A prospective randomized controlled trial of tumour chemosensitivity assay directed chemotherapy versus physician's choice in patients with recurrent platinum-resistant ovarian cancer

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The primary aim of this randomized trial was to determine response rate and progression-free survival following chemotherapy in patients with platinum-resistant recurrent ovarian cancer, who had been treated according to an ATP-based tumour chemosensitivity assay in comparison with physician's choice. A total of 180 patients were randomized to assay-directed therapy ($n=94$) or physician's-choice chemotherapy ($n=86$). Median follow-up at analysis was 18 months. Response was assessable in 147 patients: 31.5% achieved a partial or complete response in the physician's-choice group compared with 40.5% in the assay-directed group (26 versus 31% by intention-to-treat analysis respectively). Intention-to-treat analysis showed a median progression-free survival of 93 days in the physician's-choice group and 104 days in the assay-directed group (hazard ratio 0.8, 95% confidence interval 0.59–1.10, not significant). No difference was seen in overall survival between the groups, although 12/39 (41%) of patients who crossed over from the physician's-choice arm obtained a response. Increased use of combination therapy was seen in the physician's-choice arm during the study as a result of the observed effects of assay-directed therapy in patients. Patients entering the physician's-choice arm of the study during the first year did significantly worse than those who entered in the subsequent years (hazard ratio 0.44, 95% confidence interval 0.2–0.9, $P<0.03$). This small randomized clinical trial has documented a trend towards improved response and progression-free survival for assay-directed treatment. Chemosensitivity testing might provide useful information in some patients with ovarian cancer, although a larger trial is required to confirm this. The ATP-based tumour chemosensitivity assay remains an investigational method in this condition. *Anti-Cancer Drugs* **18:1093–1101 © 2007 Lippincott Williams & Wilkins.**

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Introduction

Patients who suffer a relapse during or within 6 months after first-line platinum-based chemotherapy have a poor prognosis compared with those who have one after 1 year [1]. Reported response rates and survival are low: data from subgroup analyses of large randomized trials suggest response rates of 10–20%, with a median progression-free survival (PFS) of less than 6 months and an overall survival (OAS) of less than 1 year [2–5]. In unselected patients, response rates are generally lower. In the UK, response rates for ovarian cancer are below the European average [6].

Several nonplatinum-containing drugs are commonly used in second-line therapy of ovarian cancer, including taxanes (also used first-line with platinum), anthracyclines, topotecan, gemcitabine, oral etoposide, altretamine, treosulfan, tamoxifen and megestrol acetate, producing response rates of 11–27% [1]. No evidence exists for improved survival from the use of combination therapy, compared with the

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use of single agents, in patients with platinum-resistant disease. Patients who show no response to one such agent might show a response to an alternative, suggesting that there is considerable clinical heterogeneity in tumour chemosensitivity. Any method that allows the chemosensitivity of tumours to be predicted in individual patients would therefore be welcome, as it will allow optimal treatment to be given to each patient and can be used to design better regimens without the need for large numbers of clinical trials [7–9]. Many attempts have been made to do this, but no chemosensitivity test has yet achieved widespread clinical use, with the exception of those used to guide treatment with molecularly targeted agents such as herceptin [10] or endocrine agents. The ATP-based tumour chemosensitivity assay (ATP-TCA) has been developed over the last decade as a possible solution to this problem [11]. Initial results of this assay in breast and ovarian cancer were encouraging [12–17]. Correlation with clinical outcome was demonstrated in multiple studies from several centres [11,13,14]. In primary ovarian cancer, Konecny *et al.* [14] demonstrated sensitivity, specificity and positive and negative predictive values of 95, 44, 66 and 89%, respectively, for recurrence in less than 1 year. This work culminated in a prospective case-control study of 25 patients and 30 controls [16], in which ATP-TCA-directed therapy produced a 64% response rate (37% in controls) with PFS of 45 weeks (20 weeks in controls). The majority of responses were achieved with experimental combinations rather than the more commonly used single agents, but the diversity of different combinations used made it likely that the ATP-TCA had made a contribution. As there was some indication of a particularly strong effect in the small number of platinum-resistant cases included in the earlier study, we decided to perform a follow-up trial in this poor-prognosis group, which stood to benefit the most.

The primary aim of the TCA Ovarian Cancer Trial was therefore to determine the response rate and PFS following chemotherapy in patients with platinum-resistant recurrent ovarian cancer, who were being treated according to ATP-TCA in comparison with those receiving physician's choice of treatment. Secondary aims included assessment of OAS and toxicity.

Patients and methods

Patients were enroled in the study only after a discussion of their treatment options, including chemotherapy. Only those who wished to undergo further chemotherapy were offered entry into the trial. Partners were involved in the consent process at the patient's discretion.

Entry criteria

Only those patients with cytologically and/or histologically proven platinum-resistant recurrent ovarian adenocarcinoma (locally advanced or metastatic) from whom sufficient tissue/cells could be obtained for ATP-TCA (>2 million cells) by paracentesis or surgery were included in the study. All patients had suffered relapses within 6 months of platinum-based chemotherapy before entry into this study. Clinical suspicion of early relapse on the basis of rising CA125 within 6 months, with subsequently proven recurrent ovarian cancer within 1 year of primary treatment sufficient to obtain tissue or ascitic fluid for testing, was accepted: these groups showed no difference in their sensitivity to platinum in the ATP-TCA (data not shown). No surgery was performed for the trial alone – some patients required diagnostic laparoscopy to assess recurrence, whereas others required debulking or palliative bypass surgery for gastrointestinal obstruction. Patients who were rechallenged with a platinum-based regimen for suspected platinum-sensitive disease and who subsequently developed early recurrence during treatment or within 6 months of it were accepted as being eligible for the study. Patients were also required to have (1) a life expectancy of at least 12 weeks, (2) a World Health Organization performance status (PS) less than or equal to 2 or (3) a Karnofsky PS $>70\%$, (4) measurable disease on ultrasound or computed tomography/magnetic resonance imaging (CT/MRI) scan or (5) evaluable disease on the basis of rising CA125 according to the Rustin criteria [18,19], (6) age of 18 years or more, and (7) adequate bone marrow, and hepatic and renal function. Patients with serious intercurrent medical illnesses were not eligible; neither were those with diagnoses of central nervous system metastases before study entry or with second malignancies (except in the case of cured preinvasive cancer of the cervix uteri and cured nonmelanoma skin cancer). All patients of childbearing age were requested to practise adequate contraception if they had not previously undergone either a hysterectomy or a bilateral salpingo-oophorectomy. Patients were entered into the study from December 1999 to February 2003, with follow-ups till July 2004, when the database was closed and analysis of the data commenced. Patients were stratified by centre and menopausal status and allocated to the treatment groups based on a minimization protocol.

Pretreatment evaluation

A complete clinical history and physical and gynaecological examination were included in the pretreatment evaluation. Laboratory studies included a whole blood-cell count, including platelet and white blood cell counts, liver and renal function tests, urinalysis, and ECG. Tumour measurements using ultrasound, radiography, CT scans or MRI were documented for future evaluation of response and CA125 measurements were carried out to provide a baseline for the assessment of responses in units using this modality.

Randomization was performed separately for each centre using a minimization protocol, with stratification for ascites/solid tumour and for age below 45 years.

Interim and posttreatment analyses

In both study arms, therapy was monitored by determining the CA125 tumour marker preceding each treatment course. Complete physical (including gynaecological) examinations were performed every second chemotherapy cycle. Using the appropriate radiological means as indicated above, tumour imaging was performed after every third cycle. Post-treatment evaluation was performed every 3 months after the cessation of the study medication using the same procedures as those applied for interim analyses.

ATP-based tumour chemosensitivity assay

Tissue/cells for ATP-TCA assay were obtained either during the operation or from malignant effusions, according to their treatment, maintained at 4°C and sent to the central laboratory overnight by courier. The ATP-TCA is a highly standardized method that relies on selective culture conditions to measure the action of drugs or combinations of drugs on neoplastic cells within mixed tumour-derived cell populations. It was performed as described previously [11,20]. Briefly, cells were dissociated from solid tumour samples by enzymatic digestion overnight and purified by density centrifugation. Density centrifugation was also used to obtain cells from ascites. The cells were resuspended in complete assay medium CAM (DCS Innovative Diagnostic Systems, Hamburg, Germany) and were then plated at 20 000 cells/well in polypropylene 96-well plates (Corning-Costar, High Wycombe, UK). The list of drugs is shown in Table 3a. Combinations were tested by simultaneous addition of both drugs included in the combination (Table 3a), as reported previously [11,20]. Cisplatin was included to confirm resistance: no patient received single-agent platinum during the trial. At the end of a 6-day incubation period, the ATP content of the cells was measured using the luciferin–luciferase assay. Results were deemed evaluable if the following criteria [11,20,21] were fulfilled:

(1) Histological and/or cytological diagnosis of ovarian carcinoma on the assay specimen with $> 20\%$ malignant cells
(2) No inhibition medium only control value > 20 nmol/l ATP and maximum inhibitor control $\leq 1\%$ of medium only
(3) Concentration responsiveness to those agents previously shown to exhibit this in the assay
(4) Absence of fungal or bacterial contamination

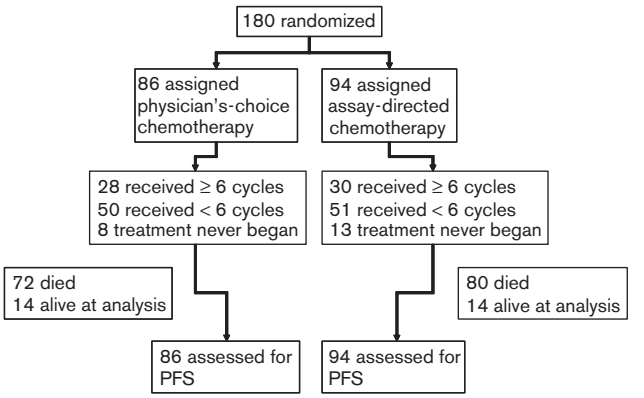
The two laboratories performing the ATP-TCA participated in an external quality control programme using cell lines supplied by the UK laboratory. The results were expressed graphically as the percentage inhibition versus a test-drug concentration derived from pharmacokinetic data. A sensitivity index (SI) was derived from these results as the sum of the percentage survival at each concentration tested, as published previously [12,17]. An SI of zero represents complete inhibition, whereas an SI > 300 represents resistance in the assay.

Treatment plan and follow-up

In the assay-directed treatment arm, ATP-TCA results were reported to the treating oncologist, within 10 days of the biopsy, in a standard format with concentration-inhibition graphs. In the physician's-choice arm, patients were allowed to start treatment immediately. In patients randomized to assay-directed chemotherapy, the ATP-TCA results were used to determine chemotherapy, chosen by the combination or single agent with the greatest activity, as measured by SI. In patients randomized to the physicians's-choice arm, treating oncologists were blind to the ATP-TCA results (Fig. 1). Patients in the latter study arm were allowed to crossover to ATP-TCA-directed chemotherapy when empirical chemotherapy was found to be ineffective. Further, the ATP-TCA results were made available, on request, to the oncologist following poststudy progression, to allow assay-directed poststudy treatment. Chemotherapy regimens used in the trial are shown in Table 3b. Supportive care was allowed, with appropriate hyperhydration, antiemesis and hypersensitivity prophylaxis, considering the diverse standards of the centres involved. Secondary use of granulocyte-colony stimulating factor, erythropoietin or amifostine was allowed, but these were rarely used.

Patients randomized to receive TCA-directed therapy were offered chemotherapy using the regimen tested in the ATP-TCA, which showed the best in-vitro chemosensitivity. In the event of two regimens producing similarly strong in-vitro sensitivity, the oncologist was requested to select the least toxic alternative. If the best regimen was contraindicated for any other reason, the next best was selected at the discretion of the individual

Fig. 1



Trial profile, showing the numbers of patients entering the study and completing treatment. PFS, progression-free survival.

oncologist. In case patients' symptoms warranted other treatment by surgery, chemotherapy, immunotherapy or radiotherapy during the study, they were withdrawn from it and their response to chemotherapy was evaluated at that time, according to ITT criteria.

Analysis of toxicity

All patients receiving at least one chemotherapy cycle according to the study protocol were considered to be eligible for assessment of toxicity. Toxicity was scored using the common toxicity criteria of the WHO.

Analysis of response

All patients receiving at least two chemotherapy courses were considered evaluable for response. The results were analyzed with respect to the proportion of patients responding [complete response (CR) plus partial response (PR)], in each group as a whole and in those who received chemotherapy with a regimen that showed strong sensitivity in the TCA. As the trial predated Response Evaluation Criteria in Solid Tumours (RECIST), patients with lesions bidimensionally measurable by ultrasound or radiological means (CT scans or MRI scans) were scored according to International Union Against Cancer criteria, as follows:

(1) CR: complete disappearance of all measurable disease and normalization of any elevated tumour marker for at least 4 weeks.
(2) PR: a decrease of 50% or more in the sum of the products of the two maximum perpendicular diameters of measurable disease for at least 4 weeks or CR with persistent elevated tumour markers.
(3) Stable disease (SD): a less than 50% decrease or a $< 25\%$ increase in the sum of the products of the two maximum perpendicular diameters of measurable disease.
(4) Progressive disease (PD): a greater than 25% increase in the sum of the products of the two maximum perpendicular diameters of measurable disease or the development of metastasis to a new site, biopsy-confirmed if possible or increasing numbers of tumour markers with or without clinical evidence of PD.

In patients with evaluable rather than measurable disease (i.e. elevated tumour markers), responses were assessed according to the Rustin criteria [18,19], as follows:

(5) CR: normalization of any elevated tumour marker (usually CA125) after two cycles of study treatment lasting for at least 4 weeks.
(6) PR: a $> 50\%$ decrease of any elevated tumour marker after two cycles of study treatment lasting for at least 2 weeks.
(7) SD: a $< 50\%$ decrease or a $< 25\%$ increase of any elevated tumour marker after two cycles of study treatment.
(8) PD: a $> 25\%$ increase of any elevated tumour marker after two cycles of study treatment.

Statistical analysis

The study was only powered to detect a substantial effect of assay-directed therapy. As the expected response rate for patients with recurrent ovarian cancer irrespective of platinum resistance was taken as 30% and the ATP-TCA false-positive rate was approximately 10%, a response rate of 50% in the TCA-selected group would be significant at the 95% confidence level if $n = 180$, with 90 patients in each group and 80% power. Survival may be influenced by poststudy therapy, but the primary statistical parameters to be evaluated were PFS and objective response rate (ORR), which are considered less likely to be affected. Preplanned analysis included a comparison of the TCA-directed and physician's-choice groups for age, CA125 levels, sample type (solid tumour or ascites), debulking, duration of platinum-based response and centre to ensure the validity of the comparison, before statistical analysis of the groups' responses proceeded further. PFS was calculated using the standard time from randomization to relapse, death, or loss to follow-up in an ITT analysis based on Kaplan–Meier statistics, although this does favour the physician's-choice arm of the study as all those receiving assay-guided therapy had to wait a minimum of 7 days for the assay results to be obtained. OAS, corresponding with the PFS, was calculated from study entry to death from any cause or loss to follow-up. Differences in response rate were assessed by χ^2 and in survival curves by log-rank analysis.

Ethics

The trial was approved by the Multicentre Research Ethics Committee (Medical Research Council), and multiple institutional ethics committees in the UK and Germany, and was conducted according to the tenets of the Declaration of Helsinki. All patients gave written informed consent before study entry for both treatment and use of tissue for research.

Results

A total of 180 patients were randomized, with 94 receiving assay-directed treatment and 86 receiving physician's-choice therapy, as shown in Fig. 1. The median ages were 59 and 61 years in the two groups.

First-line chemotherapy

Single-agent carboplatin was used in 73 patients (42%), platinum + taxane in 89 patients (51%), platinum + cyclophosphamide in four (2%), platinum + epirubicin + paclitaxel in two (1%), and carboplatin + doxorubicin + cyclophosphamide in six (3%), with uncertainty over initial treatment in six patients although this was thought to be platinum-based. For patients showing PR or CR to first-line treatment, the median times from end of

primary treatment to first relapse were 146 and 142 days, respectively, in the two treatment groups. A total of 36 patients (24 in the TCA-directed group) had PD during primary treatment, and 51 patients (32 in the TCA-directed group) showed PD or SD on primary chemotherapy Evidence of relapse within 6 months has been documented in 113 patients (63%) with strong suspicion of relapse (i.e. rising CA125) in the remaining patients before this point, although some patients delayed treatment until symptoms occurred. Such patients were regarded as eligible and randomized accordingly. All were required to have a WHO PS of 0–2 and all had previous platinum-based treatment. A few patients in each group were rechallenged, proving resistance to platinum following a previous response: they were accepted as eligible for the study. The groups were generally well balanced with respect to previous surgical intervention, previous chemotherapy and histology (Table 2). The low number of patients said to have received previous oophorectomy or hysterectomy is likely to be a reporting error, although low rates of surgery have been reported from the UK compared with other countries [22].

Table 1 Patient accrual by centre to each arm of the trial

Study centre	Physician's choice	Assay directed	Total
Airedale	3	5	8
Cheltenham	4	2	6
Koln	11	9	20
Mount Vernon	3	4	7
Poole	7	8	15
Portsmouth	0	1	1
Preston/Lancaster	15	16	31
Southend	35	37	72
Vienna	0	1	1
Wolverhampton	8	11	19
Total	86	94	180

Several centres submitted very few patients and although this led to a slight imbalance in the numbers in each group, the groups were well balanced with respect to age and type of specimen.

Table 2 Patient characteristics by group: there was no statistical difference between the groups for any of the criteria assessed

Criterion	Physician's choice	Assay directed
Age (median)	61 years	59 years
Postmenopausal	60/86	59/94
Previous laparotomy	61/86	72/94
Previous oophorectomy	58/86	58/94
Previous hysterectomy	49/86	50/94
Histology – serous type	40/86	47/94
Histology – poor differentiation	32/86	41/94
Rechallenge with platinum	10/86	9/86
Primary treatment relapse	146 days	142 days
Primary treatment response	57/86	50/94
Prior treatment with taxane	36/86	32/94
Ascites tested	57/86	61/94
Cycles given (mean)	3.7	3.7

ATP-based tumour chemosensitivity assay results

Cancer cells for testing were obtained from ascites in 118 patients and from solid tumour in 62 cases: usually at second-look laparoscopy or laparotomy, with limited surgical debulking when appropriate. The results are shown in Fig. 2 as a frequency distribution of SI, with SI > 300 representing probable resistance. This suggests that 30% of the patients in the physician's-choice arm were treated with a drug found to be inactive in the assay, whereas no active agent could be identified in only 5% of those in the assay-directed treatment arm. Only five patients showed 90% inhibition for cisplatin within the clinically achievable range of concentrations.

Second-line chemotherapy

Second-line therapy chosen for each group is shown in Table 3b. The number of cycles of chemotherapy given varied between patients, but was well matched between the trial arms, with an average of 3.7 cycles being given to each group. Combination use was higher in the assay-directed arm (72/81, 88%) than in the physician's-choice arm (50/78, 64%), but this was not statistically significant. Notably, the novel combinations treosulfan + gemcitabine and mitoxantrone + paclitaxel were the most common regimens used in either arm. No difference in outcome between patients was noted for patients who had ascites samples compared with those for whom solid tumour biopsies were submitted.

Primary end point: response

Response (Table 4) was evaluable in 147 patients: 33 patients received less than two cycles of treatment and were not evaluable for response. Evidence of a response (CR + PR) was seen in 23/73 (31.5%) of patients treated by physician's choice, and in 30/74 (40.5%) patients receiving assay-directed therapy ($P < 0.3$, not significant). Addition of age, remission duration, stage, histological classification and grade to assigned treatment group gave no factors significantly predicting response (data not shown).

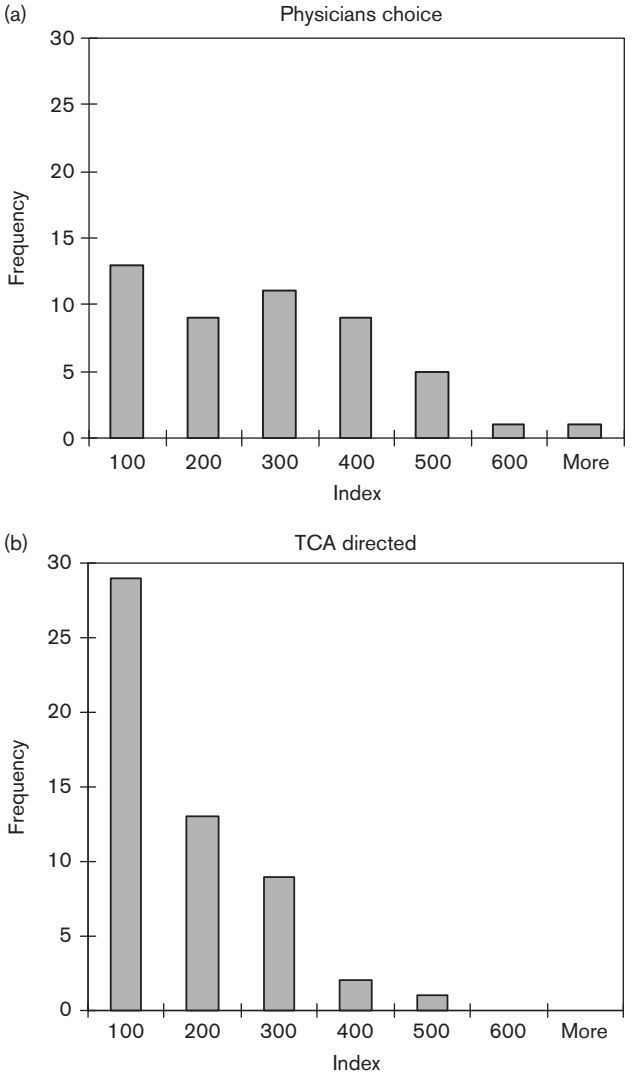
Primary endpoint: progression-free survival

The median follow-up at analysis was 546 days. At the point of analysis, 171/180 patients had progressed, with a median PFS of 93 days in the physician's-choice arm and 104 days in the assay-directed arm [hazard ratio (HR) 0.80, 95% confidence interval (CI) 0.59–1.10; $P < 0.14$, log-rank analysis], as shown in Fig. 3a. A Cox-regression model was performed to account for the influence of age, time from end of primary treatment, stage, histological classification and grade. Forcing the treatment group into this model gives HR (TCA-directed versus physician's choice) = 0.82, 95% CI = 0.6–1.1, $P < 0.2$.

Secondary endpoints

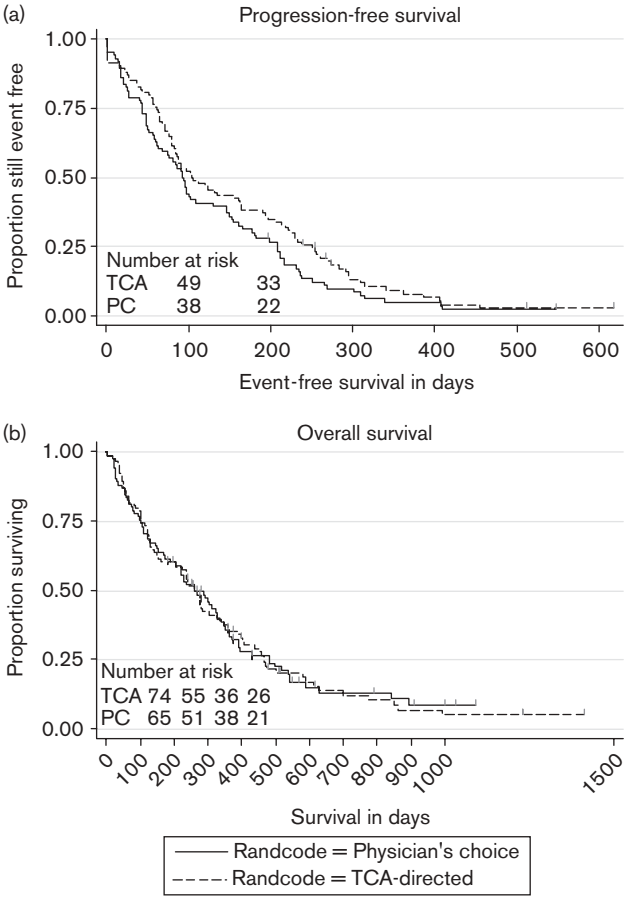
No difference was seen in OAS (time from randomization to death) between the groups, as shown in Fig. 2b. The same Cox-regression model gives HR (TCA-directed versus physician's choice) = 1.04, 95% CI 0.73–1.48, $P < 0.8$ (Fig. 3b).

Fig. 2



$Index_{Sum}$ data for each treatment group showing that 95% of those assigned tumour chemosensitivity assay (TCA)-directed treatment received therapy showing activity (sensitivity index >300) in the assay, whereas only 75% patients in the physician's-choice group received therapy suggested to be inactive by the assay.

Fig. 3



Kaplan–Meier survival curves for (a) progression-free survival and (b) overall survival, showing a trend towards improved progression-free survival (hazard ratio 0.80, 95% confidence interval 0.59–1.10) with no difference between the groups in overall survival (hazard ratio 1.01, 95% confidence interval 0.7–1.3). PC, physician's choice; TCA, tumour chemosensitivity assay.

Crossover effect

Crossover to assay-directed therapy in 29 patients from the physician's-choice group resulted in a response in 12 cases (41%), and stabilization of disease in a further two cases.

Toxicity

Side effects were reported for 99 patients, out of the 149 who received treatment (one cycle or more), and toxicity was similar between the two groups (Table 3c). The most frequent side effects were haematological or gastrointestinal, as might be expected from the frequent use of drug combinations. Only seven patients received granulocyte-colony stimulating factor, six in the assay-directed arm and one in the physician's-choice arm. Three patients were given amifostine, all of whom were receiving assay-directed treatment.

Learning effect

An analysis was performed to examine the data in the physician's-choice arm for a learning effect, as the extensive use of combinations in this arm of the study was unexpected. We suspected that this might be due to a learning effect, as oncologists treating patients with combinations in the assay-directed arm switched to the use of similar combinations in the physician's-choice arm of the study. Evidence exists of a considerable learning effect, with some apparent benefit to patients, as shown in Fig. 4 in comparisons of PFS between early and late entrants to the physician's-choice arm of the study (HR 0.44, 95% CI 0.2–0.9, $P < 0.03$, log-rank analysis). Late

Table 3a Chemotherapy regimens used during the trial

Regimen	Dosage
Doxil (Caelyx)	40 mg/m^2 day 1, IVI, q4wk
Etoposide	150 mg/m^2 day 1, 2, 3, IVI, q3wk OR oral etoposide, 50 mg/m^2/day day 1–21, q4wk
Mitoxantrone	12 mg/m^2 day 1, IVI, q3wk
Paclitaxel	175 mg/m^2 day 1, IVI, q3wk
Topotecan	1.5 mg/m^2 day 1, 2, 3, 4, 5, IVI, q3wk
Treosulfan	7000 mg/m^2 day 1, IVI, q4wk
Cisplatin + etoposide	Cisplatin 60 mg/m^2 IVI day 1,8,15,29, 36, and 43 + etoposide 50 mg oral day 1–15; 29–43, followed by: etopside 50 mg/m^2 oral daily day 1–21 q4wk (max two cycles).
Epirubicin + paclitaxel	Epirubicin 25 mg/m^2 IVI, day1 and 2 + paclitaxel 175 mg/m^2 day 3, IVI, q3wk
Cisplatin + gemcitabine	Cisplatin 75 mg/m^2 day 1 + gemcitabine 1250 mg/m^2 day 1, 8, IVI, q3wk
Mitoxantrone + paclitaxel	Mitoxantrone 4 mg/m^2 day 1, 2 + paclitaxel 175 mg/m^2 day 3, IVI, q3wk
Treosulfan + epirubicin	Treosulfan 5000 mg/m^2 day 1 + epirubicin 25 mg/m^2 IVI, day1, q3wk
Treosulfan + gemcitabine	Treosulfan 5000 mg/m^2 day 1 + gemcitabine 1000 mg/m^2 day 1, IVI, q3wk

Table 3b Chemotherapy regimens used during the trial, with the median number of cycles used

Drug/combination	Physician's choice	Assay directed
Doxil (caelyx)	9	5
Etoposide	3	0
Mitoxantrone	1	2
Paclitaxel	5	0
Topotecan	9	1
Treosulfan	1	1
Cisplatin + etoposide	1	0
Epirubicin + paclitaxel	5	5
Cisplatin + gemcitabine	10	10
Mitoxantrone + paclitaxel	13	23
Treosulfan + epirubicin	3	3
Treosulfan + gemcitabine	18	31
Combination use:	50/78	72/81

Table 3c Chemotherapy regimens used during the trial, with the maximum toxicity noted for these patients

Toxicity (grade 3/4)	Physician's choice	Assay directed
Haematological	15	10
Gastrointestinal	14	11
Pulmonary	1	1
Allergy	1	0
Hair loss	8	6
Cardiac	0	0
Hepatic	1	0
Renal	2	0
Fever/infection	7	2
Cutaneous	0	2
Neurotoxicity	0	0

randomization to the empirical arm clearly gave these patients a positive PFS advantage.

Discussion

The first randomized trial of ATP-TCA directed chemotherapy, and only the second randomized study of any TCA to complete its projected accrual, has followed the initial positive trial of a chemosensitivity assay by Von Hoff *et al.* [23]. Randomized controlled trials are rarely used to assess predictive methods. This approach is, however, becoming more common, despite the much greater costs involved in randomized than those incurred by correlation studies. The cost factor matters for the development of many diagnostic tests [24]. The recent paper by Sargent *et al.* [25] covers the options for such trials: this trial is regarded as a marker-based strategy design.

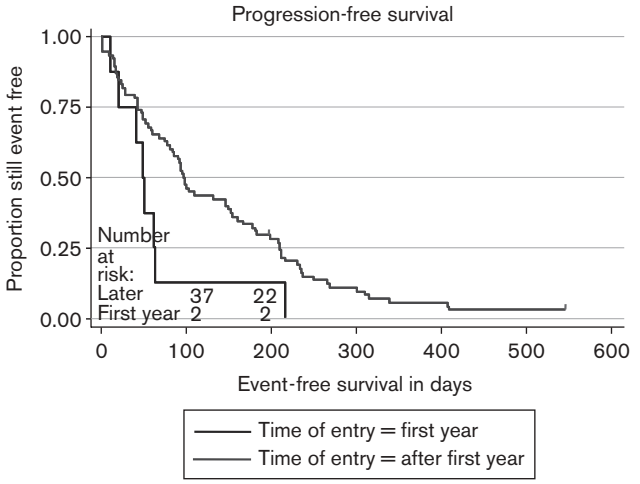
Table 4 Response data for each group, according to (a) intention to treat and (b) protocol (139 patients received two or more cycles of chemotherapy and were evaluable for response)

Best response	Physician's choice	Assay directed	Total
(a) Intention to treat			
CR	5	6	11
PR	18	24	42
SD	14	18	32
PD	31	23	54
CR + PR	23 (26%)	30 (31%)	53
CR + PR + SD	37 (43%)	48 (51%)	85
Not assessable	18	23	41
Total	86	94	139
(b) Protocol			
CR	5	6	11
PR	18	24	42
SD	14	18	32
PD	31	23	54
CR + PR	23 (34%)	30 (42%)	53
CR + PR + SD	37 (54%)	48 (68%)	85
Total	68	71	139

CR, complete response; PD, progressive disease; PR, partial response; SD, stable disease.

This trial was based on the result of our previous case–control study [16], which was designed to obtain statistical significance for differences greater than 20% between the groups. The trial was, therefore, underpowered to detect significant differences at the observed level and no statistical difference was demonstrated in response rate, PFS or OAS. The response rate, however, in those patients who received two or more cycles of chemotherapy in the assay-directed arm (40.5%) was superior to that in the physician's-choice arm (31.5%). A similar level of improvement in PFS was seen in the assay-directed treatment group. The 31.5% response rate in the physician's-choice arm was unexpectedly high and this seems to have resulted from positive bias due to a learning effect. This is shown by the fact that the majority of patients in the physician's-choice arm were treated with drug combinations rather than with single

Fig. 4



Patients entering this arm during the first year had a significantly reduced progression-free survival in comparison with later entrants (hazard ratio 0.44, 95% confidence interval 0.2–0.9, $P<0.03$, log-rank analysis).

agents, contrary to internationally accepted treatment guidelines for platinum-resistant or refractory ovarian cancer. The participating oncologists (all of whom contributed to this paper) changed their practise to the use of innovative combinations such as treosulfan + gemcitabine or mitoxantrone + paclitaxel in the physician's-choice arm on the basis of positive observations in patients randomized to the assay-directed arm. As shown in Fig. 4, late recruitment was a significant prognostic factor in the physician's-choice arm of this study. In fact, even the results achieved in the physician's-choice arm of this trial compare favourably with the best results of chemotherapy seen in any other published phase III trial in platinum-refractory/-resistant ovarian carcinoma [2–5]. The response rates and PFS demonstrated in both arms of this study are clearly outstanding.

The data suggest that at least half of the benefit observed in the previous case–control study [16] might have been due to the assay, and the remaining benefit to the use of more effective combined regimens. The data are even more encouraging, when it is considered that 70% of the cases in the physician's-choice arm were treated with agents showing activity in the assay. These patients dilute the trial's ability to discriminate between the groups, but the crossover results suggest that when patients were put onto ineffective regimens, many (40%) could be rescued by assay-directed therapy. We neither expected nor obtained an OAS difference between the groups, due to this crossover effect.

The two regimens used most in this trial were mitoxantrone + paclitaxel and treosulfan + gemcitabine, both of which were designed using the ATP-TCA [21,26]. These regimens were tested in Germany against ovarian cancer and have since then been shown to be effective even in heavily pretreated ovarian cancer patients [27]. In fact, when there was a clinical response, the different treatments in both arms produced similar PFS, suggesting that the length of PFS is a function of tumour adaptation to chemotherapy [17], rather than the regimen and that it is the achievement of a response that really matters in obtaining improved survival. This trial was balanced well for the treatments given and a rapid learning curve was present for many of the oncologists taking part: within a few months most had switched to the use of one of the regimens chosen most commonly by the assay – usually treosulfan + gemcitabine or mitoxantone + paclitaxel. The latter are also less expensive and are often better tolerated than some of the single agents [28–30]. These regimens also differ substantially in their mechanisms of action, so there was still a considerable spread in the treatments used in individual patients. We are yet not certain why treosulfan + gemcitabine has proved to be so active in this group of patients with platinum resistance, although it is possible that alterations in DNA repair associated with cisplatin resistance [31,32] might have made these patients' tumours more susceptible to this regimen. These results suggest that a randomized controlled trial of treosulfan + gemcitabine in recurrent ovarian cancer would be helpful. Mitoxantrone + paclitaxel has also proved to be a very active regimen, in this and in previous studies [21]. It is notable that toxicity was similar between the assay-directed and physician's-choice groups. Although combination regimens were less frequently used in the physician's-choice arm, this difference was not statistically significant.

Any chemosensitivity assay – molecular or cellular – is only as good as the drugs that are available. A diagnostic test might be very good; nevertheless, it will not improve the results of a poor therapeutic option. Unfortunately, cytotoxic chemotherapy is not good enough to provide a cure for very many patients with recurrent platinum-resistant ovarian cancer, particularly for those with primary platinum resistance. This almost certainly reflects the biology of the tumour and the adaptabiliy of the neoplastic cell to drug exposure [31,33]. Overcoming this adaptation is a challenge, but the development of modulating agents for drug-resistance mechanisms is feasible and can add to the therapeutic index in ovarian cancer [9,21,33].

In conclusion, this study shows a nonstatistically significant trend towards improved response rate and PFS (HR 0.8) in patients who were given assay-directed chemotherapy. Larger trials will be required to overcome the inherent dilution factors involved when treatment choices are balanced between the control and assay-directed arms of such trials. The ATP-TCA might provide useful assistance to oncologists faced with difficult

choices in some patients with recurrent ovarian cancer, but currently remains an investigational method.

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