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Research paper

Use of an *ex vivo* ATP luminescence assay to direct chemotherapy for recurrent ovarian cancer

Christian M Kurbacher, Ian A Cree,[1] Howard W Bruckner,[2] Ursula Brenne, Jutta A Kurbacher,[3] Karin Müller, Tonja Ackermann, Tobias J Gilster, Lucas M Wilhelm, Heike Engel, Peter K Mallmann and Peter E Andreotti[4]

Department of Gynecology and Obstetrics, University of Cologne Medical Center, Kerpener Strasse 34, 50931 Cologne, Germany. Tel: (+49) 221 478-4910; Fax: (+49) 221 478-4929. [1]Department of Pathology, Institute of Ophthalmology, University College London, London EC1V 9EL, UK. [2]Division of Neoplastic Diseases, Derald H Ruttenberg Cancer Center, Mount Sinai School of Medicine, New York, NY 10029, USA. [3]Department of Obstetrics and Gynecology, St Marien-Hospital, 51496 Bergisch-Gladbach, Germany. [4]Atlantic Scientific Development Inc, Boca Raton, FL 33428, USA.

Chemotherapy for recurrent ovarian carcinoma (ROC) produces response rates of 10–80% depending on the prevalence of platinum resistance. Most patients relapse within 1 year and median progression-free survival (PFS) is generally no more than 6 months. Previous pretherapeutic chemosensitivity assays mostly failed to improve the outcome of patients with ROC. Newly developed ATP assays show promising retrospective correlation with clinical outcome. We report here the first results of ATP assay-directed chemotherapy in patients with ROC. Therapy was selected by the ATP tumor chemosensitivity assay (ATP-TCA) in a prospective open-label pilot trial for ROC. Objective response rate (ORR), PFS and overall survival (OAS) of the first 25 evaluable patients were retrospectively compared with those of 30 others having similar characteristics who were treated empirically within the same period. The actuarial median observation times were 80 weeks for the ATP-TCA group and 83.5 weeks for the control group, respectively. In the control group, a 37% ORR [two complete responses (CR) and nine partial responses (PR)] was followed by a median PFS of 20 weeks and a median OAS of 69 weeks, mainly related to the use of single-agent chemotherapy. The ORR in the ATP-TCA group was 64% (eight CR and eight PR) (p=0.04) with the majority of responses (11 of 16) achieved with novel combinations. The median PFS in this group was 50 weeks (p=0.003) and the median OAS was 97 weeks (p=0.145). Survival of responding patients was similar in both groups. Chemotherapy guided by the ATP-TCA produced a greater benefit with regard to both ORR and PFS in platinum-refractory patients. ATP-TCA-directed chemotherapy for ROC compares favorably with chemotherapy chosen by a clinician and often leads to the choice of novel drug combinations. These promising results now warrant confirmation by prospective randomized trials. [© 1998 Rapid Science Ltd.]

Key words: ATP tumor chemosensitivity assay (ATP-TCA), individualized chemotherapy, platinum resistance, recurrent ovarian carcinoma.

Introduction

Epithelial ovarian cancer is the leading cause of death related to gynecological malignancies in western countries.[1] Although primary carcinomas frequently respond to post-operative platinum-based chemotherapy, the majority of patients will relapse and ultimately die from their disease. Recently, the overall 5-year survival is only 20%.[1,2] The likelihood of relapsing patients to respond to either second-line platinum- or non-platinum-based regimens depends mainly on the prevalence of inherent or acquired platinum resistance.[1,3,4] Patients progressing on or within 6 months after completion of first-line platinum must be considered refractory and their chance of benefit from any salvage chemotherapy is particularly poor with objective response rates (ORR) not exceeding 30%.[1,3–6]

Individualized selection of chemotherapy prior to salvage treatment would be desirable but requires a feasible predictive assay. A number of assays have been tested over the last 20 years but the results have been disappointing mainly due to technical limitations, particularly low evaluability rates, requirement for inappropriately large amounts of tumor material and lack of standardization.[7–11] New-generation luminescence assays such as the ATP tumor chemosensitivity

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Correspondence to CM Kurbacher

assay (ATP-TCA) compare favorably to previous methods in terms of standardization, evaluability, required tumor cell number, reproducibility, accuracy and robustness.[11,12] Due to its high sensitivity, the ATP-TCA allows testing four to six regimens with only 1×10^6 tumor cells. Therefore, solid tumors can be assayed as well as malignant effusions and needle biopsies, respectively.[11-13] We have previously obtained promising retrospective correlations between assay results and clinical outcome in ovarian cancer patients.[12-14] Furthermore, the ATP-TCA was successfully employed to preclinically screen for innovative chemotherapy regimens to be chosen for further clinical development.[15-18] High ATP-TCA response rates of around 80% achieved *ex vivo* with mitoxantrone/paclitaxel in native ovarian carcinoma specimens derived from platinum-refractory individuals were accurately reflected in a subsequent pilot trial utilizing the same drug combination in a comparable group of patients.[18] Basing on these promising results, we have now conducted an open-label pilot trial to evaluate the ability of the ATP-TCA to direct chemotherapy prospectively in individual patients with recurrent ovarian carcinoma (ROC). Treatment results of the first 25 evaluable patients are now available and were retrospectively compared with 30 others who had similar characteristics but were exclusively treated on an empirical basis.

Materials and methods

ATP chemosensitivity testing

Chemosensitivity testing was performed using commercially available ATP-TCA test kits (DCS, Hamburg, Germany). The ATP-TCA methodology has been previously described in detail.[12,19,20] Briefly, the assay utilized native tumor suspensions prepared from both solid tissue and malignant effusions. A maximum of 18 different single agents and combinations was assayed including both standard and experimental regimens: cisplatin, carboplatin, doxorubicin (DOX), mitoxantrone (MX), paclitaxel (PTX), etoposide (VP-16), 4-OOH-cyclophosphamide (4-HC), treosulfan (TREO), 4-HC+cisplatin (CP), cisplatin+VP-16 (PE), cisplatin+-TREO (PT), 4-HC+carboplatin (CC), cytosine arabinoside (ara-C), ara-C+cisplatin (aCP), ara-C+carboplatin (aCC), PTX+cisplatin (TP), DOX+PTX (AT) and MX+PTX (NT).[15-18] Each regimen was tested at six different serum adapted test drug concentrations (TDC), ranging between 6.25 and 200% TDC, allowing the identification of dose-response effects.[12,13,19,20] Assay results were available after 5-7 days of incubation using a routine cell culture equipment. Results were graphed for each regimen and tumor, respectively. Regimens tested *ex vivo* were scored considering both the 50% (IC_{50}) and 90% inhibitory concentrations (IC_{90}) and a sensitivity index (SI) given by the area under the dose-response curve.[12,20] However, due to the low internal assay error of less than 10%,[12] selection of chemotherapy was often possible by simply using the graphical representation of the curves.

Patients and treatment

Starting in February 1993, patients with histologically confirmed ROC were treated prospectively with ATP-TCA-based chemotherapy. The study was performed in accordance to the requirements of the German Drug Law (AMG) and the institutional ethical guidelines. Written informed consent was obtained from all patients. Tumor specimens were obtained by either laparotomy, if required, or puncture of ascitic/pleural effusions. The malignant nature of all specimens was confirmed by histology or cytology.

Patients were treated with the optimal protocol indicated by the ATP-TCA, i.e. the regimen with the highest *ex vivo* activity which could be tolerated by the individual patient concerned. For combinations selected for therapy, clear superiority must be indicated by the assay, as compared to the best single agent included. Supportive treatment was given according to the anticipated toxicity profile of the individual regimen.

Eligibility criteria were: bidimensionally measurable tumor before starting salvage chemotherapy, adequate bone marrow, renal and hepatic functions, Zubrod performance status of 2 or less, life expectancy of 12 weeks or more and complete recovery from surgery, if performed. Therapy was monitored by CA 125 measurements preceding each treatment course and physical examination. Tumor imaging was performed every 6 weeks. Responses were exclusively assessed by radiological and clinical means according to UICC criteria with ORR defined as the sum of complete (CR) and partial responses (PR). A significant decrease of serum CA 125 lacking a radiological correlate did not qualify a patient for CR or PR. Patients were regarded as evaluable for response if a minimum of two cycles was administered. Chemotherapy was continued for at least four cycles in both responders and in patients experiencing stable disease (SD). Patients finishing their chemotherapy were entered into a routine follow-up program with physical and gynecological checks, and CA 125 measurements repeated every 3 months. Additional tumor imaging was performed at

any time as clinically indicated. At time of relapse patients were treated either according to clinician's choice or, if feasible, retested and treated based on a second ATP-TCA.

The control group consisted of individuals with ROC treated empirically. Patient selection was not randomized but all were treated by the same department within the same period using identical therapeutic standards. In all cases, treatment choice was made by the institutional tumor board, consisting of three to five gynecological oncologists, one radiotherapist, one radiologist, and, if indicated, one pathologist and one medical oncologist. Patients were treated empirically if having no ATP-TCA performed, if the ATP-TCA was unsuccessfully attempted or if the responsible oncologist preferred another regimen as selected by the assay. Both treatment directives and follow-up for the controls were identical involving the same departmental members as compared to the ATP-TCA group. Therapy at time of relapse was guided by similar considerations as in the ATP-TCA group.

A total of 25 patients receiving ATP-TCA-directed chemotherapy between February 1993 and May 1995, and another 30 patients treated empirically were evaluable for response and survival. Both groups of patients are well balanced with respect to mean age, performance status, number of prior chemotherapies, proportion of patients with secondary cytoreductive surgery prior to chemotherapy, individuals with non-serous histology and proportion of patients with platinum-refractory disease (Table 1). Patients were defined as platinum refractory if showing progression during or within 6 months after completion of platinum-based chemotherapy.[4,21] A median of 6 chemotherapy cycles was administered in both groups [range 3-6 (ATP-TCA) and 2-9 (controls), respectively].

Table 1. Patient characteristics

Characteristic	Control group (n=30)	ATP-TCA group (n=25)
Mean age (range)	56.1 (39–71)	52.6 (28–69)
Median performance status (range)	1 (0–2)	1 (0–2)
Mean no. of prior chemotherapies	1.6 (1–4)	1.8 (1–5)
Patients with attempted cytoreduction (no.)	14 (47%)	10 (40%)
Patients with non-serous histology (no.)	4 (13%)	3 (12%)
Platinum-refractory patients (no.)	12 (40%)	14 (56%)

Statistics

Overall survival (OAS) and progression-free survival (PFS) in both groups were estimated from the start of chemotherapy (Kaplan-Meier statistics). Any cause of death was used to calculate OAS. ORR observed in both groups were compared by Fisher's exact tests, as were differences regarding the 1-year PFS and OAS, respectively. Differences in OAS and PFS were analyzed by log rank tests. For all statistical manipulations, a $p \leqslant 0.05$ was considered significant.

Results

Of a total of 31 patients from both groups tested with the ATP-TCA, 29 had evaluable results giving a 93% assay evaluability rate. In the ATP-TCA group, 25 patients were treated according to the assay, whereas in the control group, 30 others received empirical chemotherapy. The median observation periods are 80 weeks for the ATP-TCA group and 83.5 weeks for the control group, respectively. Treatments given are specified in Table 2. In the ATP-TCA group, 23 patients (92%) were treated with drug combinations and 19 (76%) received experimental protocols. Of those treated by physician's choice, 21 (70%) received single agents; experimental therapy was delivered to only three individuals (10%) in this group. As shown in

Table 2. Chemotherapy

Regime	Patients treated	
	Control group (n=30)	ATP-TCA group (n=25)
Single agents		
cyclophosphamide	4	–
epirubicin	2	–
etoposide	6	–
mitoxantrone	1	1
paclitaxel	3	1
treosulfan	5	–
Drug combinations		
carboplatin/ifosfamide	1	–
cisplatin/treosulfan	1	1
cyclophosphamide/cisplatin	5	3
cytosine arabinoside/carboplatin	1	1
cytosine arabinoside/cisplatin	–	5
doxorubicin/paclitaxel	–	6
mitoxantrone/paclitaxel	1	6
paclitaxel/carboplatin	–	1

Table 3, a 37% ORR (two CR and nine PR) was observed in this group, whereas another 11 patients (37%) progressed on therapy. In the ATP-TCA group, a significantly higher ORR of 64% was achieved (p=0.04) with eight CR (32%) and eight PR (32%); only three patients (12%) had progressive disease (PD). Eight patients in the control group and six in the ATP-TCA group had SD.

Survival curves for both groups are illustrated in Figure 1. PFS was significantly better in the ATP-TCA group compared to the controls with a median duration of 50 versus 20 weeks, a 1-year relapse-free survival of 46 versus 23%, and a hazard ratio of 0.46 (95% CI: 0.221-0.736; p=0.003). Deaths occurring within the observation period all were tumor-related except in one patient from the study group who died of thrombocytopenic cerebral hemorrhage during the sixth aCC course whilst showing durable SD. The study group also showed a longer median OAS (97 versus 69 weeks) with a 1-year survival proportion of 75 versus 62%. However, these differences did not reach statistical significance (hazard ratio: 0.607, 95% CI: 0.293-1.197; p=0.145). Kaplan-Meier plots for responding patients are shown in Figure 2. No significant differences in respect to median PFS (55 versus 58 weeks, p=0.134) and OAS (129 versus 130 weeks, p=0.980) were found between both the ATP-TCA and the empirical group.

As demonstrated in Figure 3, particularly good results were achieved in platinum-refractory patients treated on ATP-TCA (n=14) in comparison with those treated empirically (n=12) for ORR (79 versus 17%, p=0.002) and median PFS (40 versus 7 weeks; hazard ratio: 0.33, 95% CI: 0.093-0.645; p=0.003). Although a remarkable trend was obvious regarding median OAS (97 versus 39 weeks), no statistical significance was achieved (hazard ratio: 0.477, 95% CI: 0.152-1.189; p=0.104).

When analyzing the clinically active regimens in both groups, marked differences were found. In the control group, 10 of 11 remissions were produced by established regimens; seven treatments utilized single agents (i.e. 3 × PTX, 2 × VP-16 and 2 × TREO) and four others were drug combinations (i.e. 3 × CP and 1 × NT). In contrast, responses in the ATP-TCA group were predominately related to the use of experimental protocols (11 of 16). Only two remissions (both CR) were observed for single-agent chemotherapy (i.e. PTX and MX), whereas the remainder were induced by drug combinations. The most active regimens in this group accounting for more than two-thirds of responses were NT (three CR and one PR), AT (one CR and three PR) and aCP (one CR and two PR). In addition, one CR and two PR were achieved with cisplatin combined with alkylating agents.

Table 3. Results of chemotherapy

	Control group	ATP-TCA group	p^a
No. of patients	30	25	–
CR	2 (7%)	8 (32%)	0.018
PR	9 (30%)	8 (32%)	0.551
SD	8 (26%)	6 (24%)	0.536
PD	11 (37%)	3 (12%)	0.036
ORR	11 (37%)	16 (64%)	0.040
Mean PFS (weeks)	20	50	0.003
Mean OAS (weeks)	69	97	0.145

[a] Level of statistical significance calculated by Fisher's exact test (CR, PR, SD, PD and ORR) or log rank test (mean PFS and mean OAS).

Discussion

In this pilot trial, we compared the clinical outcome of patients with ROC treated on the basis of pretherapeutic ATP chemosensitivity testing with controls

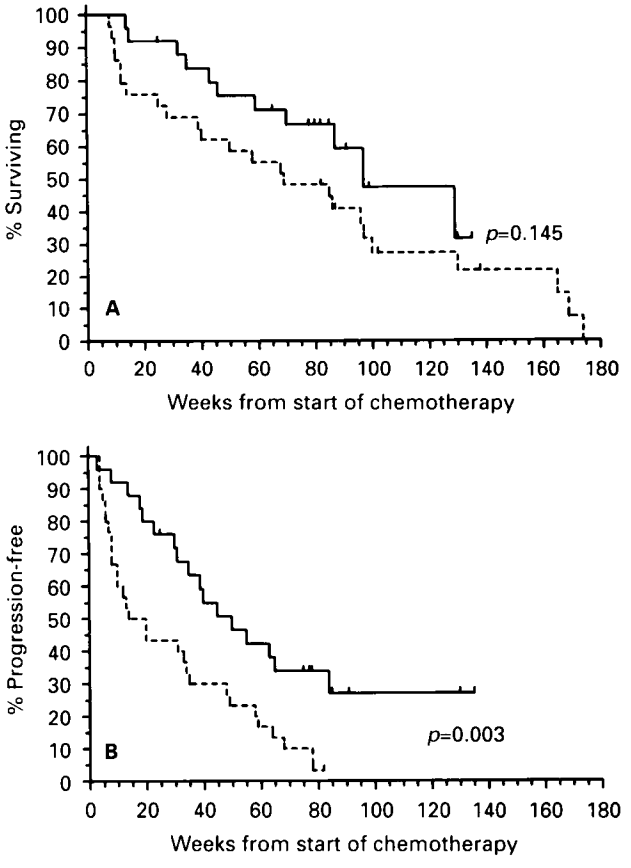

Figure 1. OAS (A) and PFS (B) of all patients with ROC calculated by Kaplan–Meier statistics. ATP-TCA group (——); control group (- - - -).

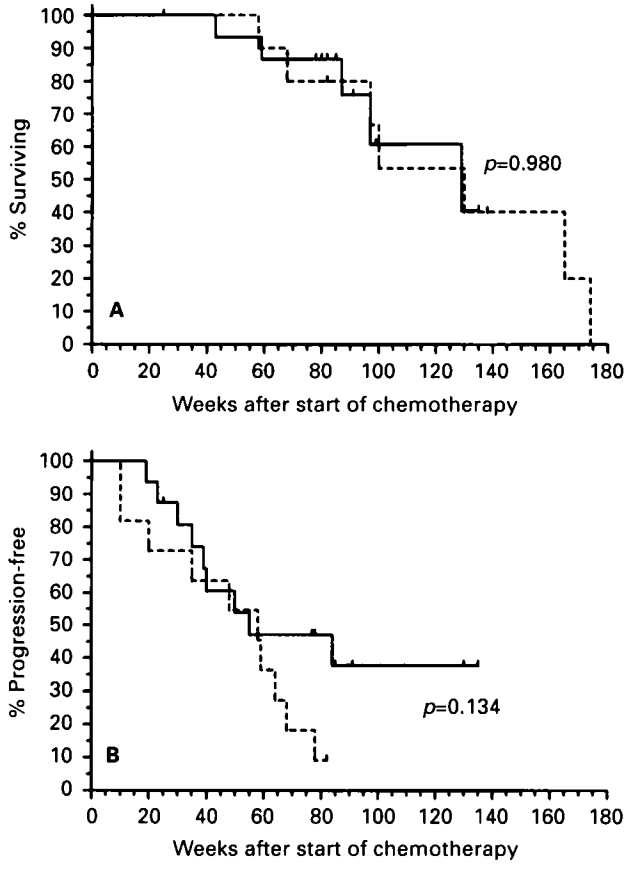

Figure 2. OAS (A) and PFS (B) of responding patients with ROC calculated by Kaplan–Meier statistics. ATP-TCA group (——); control group (- - - -).

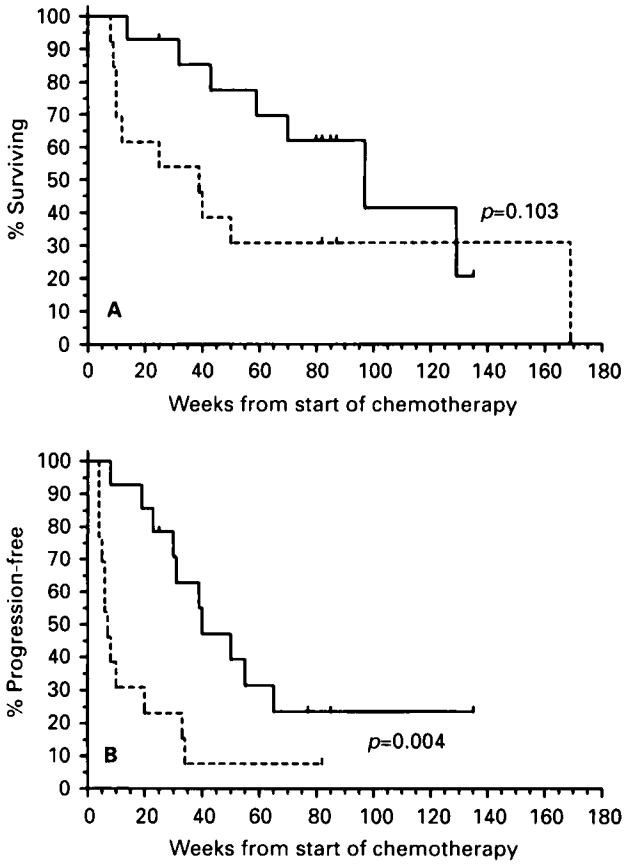

Figure 3. OAS (A) and PFS (B) of patients with platinum-refractory ROC calculated by Kaplan–Meier statistics. ATP-TCA group (——); control group (- - - -).

treated solely by physician's choice. The results in the control group in regard to ORR, median PFS, median OAS and 1-year PFS were close to those reported by others for similar populations of patients.[1,3,22] Although no randomization was performed and the results from the control group must be interpreted with caution, patients from both groups are well-matched for all criteria known to influence outcome.[1,4,5,21,22]

The concept of individualized chemotherapy for ROC has attracted clinical attention for more than two decades although previous trials using therapy directed by pretherapeutic chemosensitivity assays mostly failed to demonstrate survival benefit.[7-11,23] However, the number of regimens tested was limited by both technical problems and a relative lack of drugs with differing mechanisms of action. Many new drugs are now available allowing a wider choice and complicating the clinical decision-making process. Generally, the ATP-TCA produces results comparable to both clonogenic and other non-clonogenic assays.[24,25] However, this technique provides both improved sensitivity and standardization that allows *ex vivo* testing of many different chemotherapy regimens with a considerably lower amount of tumor cells.[11-13,25] Previously reported evaluability rates of 90% or higher achieved with the ATP-TCA in both ovarian and breast tumors,[12,13,20] were reproduced in this trial.

Use of the ATP-TCA to direct chemotherapy for ROC has enabled us to significantly improve the ORR, the PFS and the 1-year PFS. Median OAS was also better in the ATP-TCA group, but not significantly so. This might be partly due to considerable variation in subsequent therapy following relapse as well as short-term follow-up period and a relatively small number of patients. Therefore, PFS is probably a more accurate parameter of efficacy of ATP-TCA-directed chemotherapy in this study.

Since responding patients in both groups show similar survival characteristics, responses achieved with assay-directed chemotherapy appear to have the

same clinical utility as those seen with empirical protocols. These findings are extremely encouraging because recent improvements in response rates seen with empirical chemotherapy, even those achieved with high-dose protocols, have not translated into improved PFS for patients relapsing from ovarian cancer, suggesting that the majority of responses induced by empirical protocols may be of short duration.[1,3,5,22,26-28] While the improved ORR in the study group is probably related in part to the use of combination rather than single-agent chemotherapy, the inter-tumor heterogeneity of sensitivity observed in the ATP-TCA led to the use of widely differing regimens which were unlikely to be chosen clinically.[13,15-20] Previous experience with empirical combination chemotherapy in ROC has been disappointing, particularly in terms of PFS and OAS which rarely compare favorably to those achieved with mono-chemotherapy.[3,5,22] However, novel drug combinations developed on the basis of preclinical ATP-TCA drug screening produced promising rates for both response and survival.[18] This suggests that the ATP-TCA allows selection of disparate novel regimens providing superior clinical activity.[15-18]

Particularly promising results related to ATP-TCA-guided chemotherapy were achieved for platinum-refractory individuals in terms of both ORR and PFS. In this group of patients who have a extremely poor chance to benefit from any empirical chemotherapy,[3,4,22] the use of the ATP-TCA to select active regimens appears to be of particular clinical relevance.

In conclusion, our results show that use of the ATP-TCA as a predictive assay clearly is not detrimental to patients' outcome. Although the results of a retrospective analysis should always be interpreted cautiously, it seems likely that ATP-TCA-directed chemotherapy improves both response rates and patients' PFS in comparison with empirical treatment. Prospective randomized trials are in progress using the results of this study as a guide to the number of patients required. It seems possible that the ATP-TCA may provide a method for rational individualized chemotherapy of advanced or recurrent solid tumors.

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