka K, Liliemark J, Larsson R, Nygren P. Evaluation of the cytotoxic activity of gemcitabine in primary cultures of tumor cells from patients with hematologic or solid tumors. *Semin Oncol* 1995, **22**, 47–53.
16. Fridborg H, Nygren P, Dhar S, Csoka K, Kristensen J, Larsson R. In vitro evaluation of new anticancer drugs, exemplified by vinorelbine, using the fluorometric microculture cytotoxicity assay on human tumor cell lines and patient biopsy cells. *J Exp Therapeut Oncol* 1996, **1**, 286–295.
17. Jonsson B, Liminga G, Csoka K, *et al.* Cytotoxic activity of calcein acetomethyl ester (Calcein/AM) on primary cultures of human hematological and solid tumors. *Eur J Cancer* 1996, **32A**, 883–887.
18. Jonsson E, Fridborg H, Csoka K, *et al.* Cytotoxic activity of topotecan in human tumor cell lines and primary cultures of human tumor cells from patients. *Br J Cancer* 1997, **76**, 211–219.
19. Wittes R, Adrianza M, Parsons R, Felix J, Marsoni S. *Compilation of Phase II Results with Single Antineoplastic Agents*. *Cancer Treatment Symposia*, *1985:4*. Bethesda, NCI, 1985.
20. Livingston RB, Carter SK. *Single Agents in Cancer Chemotherapy*. New York, IFI/Plenum Press, 1970.
21. Wasserman TH, Comis RL, Goldsmith M, *et al.* Tabular analysis of the clinical chemotherapy of solid tumors. *Cancer Chemother Rep* 1975, **6**, 399–419.
22. Zittoun R. m-AMSA: a review of clinical data. *Eur J Cancer Clin Oncol* 1985, **21**, 649–653.
23. Civin CI, Krischer JP, Land VJ, Nitschke R, Kamen B, Vats T. Pediatric Oncology Group phase II trial of amsacrine in children with solid tumors. *Cancer Treat Rep* 1985, **69**, 335–336.
24. Kantarjian H, Barlogie B, Plunkett W, *et al.* High-dose cytosine arabinoside in non-Hodgkin's lymphoma. *J Clin Oncol* 1983, **1**, 689–694.
25. Shipp MA, Takvorian RC, Canellos GP. High-dose cytosine arabinoside. Active agent in treatment of non-Hodgkin's lymphoma. *Am J Med* 1984, 77, 845–850.
26. Davis HJ, Rochlin DB, Weiss AJ, *et al.* Cytosine arabinoside (NSC 63878) toxicity and antitumor activity in human solid tumors. *Oncology* 1974, **29**, 190–200.
27. Creech RH, Shah MK, Catalano RB, Dierks K, Dayal H, Goldberg AR. Phase II study of low-dose mitomycin in patients with ovarian cancer previously treated with chemotherapy. *Cancer Treat Rep* 1985, **69**, 1271–1273.
28. Hoskins PJ, McMurtrie E, Swenerton KD. A phase II trial of mitomycin in patients with epithelial ovarian carcinoma resistant to cisplatin or carboplatin. *Am J Clin Oncol* 1990, **13**, 416–419.
29. Oosterom AT van, Santoro A, Bramwell V, *et al.* Mitomycin C (MCC) in advanced soft tissue sarcoma: a phase II study of the EORTC Soft Tissue and Bone Sarcoma Group. *Eur J Cancer Clin Oncol* 1985, **21**, 459–461.
30. Jackson DJ, Chauvenet AR, Callahan RD, Atkins JN, Trahey TF, Spurr CL. Phase II trial of vincristine infusion in acute leukemia. *Cancer Chemother Pharmacol* 1985, **14**, 26–29.
31. Richards B, Newling D, Fossa S, *et al.* Vincristine in advanced bladder cancer: a European Organization for Research on Treatment of Cancer (EORTC) phase II study. *Cancer Treat Rep* 1983, **67**, 575–577.
32. Dombernowsky P, Hansen HH, Sorensen PG, Hainau B. Vincristine (NSC-67574) in the treatment of small-cell anaplastic carcinoma of the lung. *Cancer Treat Rep* 1976, **60**, 239–242.
33. Issell BF, Crooke ST. Etoposide (VP-16-213). *Cancer Treat Rev* 1979, **6**, 107–124.
34. Jungi WF. Etoposide single-agent chemotherapy for solid tumors. *Cancer Treat Rev* 1982, **9**(Suppl. A), 31–37.
35. Hollingshead L, Faulds D. Idarubicin—a review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in the chemotherapy of cancer. *Drugs* 1991, **42**, 690–719.
36. Gillies H, Liang P, Rogers H, *et al.* Phase II trial of idarubicin in patients with advanced lymphoma. *Cancer Chemother Pharmacol* 1988, **21**, 261–264.
37. Coonley C, Warrell R, Straus D, Young C. Clinical evaluation of 4-demethoxydaunorubicin in patients with advanced malignant lymphoma. *Cancer Treat Rep* 1983, **67**, 949–950.
38. Harper H, Kemeny N, Ahmed T, *et al.* Phase II clinical trial of 4-demethoxydaunorubicin in advanced colorectal carcinoma. *Cancer Treat Rep* 1984, **68**, 689–690.
39. Joss R, Obrecht J, Alberto P, Siegenthaler P, VanHelvoirt A, Cavalli F. Phase II trial of 4-demethoxydaunorubicin in patients with non-small cell lung cancer. *Cancer Treat Rep* 1984, **68**, 563–564.
40. Hakes T, Daghestani A, Dougherty J, Weiselberg L, Raymond V. Phase II study of 4-demethoxydaunorubicin in advanced ovarian carcinoma. *Cancer Treat Rep* 1985, **69**, 559–560.
41. Scher H, Yagoda A, Ahmed T, Budman D, Sordillo P, Watson R. Phase-II trial of 4-demethoxydaunorubicin (DMDR) for advanced hypernephroma. *Cancer Chemother Pharmacol* 1985, **14**, 79–80.
42. Milroy R, Cummings J, Kaye S, Banham S. Phase II clinical and pharmacological study of oral 4-demethoxydaunorubicin in advanced non-pretreated small-cell lung cancer. *Cancer Chemother Pharmacol* 1987, **20**, 75–77.
43. Cullen M, Smith S, Benfield G, Woodroffe C. Testing new drugs in untreated small cell lung cancer may prejudice the results of standard treatment: a phase II study of oral idarubicin in extensive disease. *Cancer Treat Rep* 1987, **71**, 1227–1230.
44. Long H. Paclitaxel (Taxol): a novel anticancer chemotherapeutic drug. *May Clin Proc* 1994, **69**, 341–345.
45. Roth B. Preliminary experience with paclitaxel in advanced bladder cancer. *Semin Oncol* 1995, **22**, 1–5.
46. Balcerzak S, Benedetti J, Weiss G, Natale R. A phase II trial of paclitaxel in patients with advanced soft tissue sarcomas. A Southwest Oncology Group Study. *Cancer* 1995, **76**, 2248–2252.
47. Hurwitz C, Relling M, Weitman S, Ravindranath Y, Vietti T, Strother D. Phase I trial of paclitaxel in children with refractory solid tumors: a Pediatric Oncology Group Study. *J Clin Oncol* 1993, **11**, 2324–2329.
48. Goa K, Faulds D. Vinorelbine—a review of its pharmacological properties and clinical use in cancer chemotherapy. *Drugs Aging* 1994, **5**, 200–234.
49. Tominaga T, Nomura Y, Furuse K, *et al.* Early phase II study of Navelbine (vinorelbine). *Ann Oncol* 1992, **3**, 127 [abstract no. 271].
50. Wilding G, Kirkwood J, Clamon G. Phase II trial of Navelbine in metastatic renal cancer. *Proc ASCO* 1993, **12**, 253 (A798).
51. Canobbio L, Boccardo F, Guarneri D. Phase II study of navelbine in advanced renal cell carcinoma. *Eur J Cancer* 1991, **27**, 804–805.
52. FASS. *The Swedish Drug Compendium*. Stockholm, LINFO, Läkemedelsinformation AB, 1997.
53. Kern D, Weisenthal L. Highly specific prediction of antineoplastic drug resistance with an in vitro assay using suprapharmacologic drug exposures. *J Natl Cancer Inst* 1990, **82**, 582–588.
54. Bosanquet A. Correlations between therapeutic response of leukaemias and in-vitro drug-sensitivity assay. *Lancet* 1991, **337**, 711–714.
55. Csoka K, Dhar S, Fridborg H, Larsson R, Nygren P. Differential activity of Cremophor EL and paclitaxel in patients' tumor cells and human carcinoma cell lines in vitro. *Cancer* 1997, **79**, 1225–1233.
56. Sparreboom A, van Tellingen O, Nooijen W, Beijnen J. Nonlinear pharmacokinetics of paclitaxel in mice results from the pharmaceutical vehicle cremophor EL. *Cancer Res* 1996, **56**, 2112–2115.
57. Beutler E. Cladribine (2-chlorodeoxyadenosine). *Lancet* 1992, **340**, 952–956.
58. Wilbur D, Camacho E, Hilliard D, Dill P, Weisenthal L. Chemotherapy of non-small cell lung carcinoma guided by an in vitro drug resistance assay measuring total tumour cell kill. *Br J Cancer* 1992, **65**, 27–32.
59. Phillips R, Bibby M, Double J. A critical appraisal of the predictive value of in vitro chemosensitivity assays. *J Natl Cancer Inst* 1990, **82**, 1457–1469.
60. Bosanquet A, Burlton A, Bell P, Harris A. Ex vivo cytotoxic drug evaluation by DiSC assay to expedite identification of clinical targets: results with 8-chloro-cAMP. *Br J Cancer* 1997, **76**, 511–518.

61. Nygren P, Kristensen J, Sundström C, Lönnerholm G, Kreuger A, Larsson R. Feasibility of the fluorometric microculture cytotoxicity assay (FMCA) for cytotoxic drug sensitivity testing of tumor cells from patients with acute lymphoblastic leukemia. *Leukemia* 1992, **6**, 1121–1128.

**Acknowledgements**—The skilful technical assistance of Dr Katalin Csoka, Mrs Carina Alvfors, Ms Christina Hedin and Mrs Lena Lenhammar, and the cytopathological expertise provided by Dr Manuel de la Torre and Dr Anna Åleskog are gratefully acknowledged. This study was supported by grants from the Swedish Cancer Society and the Lions Cancer Foundation.