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Preclinical report

The *ex vivo* effect of high concentrations of doxorubicin on recurrent ovarian carcinoma

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The cardiotoxicity of anthracyclines has largely prevented dose intensification, but the use of liposomal preparations (e.g. Caelyx/Doxil) allows much higher intra-tumoral concentrations to be achieved without cardiotoxicity. However, it is uncertain how much this will improve response rates over standard anthracycline therapy. The ATP-based chemosensitivity assay (ATP-TCA) has been used to develop new regimens for several tumor types, to investigate the molecular basis of chemosensitivity and shows considerable promise as a clinical method for individualizing chemotherapy. In this study, we have used the ATP-TCA to determine the concentration responsiveness of tumor-derived cells to concentrations of doxorubicin. The 22 tumor samples included were obtained from 20 heavily pretreated patients with recurrent ovarian cancer. Eight had previous anthracycline exposure, four as part of the CAP regimen. The results show more than 95% inhibition at clinically achievable concentrations in 11 of 22 tumors tested. Of the rest, seven showed a plateau effect between 80 and 95% inhibition, suggesting that there might be a subset of resistant cells present that is not inhibited by high concentrations of doxorubicin. Two tumors showed complete resistance and neither of these had previously received anthracycline therapy. As it has been suggested that gemcitabine might enhance anthracycline sensitivity in combination and we have had good results with gemcitabine modulation of alkylating agents in the assay, we have tested the combination of doxorubicin+gemcitabine under assay conditions in 11 tumors with little indication of improvement. In conclusion, doxorubicin at concentrations achievable with liposomal preparations shows strong *ex vivo* activity against pretreated recurrent ovarian cancer in just over half of the cases tested. [© 2000 Lippincott Williams & Wilkins.]

Key words: ATP, chemotherapy, doxorubicin, gemcitabine, liposome, ovarian carcinoma.

Introduction

Dose intensificaton of anthracyclines such as doxorubicin is an attractive proposition as these drugs are very active against some solid tumors, including ovarian cancer.[1,2] However, their toxicity has prevented their use at high dose until liposomal preparations such as Doxil/Caelyx were devised.[3] Liposomal doxorubicin (Caelyx/Doxil; Schering Plough/Sequus) enhances intra-tumoral or intra-effusion delivery of the drug to produce local concentrations of doxorubicin 4- to 16-fold greater than those achievable with the standard soluble preparation.[4–7] Liposomal doxorubicin also shows considerable reduction in cardiotoxicity, although other toxicities such as palmar–plantar erythema and dyspnea occur.[8]

Liposomal doxorubicin has been shown to be active in Kaposi's sarcoma,[9–13] and in a proportion of patients with other solid tumors.[14] While its activity in melanoma was disappointing,[15] liposomal doxorubicin has shown activity against breast carcinoma[16–18] and ovarian cancer.[1,19] Since these tumors are classically associated with anthracycline responsiveness, it seems rational to regard liposomal doxorubicin as a method of increasing the dosage of anthracycline in these patient groups. However, it is uncertain how much the use of higher-dose density of doxorubicin will improve response rates in comparison with standard anthracycline therapy used alone or in

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combination. Since single-agent anthracyclines are rarely used in the treatment of ovarian cancer, we have used an ATP-based chemosensitivity assay (ATP-TCA)[20] to determine the concentration responsiveness of tumor-derived cells to concentrations of doxorubicin achievable with liposomal preparations such as Caelyx/Doxil. In this study we have only used the standard soluble form of doxorubicin, as there is evidence that the liposomal preparation is inactive *in vitro*[12].

Materials and methods

Tumor-derived cells were obtained from patients undergoing chemosensitivity testing to guide rescue therapy for heavily pre-treated recurrent ovarian cancer. Doxorubicin was tested at 5 times the normal test drug concentration (TDC) used in the assay to mimic the concentration achieved by the liposomal preparation in patients.[6,7]

Tumors

Tumor-derived cells were obtained from 22 samples from 20 pre-treated patients with recurrent ovarian carcinoma, one of whom was thought clinically to have a Fallopian tube carcinoma treated according to ovarian cancer protocols and one an ovarian mesothelioma, treated similarly. The study included 16 ascites, two pleural fluids and four solid tumor samples. Previous treatment of these patients included first-line platinum-based therapy and second-line therapy with a variety of agents. Seven patients had previously received anthracyclines, four as part of the CAP regimen. Ascites or solid tumor was transported to the laboratory in cell culture medium (DMEM; Sigma, Poole, UK) with antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; Gibco/BRL, Paisley, UK) at 4°C. For ascites and pleural fluids, a 250 ml bottle was used containing 1 ml calcium heparin (5000 U/ml) and 25 ml culture medium (with 10 mM HEPES; Sigma) to provide support to the cells during transport and prevent clotting of any blood present in the specimen. Solid tumors were transported in 25 ml universal containers with 10 ml culture medium (DMEM) to which antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin) and HEPES (10 mM) were added.

ATP-TCA

The ATP-TCA was performed as previously described[20,22] and according to the manufacturer's instructions (TCA-100; DCS Innovative Diagnostik Systeme, Hamburg, Germany). Briefly, cells from ascites or enzymatic digestion of solid tumor were placed in 96-well polypropylene microplates at 10 000 or 20 000 cells/well, respectively, with each drug/combination at six doubling dilutions in triplicate from 200 to 6.25% TDC. The plate was then incubated at 37°C in 5% CO_2 for 6 days. The degree of cell inhibition at the end of this period was assessed by measurement of the remaining ATP in comparison with negative control (no drug, MO) and positive control (maximum inhibitor, MI) rows of 12 wells. ATP was extracted from the cells and measured by light output in a microplate luminometer (Berthold Diagnostic Systems) following addition of luciferin–luciferase. TDCs were 0.5 μg/ml for doxorubicin and 25 μg/ml for gemcitabine. Doxorubicin was used at 5 times the normal assay TDC to mimic the likely concentration achievable in patients (i.e. 2.5 μg/ml).

Data analysis

The percentage inhibition for each drug concentration was calculated as $1 - [(\text{test} - \text{MI})/(\text{MO} - \text{MI})] \times 100$ using an Excel spreadsheet (Microsoft). For each drug–concentration curve, the area under the curve ($\text{Index}_{\text{AUC}}$), the sum of the inhibition at each concentration ($\text{Index}_{\text{SUM}}$), the 50% inhibitory concentration (IC_{50}) and the 90% inhibitory concentration (IC_{90}) were calculated as previously described.[20]

Results

There is considerable heterogeneity of response to doxorubicin between different tumors, as we have observed with most chemotherapeutic agents in a variety of solid tumors. Table 1 shows the response for each of the drugs and combinations tested in each tumor, expressed as $\text{Index}_{\text{SUM}}$. Those with an $\text{Index}_{\text{SUM}}$ less than 300 would be expected to show clinical effects, if 100% inhibition is achieved at clinically relevant concentrations (i.e. 100% TDC or less). The median IC_{90} for doxorubicin was 1.38 μg/ml (55% TDC) and the median IC_{50} was 0.33 μg/ml (13% TDC).

Use of the $\text{Index}_{\text{SUM}}$ for analysis ignores the shape of the concentration–inhibition curves, which are shown in Figure 1. On this basis doxorubicin $\times 5$ shows high activity with more than 95% inhibition in 11 of 22 (50%) and more than 99% cell inhibition in six of these (28% of total), while in nine of 22 (41%) tumors the inhibition reaches a plateau at 80–95% inhibition.

Table 1. Tumors tested and their clinical characteristics [samples 3 and 4 represent the same patient, as do 6 and 8 (where there was intervening therapy with treosulfan+gemcitabine)]

TCA no.	Tumor diagnosis	Age (years)	Previous chemotherapy	Specimen type
1	ovarian carcinoma	50	1,14	ascites
2	ovarian carcinoma	50	2,5	tumor
3	ovarian carcinoma	34	1,2,3	ascites
4	ovarian carcinoma	34	1,2,3	ascites
5	ovarian carcinoma	64	1,4,13	ascites
6	ovarian carcinoma	49	3,11	ascites
7	ovarian carcinoma	65	2,3	ascites
8	ovarian carcinoma	49	3,11,12	ascites
9	ovarian carcinoma	62	3,9	tumor
10	ovarian carcinoma	64	3,5	ascites
11	ovarian carcinoma	50	1,3	ascites
12	ovarian carcinoma	66	3	ascites
13	tubal carcinoma	64	3,18	ascites
14	ovarian carcinoma	69	8	ascites
15	unknown 1 primary	60	none	pleural fluid
16	ovarian carcinoma	61	unknown	ascites
17	mesothelioma (ovarian)	28	1,7	ascites
18	ovarian carcinoma	66	2,4,5	pleural fluid
19	ovarian carcinoma	62	3,5	ascites
20	ovarian carcinoma	41	6	tumor
21	ovarian carcinoma	73	3,5	tumor
22	ovarian carcinoma	55	1,15,16,17,1	ascites

1, carboplatin (single agent); 2, CAP; 3, carboplatin+paclitaxel; 4, mitoxantrone+paclitaxel; 5, carboplatin or cisplatin+gemcitabine; 6, liposomal doxorubicin+paclitaxel+gemcitabine; 7, paclitaxel (single agent); 8, chlorambucil (single agent); 9, topotecan (single agent); 10, etoposide (single agent); 11, treosulfan (single agent); 12, treosulfan+gemcitabine; 13, vinorelbine (single agent); 14, epirubicin+paclitaxel; 15, cisplatin+treosulfan; 16, epirubicin+cisplatin; 17, cyclophosphamide+5-fluorouracil; 18, mitoxantrone (single agent).

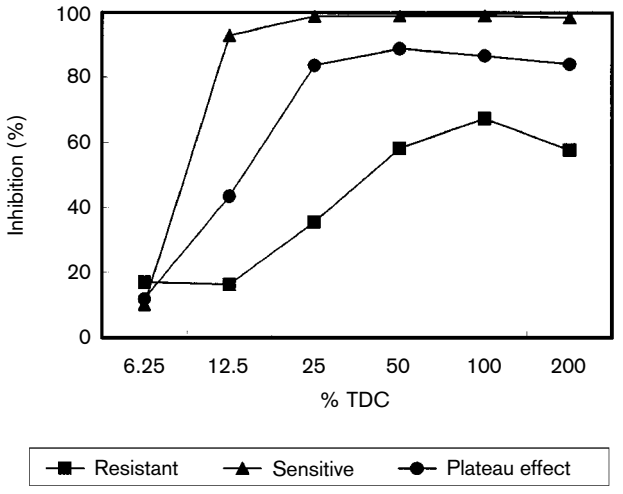

Figure 1. Example concentration–inhibition curves for doxorubicin $\times 5$ in the ATP-TCA, showing the plateau at high inhibition, resistance and sensitivity in three recurrent ovarian carcinomas.

Complete resistance with inhibition not greater than 80% at any concentration tested was only seen in two of 22 (9%) tumors tested: neither of these had received previous anthracycline therapy. All six tumors in which both ranges of doxorubicin were tested ($\times 1$ and $\times 5$) show the expected increased activity of doxorubicin $\times 5$ in comparison with doxorubicin, but in none of the four of these tumors with less than 95% inhibition did the higher concentration range lead to greater than 95% inhibition. However, in the 11 tumor samples which did achieve 95% inhibition when tested with doxorubicin $\times 5$, 95% inhibition was still present at 25% of the TDC in seven cases, suggesting that the clinical dose of liposomal doxorubicin would greatly exceed that required to inhibit greater than 95% of the cells in these tumors. This would not be achieved using the standard concentration of doxorubicin.

The combination of gemcitabine with doxorubicin $\times 5$ showed some improvement in the $Index_{SUM}$, but this only translated into an improvement in the achievable percent inhibition in one of 11 tumours in which the combination was tested (Table 2). The improvement in $Index_{SUM}$ was generally due to improvement of the percent inhibition at lower concentrations of the two drugs, where there was at least an additive effect in three of the tumors which showed a decrease in $Index_{SUM}$ of 50 points or more. In keeping with this, the median IC_{90} for the combination was 27% TDC and the median IC_{50} was 5% TDC, both of which are lower than the figures

Table 2. The chemosensitivity Index $_{SUM}$ for each patient for each of the three drugs tested, and the median and range for all four parameters measured on the series of patients included in the study

TCA no.	Doxorubicin	Doxorubicin × 5	Doxorubicin × 5 and gemcitabine	Gemcitabine
1	261**	81**	ND	170
2	164**	36**	ND	425
3	526^{R}	295*	ND	393
4	484^{R}	348^{R}	319^{R}	540
5	398^{R}	173*	60**	159
6	ND	195*	ND	ND
7	290*	177*	ND	297
8	ND	284*	186*	375
9	ND	317**	169**	243
10	ND	165^{R}	143^{R}	259
11	ND	102**	39**	208
12	ND	202*	163*	446
13	ND	82*	21**	296
14	ND	85**	27**	176
15	ND	111**	51**	155
16	ND	47**	5**	19
17	ND	154**	ND	372
18	ND	194**	ND	383
19	ND	268*	ND	160
20	ND	92**	ND	313
21	ND	183**	ND	475
22	ND	152**	ND	374
Median	344	165	60	296
Range	164–526	36–348	5–319	159–540
Description				
number	6	22	11	22
$Index_{SUM}$	344 (164–526)	165 (36–348)	60 (5–319)	305 (19–540)
$Index_{AUC}$	11116 (3737–16974)	17043 (10999–19040)	18172 (11915–19333)	11362 (3105–18988)
IC_{90}	233 (83–429)	55 (12–311)	27 (6–300)	261 (6–980)
IC_{50}	56 (10–238)	13 (4–51)	5 (3–27)	35 (3–545)

An index lower than 300 indicates strong sensitivity in the assay, if >95% inhibition is achieved. Those which achieved this are denoted by '**', those with best inhibition of 80–95% by '*' and those with evidence of resistance by '^{R}' (<80% at any concentration).

cited above for doxorubicin alone. An example of one of these tumors is shown in Figure 2.

Discussion

Increasing the concentration of doxorubicin does not always induce greater cell inhibition at the clinically relevant concentration, as the concentration–response curve tends to plateau at 80–95% cell inhibition. It is likely that the remaining cells will survive and produce resistance. The ATP assay is very sensitive and will detect less than five cells left in the well after the culture period. It is notable that total inhibition equivalent to maximum inhibitor (MI) wells was seen in 28% of the tumors tested, a figure which is remarkably similar to the response rate observed *in*

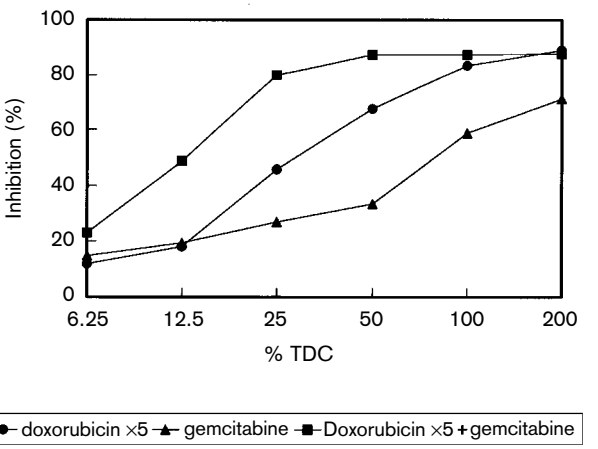

Figure 2. The influence of combining high-concentration doxorubicin with gemcitabine in an example showing an additive effect.

vivo with Caelyx/Doxil in recurrent ovarian cancer.[19] Whether this parameter will prove to be the best predictor of the clinical activity of liposomal doxorubicin remains to be seen and at present it seems sensible to regard those with a percent inhibition greater than 95% at 100% TDC for doxorubicin $\times 5$ as likely to respond to liposomal doxorubicin.

Doxorubicin is a classical topoisomerase inhibitor which acts through stabilization of the transient topoisomerase–DNA cleavable complex resulting in DNA strand breakage and cell death. Mechanisms of resistance to doxorubicin include downregulation of topoisomerase IIa, and increased expression of cell efflux proteins such as the multidrug resistance P-glycoprotein (MDR1/PgP), lung resistance protein (LRP) or multidrug resistance related protein (MRP).[23–26] Since the end result of doxorubicin exposure is DNA damage, enhancement of DNA repair is also likely to be important, together with the ability of the cell to undergo growth arrest rather than apoptosis while DNA damage is repaired.[27] The complexity of the possible cellular response to doxorubicin exposure makes it difficult to determine what combination of factors are responsible for the commonly observed plateau effect of high-dose doxorubicin. Other genes may also be involved: cellular downregulation of topoisomerase II has been associated with compensatory upregulation of topoisomerase I.[28,29]

Knowledge of the molecular response to doxorubicin and this chemosensitivity data could assist development of regimens including liposomal doxorubicin in pre-treated ovarian carcinoma, exposed to platinum $\pm$ paclitaxel.[30] Given the ability of gemcitabine to augment the effect of alkylating agents which also damage DNA, probably by interference with DNA repair,[31] we have explored the addition of gemcitabine to high concentration doxorubicin in the ATP-TCA. In contrast to alkylating agent/platinum effects, little augmentation of responses was observed. Although both IC_{90} and IC_{50} were enhanced, there was no increase in the level of the plateau effect, suggesting that no more cells were inhibited once the optimal concentration was reached. No tumors showed improvement in the plateau phenomenon. These findings are in keeping with the apparent cross-resistance previously observed *ex vivo* between gemcitabine and anthracyclines.[46]

Other options include vinca alkaloids,[33] platinum-based drugs and alkylating agents such as treosulfan. Topoisomerases are important in DNA repair and combination with DNA-damaging agents is likely to be active by analogy with existing soluble doxorubicin regimens (e.g. doxorubicin+cylophosphamide).[34,35] Most patients with recurrent ovarian cancer are likely to have already been exposed to taxanes. Nevertheless, taxanes are worthy of study with liposomal doxorubicin in other settings (including primary chemotherapy) and early results are encouraging, although there is a toxicity issue.[35,39] The option of combining liposomal doxorubicin with topoisomerase I inhibitors is interesting, but potentially toxic.[40] However, it has been reported that doxorubicin also has some activity against topoisomerase I.[41] Sequential administration is therefore a different matter, as this has been used successfully with topoisomerase inhibitors in early clinical trials.[42,43] It would be interesting to measure topoisomerase I and II activity in cells from patients treated with liposomal doxorubicin and topotecan (or a similar agent) to determine the optimal timing of this sequence. Such studies should be preceded by careful *ex vivo* studies since the ATP-TCA is capable of aiding the design of such trials.[30]

The ATP-TCA has been used to direct chemotherapy in a case control study of recurrent ovarian cancer,[44] showing doubling of progression-free survival and response rate in a cohort of patients compared with controls treated by physician's choice. Since this study was not randomized, a further study in platinum-resistant recurrent ovarian cancer with a randomized phase III design is in progress.[45,46] The option of combining this assay with molecular analysis, particularly in trials of sequential topoisomerase I and II inhibitors, should be explored.

In summary, our data suggest that single-agent liposomal doxorubicin is a good option in around a third of recurrent ovarian carcinomas. Identification of these patients is essential to improve response rates and may well be possible using the ATP-TCA to guide therapy.

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References

1. Alberts DS. Treatment of refractory and recurrent ovarian cancer. *Semin Oncol* 1999; **26**(suppl 1): 8–14.
2. Konecny G, Crohns C, Pegram M, *et al*. Correlation of drug response with the ATP tumorchemosensitivity assay in primary FIGO stage III ovarian cancer. *Gynecol Oncol* 2000; **77**: 258–63.

3. Lasic DD. Doxorubicin in sterically stabilized liposomes. *Nature* 1996; **380**: 561–2.
4. Gabizon A, Catane R, Uziely B, *et al*. Prolonged circulation time and enhanced accumulation in malignant exudates of doxorubicin encapsulated in polyethylene-glycol coated liposomes. *Cancer Res* 1994; **54**: 987–92.
5. Vaage J, Barberá-Guillem E, Abra R, Huang A, Working P. Tissue distribution and therapeutic effect of intravenous free or encapsulated liposomal doxorubicin on human prostate carcinoma xenografts. *Cancer* 1994; **73**: 1478–84.
6. Gabizon A, Goren D, Cohen R, Barenholz Y. Development of liposomal anthracyclines: from basics to clinical applications. *J Controlled Release* 1998; **53**: 275–9.
7. Symon Z, Peyser A, Tzemach D, *et al*. Selective delivery of doxorubicin to patients with breast carcinoma metastases by stealth liposomes. *Cancer* 1999; **86**: 72–8.
8. Skubitz KM, Skubitz AP. Mechanism of transient dyspnea induced by pegylated-liposomal doxorubicin (Doxil). *Anti-Cancer Drugs* 1998; **9**: 45–50.
9. Harrison M, Tomlinson D, Stewart S. Liposomal-entrapped doxorubicin: an active agent in AIDS-related Kaposi's sarcoma. *J Clin Oncol* 1995; **13**: 914–20.
10. Simpson JK, Miller RF, Spittle MF. Liposomal doxorubicin for treatment of AIDS-related Kaposi's sarcoma. *Clin Oncol (R Coll Radiol)* 1993; **5**: 372–4.
11. Northfelt DW, Dezube BJ, Thommes JA, *et al*. Efficacy of pegylated-liposomal doxorubicin in the treatment of AIDS-related Kaposi's sarcoma after failure of standard chemotherapy. *J Clin Oncol* 1997; **15**: 653–9.
12. Northfelt DW, Dezube BJ, Thommes JA, *et al*. Pegylated-liposomal doxorubicin versus doxorubicin, bleomycin, and vincristine in the treatment of AIDS-related Kaposi's sarcoma: results of a randomized phase III clinical trial. *J Clin Oncol* 1998; **16**: 2445–51.
13. Stewart S, Jablonowski H, Goebel FD, *et al*. Randomized comparative trial of pegylated liposomal doxorubicin versus bleomycin and vincristine in the treatment of AIDS-related Kaposi's sarcoma. International Pegylated Liposomal Doxorubicin Study Group. *J Clin Oncol* 1998; **16**: 683–91.
14. Gabizon A, Isacson R, Libson E, *et al*. Clinical studies of liposome-encapsulated doxorubicin. *Acta Oncol* 1994; **33**: 779–86.
15. Ellerhorst JA, Bedikian A, Ring S, Buzaid AC, Eton O, Legha SS. Phase II trial of doxil for patients with metastatic melanoma refractory to frontline therapy. *Oncol Rep* 1999; **6**: 1097–9.
16. Ranson MR, Carmichael J, O'Byrne K, Stewart S, Smith D, Howell A. Treatment of advanced breast cancer with sterically stabilized liposomal doxorubicin: results of a multicenter phase II trial. *J Clin Oncol;* 1997; **15**: 3185–91.
17. Muggia FM. Doxil in breast cancer. *J Clin Oncol* 1998; **16**: 811–2.
18. Vogel CL, Nabholtz JM. Monotherapy of metastatic breast cancer: a review of newer agents. *Oncologist* 1999; **4**: 17–33.
19. Muggia FM, Hainsworth JD, Jeffers S, *et al*. Phase II study of liposomal doxorubicin in refractory ovarian cancer: antitumor activity and toxicity modification by liposomal encapsulation. *J Clin Oncol* 1997; **15**: 987–9.
20. Andreotti PE, Cree IA, Kurbacher CM, *et al*. Chemosensitivity testing of human tumors using a microplate adenosine triphosphate luminescence assay: clinical correlation for cisplatin resistance of ovarian carcinoma. *Cancer Res* 1995; **55**: 5276–82.
21. Wang Y, Eksborg S, Lewensohn R, Lindberg A, Liliemark E. *In vitro* cellular accumulation and cytotoxicity of liposomal and conventional formulations of daunorubicin and doxorubicin in resistant K562 cells. *Anti-Cancer Drugs* 1999; **10**: 921–8.
22. Cree IA. Luminescence-based cell viability testing. *Methods Mol Biol* 1998; **102**: 169–77.
23. Nielsen D, Maare C, Skovsgaard T. Cellular resistance to anthracyclines. *Gen Pharmacol* 1996; **27**: 251–5.
24. Wang JC. DNA topoisomerases. *Annu Rev Biochem* 1996; **65**: 635–92.
25. Yamada S, Ando K. The role of topoisomerase II in multidrug resistance. In: Gupta S, Tsuoro T, eds. *Multidrug resistance in cancer cells*. London: Wiley 1996; 1: 137–50.
26. Satherley K, De Souza L, Neale MH, *et al*. Relationship between expression of topoisomerase II isoforms and chemosensitivity in choroidal melanoma. *J Pathol* 2000; in press.
27. Fink D, Aebi S, Howell SB. The role of DNA mismatch repair in drug resistance. *Clin Cancer Res* 1998; **4**: 1–6.
28. Bonner JA, Kozelsky TF. The significance of the sequence of administration of topotecan and etoposide. *Cancer Chemother Pharmacol* 1996; **39**: 109–12.
29. Masumoto N, Nakano S, Esaki T, *et al*. Sequence-dependent modulation of anticancer drug activities by 7-ethyl-10-hydroxycamptothecin in an HST-1 human squamous carcinoma cell line. *Anticancer Res* 1995; **15**: 405–9.
30. Cree IA, Kurbacher CM. ATP-based tumour chemosensitivity testing: assisting new agent development. *Anti-Cancer Drugs* 1999; **10**: 431–5.
31. Neale MH, Myatt N, Cree IA, *et al*. Combination chemotherapy for choroidal melanoma: ex vivo sensitivity to treosulfan with gemcitabine or cytosine arabinoside. *Br J Cancer* 1999; **79**: 1487–93.
32. 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 and solid tumors. *Semin Oncol* 1995; **22**(suppl 11): 47–53.
33. Burstein HJ, Ramirez MJ, Petros WP, *et al*. Phase I study of Doxil and vinorelbine in metastatic breast cancer. *Ann Oncol* 1999; **10**: 1113–6.
34. Holder L, Overmoyer B, Silverman P, Tripathy D, Marrs N, Teitelbaum AH. Doxil and oral cyclophosphamide as first-line therapy for patients with metastatic breast cancer (MDC): preliminary results of a pilot trial. *Proc Am Soc Clin Oncol* 1998; **17**: 146a (abstr 556).
35. Klein P, Lyass O, Sorich J, *et al*. Doxil plus cisplatin: combined results of two phase I studies. *Proc Am Soc Clin Oncol* 1998; **17**: 246a (abstr 942).
36. Langley RE, Carmichael J, Woll PJ, Mason E, Welbank H. Phase II trial to evaluate the safety and tolerability of Caelyx (Doxil) in combination with paclitaxel in the treatment of metastatic cancer. *Proc Am Soc Clin Oncol* 1998; **17**: 246a (abstr 943).
37. Moore MR, Srinivasiah J, Feinberg BA, *et al*. Phase II randomized trial of doxorubicin plus paclitaxel (AT) versus doxorubicin HCl liposome injection (Doxil) plus paclitaxel (DT) in metastatic breast cancer. *Proc Am Soc Clin Oncol* 1998; **17**: 160a (abstr 614).
38. Sparano JA, Malik UR, Einzig A, *et al*. Phase I trial of liposomal doxorubicin (Doxil) and docetaxel (Taxotere) in patients (pts) with advanced breast cancer (ABC). *Breast Cancer Res Treat* 1998; **50**: 264 (abstr 235).

39. Schwonzen M, Kurbacher CM, Mallmann P. Liposomal doxorubicin and weekly paclitaxel in the treatment of metastatic breast cancer. *Anti-Cancer Drugs* 2000; **11**: 681–5.
40. Yeung AW, Wong A, Ali M. Phase 1 trial of doxil and topotecan in solid tumors. *Proc Am Soc Clin Oncol* 1998; **17**: abstr 979.
41. Foglesong PD, Reckord C, Swink S. Doxorubicin inhibits human DNA topoisomerase I. *Cancer Chemother Pharmacol* 1992; **30**: 123–5.
42. Hammond LA, Eckardt JR, Ganapathi R, *et al*. A phase I and translational study of sequential administration of the topoisomerase I and II inhibitors topotecan and etoposide. *Clin Cancer Res* 1998; **4**: 1459–67.
43. McGuire WP, Brady MF, Ozols RF. The Gynecologic Oncology Group experience in ovarian cancer. *Ann Oncol* 1999; **10**(suppl 1): 29–34.
44. Kurbacher CM, Cree IA, Bruckner HW, *et al*. Use of an *ex vivo* ATP luminescence assay to direct chemotherapy for recurrent ovarian cancer. *Anti-Cancer Drugs* 1998; **9**: 51–7.
45. Kurbacher CM, Untch M, Cree IA. A randomised trial of chemotherapy directed by a tumour chemosensitivity assay versus physician's choice in patients with recurrent platinum-resistant ovarian adenocarcinoma. *Lancet* Internet publication 1997: http://www.thelancet.com/newlancet/sub/author//menu_NOD7.html
46. Cree IA, Kurbacher CM. Individualizing chemotherapy for solid tumors—is there any alternative? *Anti-Cancer Drugs* 1997; **8**: 541–8.

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