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Chemotherapy with Maximally Tolerable Doses of VP 16–213 and Cyclophosphamide Followed by Autologous Bone Marrow Transplantation for the Treatment of Relapsed or Refractory Germ Cell Tumors

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Abstract—*Eleven patients with advanced nonseminomatous germ cell tumors (NSGCT), who relapsed after or were refractory to standard dose cisplatin-based remission induction chemotherapy, were treated in a phase II clinical trial with VP* 16–213 2500 mg/m^2 *and cyclophosphamide* 7 g/m^2. *Both drugs were given in maximally tolerable doses regarding extramedullary toxicity. Urothelial damage due to cyclophosphamide was prevented by the administration of mesnum. Autologous bone marrow was infused on day* 7 *to prevent long lasting medullary toxicity. Because of the disappointing results in the first three patients, a second treatment step was added. The next eight patients were treated with* 2500 mg/m^2 *VP* 16–213 *divided and given on days* 1–2–3 *and after full bone marrow recovery with total doses of VP* 16–213 2000 mg/m^2 *plus cyclophosphamide* 7 g/m^2 *divided and given on days* 29–30–31, *followed by autologous bone marrow transplantation (ABMT) on day* 35. *Toxicity to high-dose VP* 16–213 *plus cyclophosphamide followed by ABMT consisted of mucositis, nausea, vomiting and diarrhea. No cardiac toxicity or hemorrhagic cystitis occurred. The mean duration of leukopenia and thrombopenia was* 14 *and* 13 *days respectively. The additional, preceding treatment with VP* 16–213 *as a single agent caused mucositis, and leukopenia and thrombopenia for a mean number of* 9 *and* 6 *days respectively. Seven responses were obtained: two complete responses of* 46 *and* 66+ *weeks respectively and five partial responses with a median response duration of* 12 *weeks. The median survival time was* 40 *weeks. This regimen of one or two courses with maximally tolerable doses of VP* 16-213 *plus cyclophosphamide and ABMT is not sufficient to salvage a substantial number of patients with relapsing or refractory NSGCT.*

INTRODUCTION

THE PROGNOSIS of patients with disseminated nonseminomatous germ cell tumors (NSGCT) has been remarkably improved by cisplatin-based combination chemotherapy regimens. The chance of reaching a complete remission (CR) on standard dose remission induction chemotherapy with cisplatin plus bleomycin plus either vinblastine (PVB) or VP 16–213 (BEP) is 70% while an additional number of patients can achieve a disease free status with chemotherapy plus surgical resection of residual tumor [1, 2]. Patients who relapse are not expected to have long-term survival with reinduction regimens. The prognosis of patients who reach only a partial remission or less after conventional induction chemotherapy is also dismal.

Other therapeutic approaches are being investigated for relapsing or refractory NSGCT. Available phase II data indicate that the epipodophyllotoxin VP 16–213 is an active drug in germinal neoplasms [3] and an important component of salvage regimens [4]. Also cyclophosphamide has been con-

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Table 1. Patient characteristics and results

Patient No.	Age (years)	Serum level at diagnosis (ng/ml)		Prior treatment	Initial CR duration (months)	Extent of disease at presentation for ABMT program*		Response to ABMT program	Response duration (weeks)	Survival (weeks)
1	35	HCG	1668	4PVB,P,VP-16,A	N	IV		SD	—	30
2	19	HCG	2505	4PVB+surgery	N	III		SD	—	47
3	40	AFP	1600	4PVB,1PV,Do	N	IV		PR	6	32
4	22	AFP	12,980	4PVB	2	IIc		PR	21	60
		HCG	1505							
5	29	HCG	10,000	4PVB+surgery	N	IV		PR	10	33
6	27	—		4BEP	8	IIc		CR	46	59
7	49	AFP	24,497	4PVB+1PV	4	IV		SD	—	72+
8	40	—		4PVB	11	IV	primary extragonadal	PR	12	20
9	28	HCG	12	4PVB	15	IIc		PR	22	84
10	24	HCG	901,400	2BEP,2PVB,P, VP-16,Do	N	IV		CR	66+	66+
11	26	HCG	600,000	4PVB,1BEP,P, VP-16,Do	N	IV		SD	—	14

Abbreviations: AFP = α-fetoprotein, normal value; <16 ng/ml; HCG = β-human chorionic gonadotrophin, normal value: <2 ng/ml; CR = complete remission; PR = partial remission; SD = stable disease; N = none; P = platinum; V = vinblastine; B = bleomycin; E and VP-16 = etoposide; Do = doxorubicin; A = actinomycin D.

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

sidered to be of value in the treatment of metastatic testicular neoplasms [5]. The response rate of NSGCT increases with intensification of chemotherapy [6] but high-dose chemotherapy may cause severe toxicity. VP 16-213 and cyclophosphamide are suitable for considerable dose escalation until dose-limiting extramedullary toxicity ensues. Due to oropharyngeal mucositis the highest dose of VP 16-213 that can be given is 3500 mg/m^2 as a single agent [7] and 2500 mg/m^2 in combination with cyclophosphamide [8]. The dose limit of cyclophosphamide for cardiac toxicity is 7 g/m^2 [9]. Urothelial damage can be prevented by administration of protective agents such as mesnum [10]. As single agents VP 16–213 and cyclophosphamide in maximally tolerable doses are not absolutely marrow ablative, but when used in combination in pretreated patients they most probably are.

Based on these considerations we performed a phase II study with a combination of high-dose VP 16-213 and cyclophosphamide, in doses limited by extramedullary toxicity, for the treatment of relapsing or refractory NSGCT. Autologous bone marrow was reinfused to prevent long lasting aplasia.

Preliminary results of some of these patients have been reported earlier [11].

MATERIALS AND METHODS

Patients

From June 1982 to December 1985 11 male patients with disseminated NSGCT were included in this study. Patient characteristics are given in Table 1.

Nine patients had initially received PVB, consisting of courses with cisplatin 20 mg/m^2/d × 5, vinblastine 0.15 mg/kg body wt on days 1 and 2, and bleomycin 30 mg on days 2, 9 and 16. Two patients had received BEP, consisting of bleomycin 30 mg on days 2, 9 and 16, etoposide 120 mg/m^2 on days 1, 3 and 5, and cisplatin 20 mg/m^2/d × 5. Courses were repeated every 3 weeks four times.

On this initial therapy five patients reached a complete remission but relapsed after a median response duration of 8 months (range 2–15 months). Six patients had not reached a complete remission. Patient 1, 3, 10 and 11 (Table 1) showed regression of evaluable tumor and a decrease in tumor marker level, but normal values at the end of remission-induction chemotherapy were not reached. These patients received second line chemotherapy, containing standard dosages of cisplatin, VP 16-213 plus doxorubicin or actinomycin D. Patients 1 and 3 had progressive disease and patients 10 and 11 had reached no complete remission. Patients 2 and 5 also showed regression of evaluable tumor and normalization of elevated marker levels. Because of residual disease, surgery was performed in both. In patient 2 mature teratoma was found. However, 2 weeks after surgery HCG levels increased. In patient 5 vital tumor was found in a resected pulmonary metastasis.

At the start of high-dose chemotherapy patients were classified according to the Peckham staging system [12] into stage IIc (three patients), III (one patient) and IV (seven patients). The poor prognosis is expressed by bulky metastatic disease and/or initial high serum levels of tumor markers [13]. Two

patients had no initial marker elevation (Table 1).

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

Chemotherapy

Patients 1–3 received infusions of cyclophosphamide in a total dose of 7 g/m^2 and VP 16-213 in a total dose of 2500 mg/m^2, divided and given over 3 consecutive days. Mesnum was given intravenously in a total dose of 4 g/m^2 to prevent hemorrhagic cystitis. On day 7 autologous bone marrow was reinfused. Based on the disappointing results of these patients a second treatment stage was added in the next eight patients. Patients 4–11 received a total dose of 2500 mg/m^2 VP 16-213 divided over 3 consecutive days plus, divided over days 29, 30 and 31, a total dose of VP 16-213 2000 mg/m^2 and cyclophosphamide in a total dose of 7 g/m^2, followed by ABMT on day 35.

Supportive care

Patients were treated in a single bedroom. Selective decontamination of the digestive tract [14] with oral antibiotics and oral amphotericin B was administered to prevent gram negative or mycotic infection. Patients were fed by parenteral nutrition alone or in combination with enteral tube feeding. Platelets were transfused when bleeding was present or prophylactically if the thrombocyte count was below 15 × 10^9/l. A number of patients received autologous platelets collected and cryopreserved prior to high-dose chemotherapy, otherwise allogeneic platelets were used. In case of fever above 38°C axillary intravenous antibiotics were administered after blood sampling for microbial culture.

Bone marrow collection, cryopreservation and reinfusion

The technique of bone marrow collection without general anaesthesia and of cryopreservation has been reported previously [8]. Before marrow reinfusion patients received prednisolone 60 mg i.v.and clemastine 2 mg i.v. Reinfusion was done after rapid thawing without washing out of cryoprotectant over a standard blood filter into a central venous (Hickman) catheter or into a needle inserted in an arteriovenous fistula.

Response and toxicity

A complete response (CR) was defined as complete disappearance of all objective parameters, determined by two observations not less than 4 weeks apart. Elevated levels of beta human chorionic gonadotrophin (HCG) or alpha fetoprotein (AFP) should be normal in separate blood tests not less than 4 weeks apart. A partial response (PR) was defined as at least a 50% reduction of the sum of the products of the two largest perpendicular diameters of all measurable lesions plus a 90% or greater reduction in the pretreatment level of HCG or AFP over a period of at least 2 weeks. Stable disease (SD) was defined as a less than 50% response without signs of progression and a decrease of less than 90% or an increase less than 25% of the pretreatment level of biochemical markers. Response duration was measured from the first day of treatment until progression of the tumor or rise of serum markers. Survival time was measured from the first day of treatment in the ABMT program until death.

Drug toxicity was graded according WHO criteria [15]. Special attention was paid to mucositis, vomiting, diarrhea, hemorrhagic cystitis and congestive heart failure.

RESULTS

There were no treatment related deaths and all patients were evaluable for response and toxicity. Responses, response duration and survival of individual patients are shown in Table 1. Seven patients responded to treatment, three in the group with refractory disease and four in the group with relapsing disease. Two patients achieved a CR of 46 weeks and 66+ weeks respectively. Five patients achieved a PR, with two PRs based on tumor markers only, with a median response duration of 12 weeks (range 6–22 weeks). The four patients who did not respond had shown refractory disease to first line therapy (three patients) and also to second line therapy (two patients). They reached at best a transient period of stable disease from 2 to 14 weeks. Two patients remain alive, one still in CR for 66 weeks and one for 72 weeks with slowly progressive disease after a short period of SD. The median survival time was 40 weeks.

Toxicity

Extramedullary toxicity of VP 16-213 plus cyclophosphamide consisted of mucositis grade 1 in one patient, grade 2 in eight patients and grade 3 in two patients. Gastrointestinal toxicity included nausea, vomiting and diarrhea. Two patients had vomiting grade 3 and nine had grade 2. Also diarrhea grade 3 was seen in two patients, the others had grade 2. No congestive heart failure or hemorrhagic cystitis occurred.

In all patients hematologic toxicity grade 4 occurred. In the treatment stage with high-dose cyclophosphamide plus VP 16-213 followed by ABMT the mean number of days with leukopenia $<1.0 \times 10^9$/l was 14 (range 8–20) and with thrombopenia $<40 \times 10^9$/l was 13 (range 5–18). No bleeding episodes occurred. Febrile days were scored in every patient with a mean number of nine (range 5–13). Septicemia occurred in six of the 11 patients (54%) and was caused by *Staphylococcus*

epidermidis and viridans streptococci. No gram negative bacterial or fungal infection was seen. Three patients had a reactivation of herpes simplex virus infection.

The additional, preceding treatment with VP 16-213 as a single agent caused mucositis grade 2 in all patients and leucopenia and thrombopenia for a mean number of 9 (range 6–18) and 6 days (range 3–10) respectively.

DISCUSSION

The aim of this study was to achieve cure through durable CRs in patients with relapsing or refractory NSGCT. Patients who fail to be cured on standard dose chemotherapy may respond to massive dose treatment. An important dose–response relationship with VP 16-213 in refractory germ cell tumors was suggested by Wolff *et al.* who reported in 10 evaluable cisplatin refractory patients two CRs and four PRs after high-dose VP 16-213 monotherapy with two cycles of 2400 mg/m^2 with ABMT every 3–4 weeks, followed by 1200 mg/m^2 without marrow infusion. They observed also responses in two patients who had failed standard VP 16-213 dose [16].

A combination regimen with VP 16-213 600 mg/m^2 and cyclophosphamide 4.5 g/m^2 plus ABMT in 10 evaluable patients (nine with refractory and one with relapsing disease) resulted in four CRs and three PRs with a median duration of 15 weeks [17]. Other reports on high-dose VP 16-213 combination chemotherapy followed by ABMT described 13 responses (eight CRs and five PRs) in 15 evaluable patients treated with VP 16-213 350 mg/m^2/d × 5, cisplatin 40 mg/m^2/d × 5, and cyclophosphamide 1.4 g/m^2/d × 4 [18].

The disappointing results of the first three patients treated with one course of high-dose VP 16-213 and cyclophosphamide plus ABMT in this study could partly be explained by refractoriness of the disease to cisplatin based induction chemotherapy. Patients with refractory NSGCT are reported to have a lower response rate on reinduction or salvage therapy than patients who relapse after an initial CR [19, 20]. However none of our first three patients had ever received treatment with VP 16-213 or cyclophosphamide and their tumors were therefore not expected to be refractory to these agents.

In order to improve on the therapeutic efficacy of the regimen a course of high-dose VP 16-213 as single agent was inserted before the course of cyclophosphamide with VP 16-213 and ABMT. This regimen was active as indicated by a response rate of 75% but failed the primary aim of the study, to induce cure, as only two of the eight patients achieved a CR.

Our treatment results are not superior to the results of other salvage treatments [21] and are in agreement with the reports that high-dose cyclophosphamide, although active in non-pretreated germ cell tumors (GCT) [5], does not produce long standing responses in patients who failed to be cured on cisplatin based induction therapy.

The toxicity of the regimen was considerable but could be managed without overwhelming problems. There were no treatment related deaths. The questions whether the reinfused autologous bone marrow added to the speed of recovery is difficult to answer. We feel that, as our harvesting procedure does not require general anesthesia and is a limited burden to the patient, it would hardly be ethical to omit ABMT.

From our clinical trial it is clear that one or two courses of maximally tolerable chemotherapy with VP 16-213 and cyclophosphamide are not sufficient to salvage a substantial number of these patients. Multiple courses of chemotherapy, closely spaced, are probably needed. New active agents should be applied in these courses. Carboplatin (CBDCA), an analog of cisplatin, is active in patients with GCT no longer responsive to cisplatin [22] and ifosfamide, a cyclophosphamide analog, also demonstrates activity in such patients [23]. Both drugs are in particular bone marrow toxic and can probably be used in a marrow ablative regimen aimed at increasing CR rates of relapsing or refractory GCT.

Besides salvage treatment, another primary approach could be the use of these marrow ablative regimens, in the treatment of patients initially presenting with bad prognostic characteristics [13], as up front chemotherapy or as late intensification after standard remission induction chemotherapy.

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