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Original Paper

An I.T.M.O. Group Study on Second-line Treatment in Advanced Epithelial Ovarian Cancer: an Attempt to Identify Clinical and Biological Factors Determining Prognosis

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The aim of the present study was to determine the activity of a combined regimen of mitoxantrone (DHAD) and ifosfamide (IFO) and identify clinical and biological factors with prognostic importance for the second-line treatment of ovarian cancer. The following factors were investigated for their prognostic importance: age, disease sites, platinum responsiveness, histological grade, the presence of clinically/radiologically detectable versus not detectable disease, residual disease volume after first surgery, p53 protein, c-erbB-2 oncoprotein and laminin receptor. 72 patients entered the trial. DHAD and IFO therapy led to a 15% response rate among the 47 cases with clinically/radiologically detectable disease (1 complete and 6 partial responses), with a median response duration of 4 months. The response rate was significantly different according to platinum responsiveness (4% objective responses in platinum-resistant versus 27% in platinum-sensitive disease). The time to treatment failure (TTF) and overall survival (OS) were affected by the presence of clinically detectable disease at study entry (median TTF 4 months in the presence of clinically/radiologically detectable disease versus 9 months if the disease was not similarly detectable, $P = 0.02$; median OS 10 months versus 21 months, $P = 0.01$). Initially overexpressed in only a few tumours, the c-erbB-2 oncoprotein became overexpressed in 36% of platinum-resistant tumours; this modulation did not occur in platinum-sensitive tumours. Furthermore, laminin receptor was expressed in 77% of platinum-sensitive versus 39% of platinum-resistant patients. There were no differences in p53 protein expression according to drug responsiveness.

Key words: ovarian cancer, second-line treatment, prognostic factors
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INTRODUCTION

APPROXIMATELY 4000 new cases/year of ovarian cancer are reported in Italy and approximately half of these will receive second-line chemotherapy due to the failure of first-line treatment [1, 2]. Over the last few years, mitoxantrone (DHAD) and ifosfamide (IFO) have been tested as single agents in the second-line treatment of advanced ovarian cancer [3–6]. The results have been interesting, particularly in patients who have not been heavily pretreated [3, 5, 6]; therefore, from March 1990, the I.T.M.O. (Italian Trials in Medical Oncology) group decided to evaluate the combination of these drugs in an attempt to produce better results than those observed when they are used as single agents. A further aim of this trial was to define the prognostic role of some clinical and biological factors in patients undergoing second-line therapy. Such patients are quite heterogeneous, and responses to the same agent can be dramatically different in different studies [7, 8]. It, therefore, appeared meaningful to try to determine which clinical or biological factors may play a role in conditioning the results of second-line therapy.

Given that their prognostic role in patients undergoing first-line therapy is well documented [9–11], histological grading and the volume of residual disease after first surgery were investigated in order to discover whether they have any influence on the activity of second-line treatment. Age, platinum responsiveness, radiologically detectable disease and disease sites at study entry were investigated because they are more representative of both the patient and tumour status at the time of starting second-line therapy; it has already been proposed that the previous response to platinum compounds may influence the results of second-line therapy [8], as well as that patients' age may be a prognostic factor in ovarian cancer [12].

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In addition to clinical parameters, the overexpression of proteins coded by the *TP53* and *ERBB-2* genes and laminin receptor were examined to assess their prognostic role in patients undergoing second-line therapy [13–17]. So far, no clinical parameters can be considered as prognostic factors for patients undergoing second-line treatment in advanced ovarian cancer; therefore, the search for laboratory data with prognostic significance is justified.

Since platinum responsiveness (determined on the basis of response to first-line treatment) was assessed for all patients entered, it also appeared meaningful to verify whether there was any correlation between the response to platinum compounds and the biological parameters investigated.

MATERIALS AND METHODS

Eligibility criteria

This multicentre phase II trial involved patients with initial stage III–IV epithelial ovarian cancer, persistent or relapsed (within 1 year) after previous chemotherapy containing platinum compounds (cisplatin, carboplatin). The following inclusion criteria had to be met: the presence of clinically, radiologically or surgically detectable disease, an age of between 18 and 75 years, a performance status of 0–2 (ECOG), adequate bone marrow reserve and serum biochemistry within normal limits. The approval of the local Ethics Committee, as well as witnessed informed consent from each subject, were obtained.

Staging procedures and treatment scheme

Baseline staging included a physical and gynaecological examination, CA125 assessment, a chest X-ray and radiological examination of the abdomen (CT scan, NMR, echography). Those patients who did not show any evidence of clinically/radiologically detectable disease (i.e. disease documented only at laparotomy) did not receive laparoscopy, because in the authors' experience the sensitivity of this examination was comparable to the radiological examination. Laparoscopy was performed only in those cases in which radiological examination revealed some doubtful lesions, in order to assess whether these lesions were malignant. These examinations were repeated at the time of response evaluation and every 2 months after treatment discontinuation. The eligible patients were treated according to the following scheme:

—DHAD 10 mg/m^2 intravenous (i.v.) bolus, on day 1
—IFO 4000 mg/m^2 i.v. through a 6 h infusion, on day 1
—mesna 800 mg/m^2 i.v. bolus, three times during the IFO infusion at hours 0–4–6, on day 1
The cycle was repeated every 21 days.

Treatment plan and side-effects

In patients with clinically or radiologically detectable disease, response was evaluated according to WHO criteria [18], after every three cycles of therapy; in the case of complete response (CR) or partial response (PR), treatment was continued. In patients who stabilised after six cycles of chemotherapy, treatment was stopped. In cases with disease documented only through laparotomy, treatment was given for three cycles and then, after excluding clinical or radiological progression, a further three cycles were given; treatment was then stopped without any further surgical evaluation. Therefore, in these patients, tumour response was not assessable. Side-effects were reported according to WHO criteria [18].

Prognostic factors

Clinical. The following parameters were investigated: (a) tumour imaging at study entry, determined after staging procedures, with the patients being divided into those with and those without clinically/radiologically detectable disease; (b) histological grading of primary tumours according to FIGO classification (G1: well differentiated; G2: moderately differentiated; G3: poorly differentiated); (c) residual tumour volume $<$ or >2 cm after initial surgery (in some patients who received chemotherapy before initial surgery, these data were unavailable); (d) age at the moment of study entry ($\leq$64 years of age or $>$64 years of age); (e) disease sites at the moment of study entry, classified as abdominal disease (except liver) and extra-abdominal disease; (f) tumour responsiveness to previous treatment with platinum compounds. This last parameter was assessed according to the following empirical rules: a platinum-resistant tumour was defined as a tumour which had progressed during platinum-containing chemotherapy or which had stabilised–regressed $<$50% after previous platinum compounds treatment, given until the total cumulative doses of cisplatin and carboplatin had reached, respectively, 450 mg/m^2 or 1800 mg/m^2. A platinum-sensitive tumour was defined as one which had regressed completely or by at least 50% after previous treatment with platinum compounds. The status of patients for whom information on previous treatments was incomplete was defined as undetermined. The duration of response was not considered as determining platinum responsiveness. In the case of patients receiving two lines of platinum-containing chemotherapies before study entry, platinum responsiveness was determined by taking into account the results achieved after the last platinum-containing regimen given to the patient.

Tumour responsiveness to a previous treatment with platinum compounds was generally determined at the moment of second-look (i.e. after five or six cycles of first-line chemotherapy); the only cases who were classified as platinum-resistant before treatment termination were those who clearly showed progressive disease at the moment of the initially planned debulking surgery (i.e. after three cycles of first-line chemotherapy).

Biological. The overexpression of proteins encoded by the *TP53* and *ERBB-2* genes and laminin receptor expression was assessed by means of immunocytochemistry, applying MAb D07 (Novocastra Lab. 1:1000 diluted), c-erbB-2 (pAb1) (Triton Diagnostics 1:200 diluted) and MLuC5. Immunostaining was performed as already described elsewhere [19, 20]. Samples were defined as positive if staining of at least 10% was demonstrated.

The immunocytochemical analyses were carried out at the Departments of Anatomical Pathology and Experimental Oncology at Milan's Istituto Nazionale Tumori. In both the departments, the observers were blinded as to clinical outcome.

Tissue samples were obtained on the occasion of the first surgery (baseline data); further samples were available for some patients who underwent debulking surgery before second-look laparotomy (i.e. after three cycles of first-line chemotherapy with platinum compounds).

Statistical analysis

Response duration was calculated from the time the tumour became evident to the time of progression. The time to treatment failure (TTF) and overall survival (OS) were calculated for all entered patients as the time elapsing between the beginning of the treatment and the event (progression or death).

The study was planned according to the Simon optimal two-stage design to test the null hypothesis that the true response probability was less than 20%, against the alternative hypothesis that this probability was greater than 40% [21]. By adopting type I and type II error probability levels of 0.05 and 0.10, respectively, it was estimated that the treatment had to be rejected if no more than four and 15 responses were observed at the end of the first (19 patients) and second stage (54 patients overall).

The probabilities of response to treatment in the different categories of patients, as well as the expression of biological parameters according to platinum responsiveness, were compared using Fisher's exact test. The TTF and OS curves were drawn as Kaplan–Meier plots. The TTF and survival curves were compared using the log-rank test [22]. Multivariate analyses were performed using Cox's regression model [23] or by means of recursive partitioning techniques [24].

RESULTS

Patient characteristics

Between March 1990 and March 1993, 78 patients were enrolled in this multicentre trial co-ordinated by the I.T.M.O. group; five Centres participated in the accrual. 6 patients were considered ineligible before study entry and were, therefore, not enrolled, leaving a total of 72 patients. 6 patients received only two cycles of therapy due to progression (4 cases) or side-effects (2 cases); all of the other patients received at least three cycles of chemotherapy.

The main characteristics of the patients are shown in Table 1. It should be emphasised that 25 of the 72 patients had no clinically or radiologically detectable residual disease; in these cases, no assessment could be made of tumour response. Platinum responsiveness was undetermined in 8 cases, one of whom had clinically/radiologically detectable disease. In 14 cases, FIGO histological grading was uncertain; 15 cases had an undetermined residual tumour volume after initial surgery because they had previously received chemotherapy. In the remaining 57 patients, initial surgery was performed before first-line chemotherapy and consisted of hysterectomy, bilateral oophorectomy-salpingectomy and omentectomy. Furthermore, assessment of peritoneal fluid volume and cytology, pelvic and para-aortic lymph node sampling and random biopsies were performed.

In the 42 cases who, after initial surgery, showed a disease volume ≥ 2 cm, a debulking surgery was performed after the third cycle of chemotherapy; in the 15 cases who had a disease volume <2 cm after initial surgery, no debulking was performed.

Table 1. Patient characteristics

No. entered/evaluable	72/72
Median age (range)	57 (39–74)
≤64 years	55
>64 years	17
Performance status (ECOG)	
0	29
1	35
2	8
Histotype	
papillary serous adenocarcinoma	60
mucinous adenocarcinoma	6
endometrioid adenocarcinoma	4
clear cell carcinoma	2
Histological grading	
well differentiated	11
moderately differentiated	20
poorly differentiated	27
unknown	14
Extra-abdominal disease	20
liver	9
pleural	5
subcutis/nodes/bone	6
Responsiveness to platinum compounds	
sensitive	27
resistant	37
undetermined	8
Tumour evidence at study entry	
clinically/radiologically detectable	47
detectable only at laparotomy	25
Disease volume after first surgery	
≥2 cm	42
<2 cm	15
undetermined	15

Expression of biological parameters

Table 2 reports the data concerning the expression of the investigated biological parameters in 28–42 of the 72 patients who entered the trial. This subgroup was represented by the patients treated at the National Cancer Institute of Milan, because it was not possible to have tumour samples from the other institutions involved in the trial. Nevertheless, patients' characteristics in Milan and in the other institutions were comparable. These data refer to tissue samples obtained at the time of initial surgery (performed before first-line treatment with platinum compounds) from patients with initial stage III–IV disease. In some cases, it was possible to obtain further tissue samples from the same patient (generally at the moment of debulking surgery, i.e. after three cycles of first-line treatment), and, therefore, to determine any changes in the investigated biological parameters. There was an overexpression of p53 in most of the primary tumours, with no significant modulation of this parameter occurring during treatment. Laminin receptor was detected in 51% (21/41) of the evaluated cases, modulation during treatment being observed in 20% (5/25) of tumours, either as a decrease in or increase of expression. Overexpression of the c-erbB-2 oncoprotein was found in only 14% (6/42) of cases, but after three cycles of platinum therapy, an additional 19% (5/26) overexpressed the c-erbB-2 oncoprotein.

Activity and tolerability of mitoxantrone plus ifosfamide

The response rate was calculated in the 47 patients with clinically/radiologically detectable disease. 2 of the 47 patients received fewer than three cycles of therapy due to progressive disease; all the other patients were treated with at least three cycles. One CR and 6 PRs were observed for an overall response rate of 15% (exact 95% C.L.: 4–25%). Among the remaining 40 patients, 19 stabilised and 21 progressed. The CR was observed in a 54-year-old patient with pleural metastases, who had previously responded to platinum compounds. In the 6 PRs, the responsive sites were skin, pelvic mass, superficial lymph nodes, abdominal mass and pleura. The median response duration was 4 months (range 1–19). The median TTF for the 47 patients with detectable disease was 4 months (6 months if all the 72 patients who entered the study are considered). The median survival of patients with detectable disease was 10 months (11 months if all 72 patients are considered).

Table 2. Expression of biological parameters in advanced epithelial ovarian cancer

Parameter	No. of patients			No. of patients with multiple evaluations	No. of patients with parameter modulation (%)	Type of modulation	
	Positive (%)	Negative	Evaluated			neg→pos	pos→neg
p53 protein	19 (68)	9	28	19	1/19 (5)	1	–
Laminin receptor	21 (51)	20	41	25	5/25 (20)	2	3
c-erbB-2 oncoprotein	6 (14)	36	42	26	5/26 (19)	5	–

Side-effects were evaluated in all patients who entered the study. The following grade 3–4 side effects were observed: leucopenia in 10 cases (4 cases of grade 4), thrombocytopenia in 3 cases (1 grade 4), nausea and vomiting in 4 cases, cystitis in 3 cases, mucositis in 2 cases, anaemia in 3 cases and a worsening of platinum-related peripheral neurotoxicity in 2 cases. Pronounced hair loss occurred in 17 patients. Rises in serum creatinine and in transaminases were observed in one case each.

Analysis of prognostic factors

The effect of the clinical and biological factors described in the "Materials and Methods" section on the response rates, TTF and OS of patients undergoing second-line therapy was investigated with the following results.

Response rates. This analysis was carried out in the 47 patients with clinically or radiologically detectable disease; in the remaining 25 patients with residual disease documented only by means of laparotomy, no response evaluation was feasible because the patients did not undergo a further laparotomy. Some of the 47 patients with clinically or radiologically detectable disease were not evaluable for this analysis due to the lack of data concerning the investigated clinical and biological factors. Table 3 reports response rates according to the different putative prognostic factors. In the case of multiple evaluations of biological parameters at different times, the assay considered for this analysis was the last performed before starting second-line treatment.

It can be seen that the only factor capable of influencing the probability of response to second-line treatment was platinum reponsiveness: 27% of platinum-sensitive patients responded as opposed to 4% of platinum-resistant tumours ($P = 0.03$). Although age was not statistically significant as a predictive factor for response, it is worth noting that all the responses were achieved in patients aged 64 years or less.

Time to treatment failure (TTF). A univariate analysis was made of the 72 evaluable patients in order to discover whether there was any significant variation in TTF according to the putative prognostic factors. The TTF in patients with clinically or radiologically detectable disease before study entry was significantly worse than in those whose disease was demonstrated only by means of laparotomy (Figure 1). It is worth noting that, among the group of patients with detectable disease, no differences in TTF were observed according to platinum responsiveness, although five objective responses were observed in the 18 platinum-sensitive patients versus one response in the 28 resistant cases (Figure 2). None of the other factors investigated were capable of significantly influencing TTF, although a slight advantage was observed for patients with platinum-sensitive as opposed to platinum-resistant tumours (median TTF 6 versus 4 months). Multivariate analysis failed to reveal any other prognostic factor.

Table 3. Responses according to clinical and biological factors

Proposed prognostic factor (No. of patients)	Response to treatment	
	CR + PR (%)	NC + PD (%)
Age (47)		
≤64 years (36)	7 (19)	29 (81)
>64 years (11)	– –	11 (100)
Disease sites (47)		
extra-abdominal (20)	3 (15)	17 (85)
abdominal except liver (27)	4 (15)	23 (85)
Platinum responsiveness (46)		
Sensitive (18)	5 (27)	13 (73)
Resistant (28)	1 (4)	27 (96)
Histological grade (37)		
G1 (8)	1 (12)	7 (88)
G2 (10)	2 (20)	8 (80)
G3 (19)	3 (16)	16 (84)
Residual disease volume after first surgery (36)		
<2 cm (9)	1 (11)	8 (89)
≥2 cm (27)	4 (15)	23 (85)
p53 protein (16)		
negative (5)	1 (20)	4 (80)
positive (11)	3 (27)	8 (73)
c-erbB-2 oncoprotein (22)		
negative (14)	2 (14)	12 (86)
positive (8)	2 (25)	6 (75)
Laminin receptor (22)		
negative (12)	2 (17)	10 (83)
positive (10)	2 (20)	8 (80)

CR, complete response; PR, partial response; NC, no change; PD, progressive disease.

Overall survival (OS). The same univariate analysis was also carried out for OS, and reflected the significant prolongation of TTF in patients with no clinically or radiologically detectable disease (Figure 3). Among the patients with detectable disease, the survival of platinum-sensitive cases was comparable with that of resistant patients (Figure 4). As for TTF, no influence on OS was observed for the other investigated factors. Again, multivariate analysis emphasised the importance of a radiologically detectable tumour as the only parameter capable of significantly influencing overall survival.

Correlation between biological parameters and platinum responsiveness

The results of our attempt to correlate biological data with platinum responsiveness are given in Table 4. The biological data refer to the assay performed at the time of first surgery, before first-line medical treatment. Changes in these biological parameters were investigated by comparing baseline expression

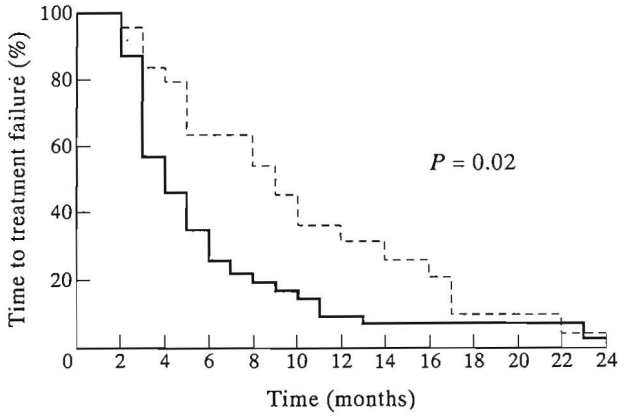

Figure 1. TTF according to method of detection of tumour at study entry. – – – 25 patients, tumour not clinically or radiologically detectable; —— 47 patients, tumour clinically or radiologically detectable.

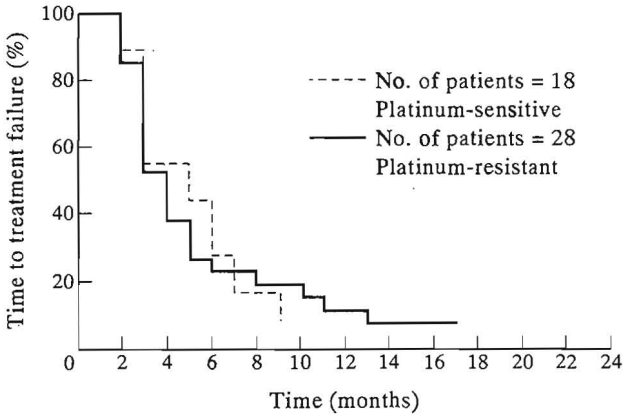

Figure 2. TTF of patients with radiologically detectable disease, according to platinum responsiveness.

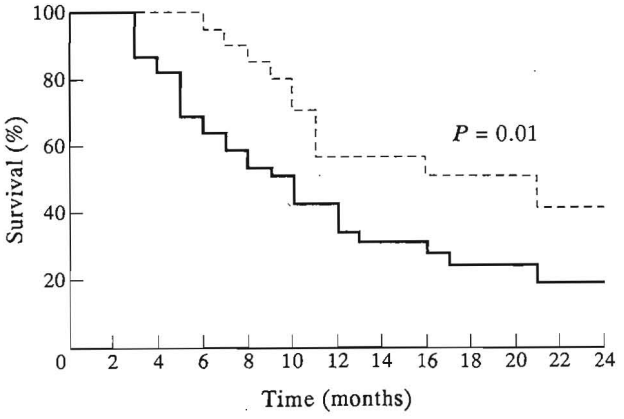

Figure 3. OS according to method of detection of tumour at study entry. – – – 25 patients, tumour not clinically or radiologically detectable; —— 47 patients, tumour clinically or radiologically detectable.

with a further analysis performed at the moment of debulking surgery (i.e. after three cycles of first-line treatment).

The overexpression of p53 was found to be independent of tumour drug sensitivity, whereas laminin receptor was more frequently expressed in platinum-sensitive tumours ($P = 0.03$).

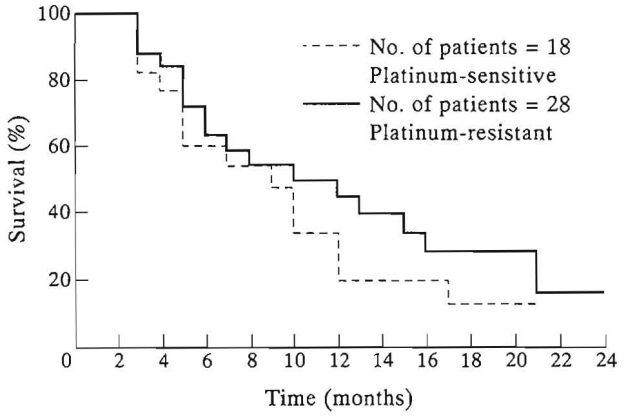

Figure 4. OS of patients with radiologically detectable disease, according to platinum responsiveness.

The modulation of laminim receptor expression also occurred more frequently in platinum-sensitive patients ($P = 0.056$), although the type of modulation did not correlate with platinum responsiveness (from positive to negative in three cases, one platinum-resistant and two platinum-sensitive; from negative to positive in two cases, both platinum-sensitive). The overexpression of c-erbB-2 did not correlate with drug responsiveness, but it is worth noting that an overexpression appeared during treatment only in patients who later showed platinum resistance ($P = 0.05$).

In an attempt to improve prediction of the response to first-line treatment, the combination of baseline expression of laminin receptor and c-erbB-2 modulation was investigated in those patients who were evaluable for both. In 12/14 platinum-resistant patients (86%), either a negative to positive c-erbB-2 modulation or the absence of laminin receptor expression was observed, whereas this phenotype was seen in only 2/9 platinum-sensitive cases (22%).

DISCUSSION

The identification of an effective medical therapy for patients with persistent or relapsing (within 1 year) advanced epithelial ovarian cancer after first-line chemotherapy is problematic [2], although there is a full convergence of opinion concerning the usefulness of retreating all patients who achieve CR after first-line chemotherapy and relapse after 1 year with platinum compounds [25].

The combination of DHAD and IFO in such patients led to a 15% response rate, with a median response duration of 4 months. These data do not support the use of this regimen as second-line therapy, because better results might be obtained by retreating patients with platinum compounds or by administering paclitaxel or docetaxel [26–28]. Nevertheless, some important observations can be made concerning the significant variations in treatment efficacy observed when the results of our study were analysed according to certain factors. These variations support the hypothesis already postulated by other authors of a significant heterogeneity among patients undergoing second-line therapy [7, 8].

The response rate was significantly influenced by tumour platinum responsiveness: 27% of responses were observed in the group of platinum-sensitive versus 4% in the group of platinum-resistant tumours. This difference is especially evident if those patients with highly platinum-sensitive tumours (i.e. complete

Table 4. Correlation of biological parameters with platinum responsiveness

Parameter	Platinum-resistant patients		Platinum-sensitive patients	
	No. positive	No. evaluated (%)	No. positive	No. evaluated (%)
p53 protein	11	17 (65)	5	8 (62)
Laminin receptor	9	23 (39)	10	13 (77)
c-erbB-2 oncoprotein	3	23 (13)	1	13 (8)
c-erbB-2 oncoprotein modulation (neg.→pos.)	5	14 (36)	0	9 (–)
Laminin receptor modulation	1	14 (7)	4	9 (44)

remission after platinum compounds lasting 1 year or more), who were excluded from this trial, are taken into account. The population of platinum-resistant patients is the best for testing new drugs in advanced ovarian cancer.

It is worth noting that all the tumour regressions were observed in patients aged 64 years or less, although it is unclear whether this may be related to the fact that older patients cannot adequately receive the planned treatment or whether there are some biological reasons for the different outcome. In our trial, the treatment dose intensity was not significantly compromised: only 2% of cycles were delayed for more than 2 weeks and drug dosages were reduced by 25% in only 4% of cycles. It, therefore, seems that the worse response rate to second-line therapy in older patients cannot be attributed to any compromised dose intensity of the treatment delivered.

Both TTF and OS were significantly better in patients without any clinically or radiologically detectable disease than in those with detectable lesions, a finding which was confirmed by both univariate and multivariate analysis. It is worth noting that, among the patients with clinically detectable disease, those who were platinum-sensitive had a better response rate than those with platinum-resistant tumours, although no differences were observed in terms of TTF and OS. This suggests that the responses achieved did not have any effect on prolonging the TTF or the survival of treated patients.

It would have been interesting to have evaluated the prognostic role of clear cell histology and the interval elapsing from the end of first-line treatment to the second-line therapy (< or >12 months). The latter has been clearly demonstrated to determine the probability of response to second-line treatment [29]. Unfortunately, clear cell histology was found in only 2 of our 72 patients and, therefore, no analysis of this factor was feasible. With regard to the time to relapse, this factor was not evaluated because in all the relapsing patients, the interval was shorter than 12 months, as stated in the trial eligibility criteria.

The biological parameters did not predict response to the second-line treatment and accordingly, did not seem to have any effect on overall survival. However, as the response rate was very low, the examined series was probably too small to verify the relevance of these factors appropriately. When first-line treatment is considered, two of these parameters (the c-erbB-2 oncoprotein and laminin receptor), determined before or during platinum compound therapy, can predict final platinum responsiveness defined according to clinical criteria. The assessment of p53 using immunohistochemical techniques has not been helpful and this could be related to the fact that this method of measurement does not provide enough data on the functional status of this protein. The increase of c-erbB-2 expression during treatment suggests a selection of c-erbB-2-positive cells that are more drug-resistant. The expression of this oncoprotein has already been reported to be associated with a resistance to platinum therapy [17], whereas the observation of the influence of laminin receptor as a predictor of drug sensitivity is new. Associated with the metastatic potential of cancer cells [15], laminin receptor has been shown to indicate a poor prognosis in breast carcinomas not treated with chemotherapy [19]. The influence of the receptor on drug sensitivity is probably due to the mediated interaction of tumour cells with the extracellular matrix, already suggested as playing a role in conditioning drug sensitivity [30]. The prognostic potential of receptor expression will, therefore, be an increase in metastatic capability [15] balanced by an increase in drug sensitivity. The use of these biological parameters should aid the identification of patients before or during therapy, who are more likely to be responsive.

However, although these data suggest the possibility of predicting platinum responsiveness after first-line therapy, they must be taken as preliminary and need to be verified in a larger patient population. Furthermore, the investigated biological markers would present a problem in items of costs if employed as "standard" tests in the management of ovarian cancer.

In conclusion, this paper suggests that DHAD plus IFO is not an effective second-line treatment for advanced ovarian cancer. Furthermore, it has been observed that the results of second-line therapy can change according to the presence or absence of radiologically detectable disease at the time of starting therapy and the previous response of the tumour to platinum.

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