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High-dose Chemotherapy with Autologous Bone Marrow Transplantation in Patients with Refractory Ovarian Cancer

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Abstract—*Eleven patients with persistent ovarian cancer after remission-induction chemotherapy were treated with high-dose cyclophosphamide and etoposide followed by autologous bone marrow transplantation (ABMT). Six complete responses (CR), of which five were pathologically confirmed, were achieved in eight patients who had microscopic or residual disease* ≤2 cm *at the start of high-dose chemotherapy. The median duration of response was* 15 *months with two sustained CRs after respectively* 43 *and* 75 *months. None of the three patients with residual disease* >2 cm *responded. The median survival measured from the start of the ABMT regimen was for all patients* 23 *months.*

These results suggest that high-dose systemic chemotherapy followed by ABMT is a therapeutic option in patients with refractory ovarian cancer deserving further investigation.

INTRODUCTION

EPITHELIAL OVARIAN CANCER, relapsing after first-line chemotherapy, or not responding to such therapy, is not curable by standard regimens. Even induction of responses after previous therapy with cyclophosphamide and *cis*-platinum by a second-line regimen is only occasionally reported [1–3].

In most tumor types a dose–response relationship has been demonstrated for a number of agents, especially alkylating cytotoxic drugs [4]. For ovarian cancer this has been demonstrated for *cis*-platinum [5, 6]. Although a continuing interest exists for high-dose chemotherapy with autologous bone marrow rescue, its place in the treatment of common solid tumors has not been established.

In a previously reported phase I study using cyclophosphamide and increasing doses of etoposide [7] we saw complete remissions in two patients who were previously unsuccessfully treated with cyclophosphamide, adriamycin and *cis*-platinum. We now report our experience with high-dose chemotherapy and autologous bone marrow transplantation (ABMT) in 11 patients with refractory ovarian cancer.

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MATERIALS AND METHODS

Patients

All patients entering the ABMT program had persistent ovarian cancer, on second look laparotomy, after treatment with conventional doses of remission-induction chemotherapy and cytoreductive surgery. Entry criteria were: age below 60 years, a pretreatment Karnofsky performance status of 80 or more, serum bilirubin levels below 30 μmol/l, serum creatinine below 120 μmol/l and no clinical signs of cardiac failure.

Informed consent was obtained in all patients and the study was approved by the local medical ethical committee.

Bone marrow collection, cryopreservation and reinfusion

Methods of bone marrow collection without general anesthesia and of cryopreservation have been reported previously [8]. In short, bone marrow was recovered from the iliac crest. Patients received a local anaesthetic and 100 mg meperidine and 20 mg diazepam i.m. The marrow was collected in Hanks solution with hepes buffer and heparin, final concentration of heparin 150,000 U/l. The marrow was processed in a Haemonetics model 30 cell separator. The buffy coat was resuspended in Hanks balanced solution to a final concentration of 200×10^6 cells per ml. This solution was dissolved 1:1 in 20% DMSO in autologous plasma. The

marrow was then placed in 5 ml ampoules (Nunc), these were frozen in a Cryoson BV-4 liquid nitrogen controlled freezer at a rate of 1 grade per minute until −40°C. Ampoules were then stored in liquid nitrogen. Reinfusion of marrow was done after rapid thawing and without washing out of the cryoprotectant dimethyl sulfoxide (DMSO). To prevent allergic reactions due to DMSO patients received before marrow infusion prednisolone 60 mg i.v. and clemastine 2 mg i.v.

Chemotherapy

The high-dose chemotherapy infused consisted of cyclophosphamide in a total dose of 7 g/m^2, etoposide total dose 0.9 or 1.0 g/m^2. Both drugs were divided and given over 3 consecutive days. Mesnum was given over the same days to prevent hemorrhagic cystitis in a total dose of 4 g/m^2 i.v. The bone marrow was reinfused on day 7.

Supportive care

All patients received parenteral nutrition alone, or in combination with enteral tube feeding. Prophylactic oral antibiotics and amphotericin B against potential pathogenic intestinal flora were administered. In case of fever >38°C axillary i.v. antibiotics were given after appropriate culturing. A number of patients seropositive for Herpes simplex virus received prophylactically Acyclovir. Cryopreserved (DMSO) autologous platelets or allogeneic single donor platelets were transfused in case of hemorrhagic diathesis or prophylactically at a platelet level below 15 × 10^9/l.

Response and toxicity

To assess therapeutic responses to high-dose chemotherapy, an intraperitoneal inspection with biopsies and peritoneal washing was performed prior to and after the ABMT program. Patients were considered to have microscopic disease when at laparotomy no macroscopic tumor was found, but when biopsies or peritoneal washings revealed tumor cells. Minimal disease was defined as the largest lesion of residual disease after surgery being ≤2 cm. In case of bulky disease the largest lesion was >2 cm after surgery. Response and toxicity were graded according to WHO criteria [9].

The duration of response in months was measured from the onset of high-dose chemotherapy until signs of recurrent disease on physical and gynecological examination or increasing serum levels of CA 125 [10]. Follow-up examination was every 4 weeks and after 1 year at 3 monthly intervals.

The survival was also determined from the onset of high-dose chemotherapy. The cut off date for analysis was 1 August 1988. The survival was analyzed by the life-table method [11].

RESULTS

A total of 11 patients, median age 46 years (range 29–57) with persistent ovarian cancer after conventional doses of chemotherapy and cytoreductive surgery entered the ABMT program. Microscopic disease was present in three patients, minimal disease in five patients and bulky disease in three patients, two of whom had lesions with a diameter of more than 7 cm not amenable for surgical debulking. Bone marrow was collected successfully as soon as possible after the positive second look laparotomy and high-dose chemotherapy was started within 6 weeks after surgery. All patients were evaluable for response and toxicity. Reevaluating laparotomy was performed in seven patients and laparoscopy in three patients. Age, histological tumor type and differentiation, initial surgical staging, previous chemotherapy, response to previous chemotherapy, residual tumor size at the start of high-dose chemotherapy, response, response duration and survival of individual patients are shown in Table 1.

Six out of 11 patients achieved a complete response (CR), pathologically confirmed in five patients (95% confidence level 16.75–76.62). In the sixth patient a reassessment peritoneal inspection was not performed and her clinical CR was evaluated by noninvasive techniques. The median duration of CR was 15 months. Two patients have been in sustained CR for respectively 43 and 75 months. All CRs were attained in patients with minimal or microscopic disease. No response was observed in patients with bulky disease, they died respectively 3, 11 and 14 months after the start of high-dose chemotherapy. Life table survival of all patients is shown in Fig. 1.

Toxicity and complications

The extramedullary toxicity of cyclophosphamide and etoposide consisted of mucositis, nausea, vomiting and diarrhea (Table 2). A polyneuropathy already existing after prior cisplatin treatment wors-

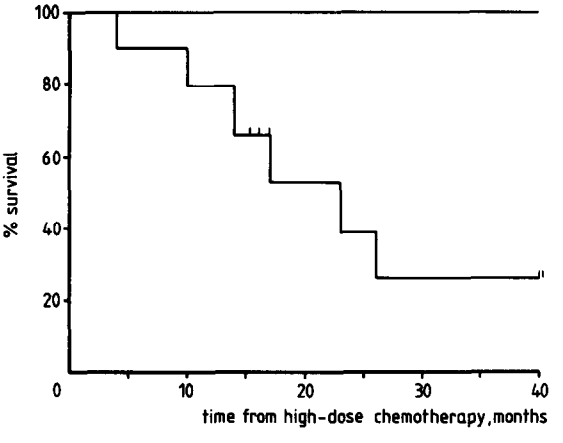

Fig. 1. Survival of 11 patients with refractory ovarian cancer after high-dose cyclophosphamide and etoposide followed by ABMT.

Table 1. Patient characteristics and responses

Age (years)	Histological type	Differentiation	FIGO*	Previous therapy	Response to previous therapy	Residual disease† (cm)	High-dose Cy + etoposide response	Response duration (months)	Survival (months)
29	pca	well	III	Cy, HMM, ADR, CDDP	PD	<2	PCR	75+	75+
42	pca	moderate	III	Cy, ADR, CDDP	PR	<2	CCR	19	26
34	pa	moderate	IV	Cy, HMM, ADR, CDDP	PD	7	PD	—	11
46	pca	moderate	IV	Melphalan	PR	8	PD	—	3
46	pa	poor	IV	Cy, ADR, CDDP	PD	3	PD	—	14
47	pca	poor	IV	Cy, ADR, CDDP	PR	microsc.	PCR	12	22
46	pa	moderate	III	Cy, ADR, CDDP	PR	microsc.	PCR	43+	43+
36	mca	moderate	IIc	Cy, ADR, CDDP	PR	microsc.	PCR	5	8
52	pa	poor	III	Cy, ADR, CDDP	PR	<2	PCR	10	17+
57	mca	moderate	III	Cy, CBDCA	PR	<2	SD	—	16+
47	pa	moderate	III	Cy, VCR, CDDP	PR	<2	SD	—	15+

Abbreviations: pca = papillary cystadenocarcinoma; pa = papillary adenocarcinoma; mca = mucinous cystadenocarcinoma; Cy = cyclophosphamide; HMM = hexamethylmelamine; ADR = Adriamycin®; CDDP = cisplatin; CBDCA = carboplatin; VCR = vincristine; PR = partial response; PCR = pathologically confirmed complete response; CCR = clinically complete response; PD = progressive disease; SD = stable disease; microsc. = microscopic.

*Extent of disease according to the FIGO classification [12].

†Residual disease at start of high-dose chemotherapy.

Table 2. Toxicity

Patient	Mucositis grade	Vomiting grade	Diarrhea grade	Leukocyte $\leq 1.0 \times 10^9$/l (days)	Thrombocytes $\leq 40 \times 10^9$/l (days)
1	3	1	2	9	13
2	2	2	2	10	16
3	1	3	2	19	17
4	1	4	3	12	9
5	2	3	3	16	12
6	3	2	2	18	16
7	3	2	2	22	18
8	2	2	2	16	16
9	2	2	3	17	10
10	1	1	2	15	8
11	2	1	2	18	13

ened in one patient, probably due to etoposide. One patient with bulky disease had transient gross hematuria possibly because of hemorrhagic cystitis. No congestive heart failure occurred. Autologous bone marrow infusion was complicated once by cardiopulmonary arrest possibly mediated by DMSO, despite prior treatment with prednisolone and clemastine. Resuscitation was successful.

Grade 4 hematologic toxicity occurred in all patients (Table 2). The mean number of days with leukopenia $<1.0 \times 10^9$/l was 16 (range 9–22) and with thrombopenia $<40 \times 10^9$/l was 13 (range 8–18). Bleeding episodes occurred in two patients as hematuria and gastrointestinal bleeding. Septicemia was seen in four of the 11 patients and was caused by *Staphylococcus epidermidis* in three cases and *Klebsiella pneumoniae* in one. Three patients without Acyclovir prophylaxis had reactivated Herpes simplex virus infection. The mean duration of hospitalization was 23 days (range 19–29). All patients were ambulant when they left the hospital. One patient had a primary cytomegalo virus infection a few months after ABMT.

DISCUSSION

Standard dose systemic chemotherapy after failure of a primary attempt at remission-induction for advanced epithelial ovarian cancer is hardly ever followed by a substantial number of responses and never by long-term disease-free survival [1, 3]. Ovarian cancer, however, is a tumor with an established relation between dose intensity and response rate [5, 6]. On this premise we studied the potential of a salvage regimen of a combination of drugs in high doses with the use of ABMT.

Cyclophosphamide, the alkylating agent in the regimen, has a long history in the treatment of ovarian cancer [13]. High-dose cyclophosphamide as single-agent treatment is mainly myelotoxic. The dose limit for cardiac toxicity is reached at 7 g/m^2 [14] and urothelial toxicity can be prevented by prophylaxis with mesnum [15].

The second drug, etoposide, is an active agent in a number of solid tumors [16]. No clear consensus is presently available on the activity of etoposide in epithelial ovarian tumors [17]. It is therefore of interest that recently a phase II study of etoposide as single agent in advanced ovarian cancer with primary or secondary resistance to high-dose cisplatin containing regimens was reported [18].

In 66 patients treated with etoposide 150–200 mg/m^2/d × 3 i.v. every 4 weeks, eight CRs and 19 PRs were attained. Less favorable results were observed by Lele *et al.* [19]. These investigators treated 25 patients with FIGO stage III and IV ovarian carcinoma with etoposide 100 mg/m^2/d × 3 or 150 mg/m^2/d × 2 every 4 weeks in combination with cisplatin 50 mg/m^2 on day 1. Two patients achieved a PR, 18 had no change and five showed progressive disease. De Lena *et al.* treated 25 patients with bulky disease who failed prior treatment with cyclophosphamide and doxorubicin with a second-line regimen with 3-week intervals consisting of etoposide 100 mg/m^2/d × 3 orally, in combination with cisplatin 100 mg/m^2 on day 1. This resulted in one CR and nine PRs [20].

It is possible that higher doses of etoposide, as used in our regimen, have a higher response rate than the normal dose in the regimens cited above, as Reichman *et al.* found superior activity of its application as an intraperitoneal agent [21], a method that results in a high local drug concentration. The small number of patients entered into our study limits the conclusions that can be extracted. However, the response rate of 55% with two long-term disease-free survivors compares favorably to other forms of systemic second-line chemotherapy. The toxicity of the regimen was acceptable.

Although no treatment related deaths occurred in this study, nor in patients with breast cancer or germ cell cancer treated with the same regimen of high-dose cyclophosphamide and etoposide [22, 23], the severe bone marrow aplasia limits its applicability to relatively young patients as selected for this study with a good organ function and performance score. Such patients are limited in number because ovarian cancer occurs in an older age group.

Also, unless the intensive chemotherapy procedure could be repeated, which would substantially increase its toxicity, all clinical advantage should result from one single course of treatment. This would indicate that especially patients with a limited tumor burden can benefit from this treatment. In agreement, in our study the patients with tumor size less than 2 cm seemed to have the best results.

We conclude that high-dose chemotherapy and ABMT deserves further investigation as a therapeutic option in selected patients with refractory ovarian cancer.

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