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Long-term Survivors after Salvage High Dose Chemotherapy with Bone Marrow Rescue in Refractory Germ Cell Cancer

J.P. Droz, J.L. Pico, M. Ghosn, A. Gouyette, D. Baume, G. Piot, M. Ostronoff, C. Theodore, F. Beaujean and M. Hayat

Between April 1984 and May 1985, 17 heavily pretreated patients with relapsing or refractory germ cell tumours were treated with cisplatin 40 mg/m²/day, days 1–5; etoposide 350 mg/m²/day, days 1–5; cyclophosphamide 1600 mg/m²/day, days 2–5 and autologous bone marrow transplantation on day 8 as consolidation of conventional salvage chemotherapy. None of the 11 refractory patients and 4 of the 6 responders to prior salvage treatment are long-term survivors at 68, 72, 74 and 74 months. Mean aplasia duration was 17 days and there were 7 documented episodes of septicaemia in 17 febrile patients. 1 patient died of treatment. Among the 4 survivors, 2 patients have a sustained grade II invalidating neuropathy. We conclude that this regimen is not recommended as salvage therapy in refractory patients but may be a useful consolidation treatment in patients responding to conventional salvage chemotherapy.
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INTRODUCTION

THE PROGNOSIS of advanced stage non-seminomatous germ cell tumours has been greatly improved by the introduction of cisplatin in chemotherapeutic regimens. Nevertheless in approximatively 20% [1] of the whole population and 40–50% of poor risk patients [1, 2] a complete remission cannot be obtained. Simultaneously 10% of those who achieve a primary complete remission relapse [3]. Several salvage conventional chemotherapy regimens have been previously reported with objective responses in up to 40% [4, 5].

In order to achieve long-term complete remission in relapsed and refractory patients we developed a high dose chemotherapy regimen followed by autologous bone marrow transplantation (ABMT) as part of their salvage treatment. Preliminary studies

Correspondence to J.P. Droz.
J.P. Droz, J.L. Pico, M. Ghosn, D. Baume, G. Piot, M. Ostronoff, C. Theodore and M. Hayat are at the Department of Medicine, and A. Gouyette is at the Department of Pharmacology, Institut Gustave-Roussy, 94800 Villejuif; and F. Beaujean is at the Centre de Transfusion Sanguine, Hôpital Henri-Mondor, Créteil, France.
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Table 1. Patients' characteristics

No. of patients	17
Median age (range)	27 (16–43)
Sex: male/female	17/0
Primary site	
Testis	16
Abdomen	1
Previous treatment	
Number of drugs ≥ 4	17
Mean cisplatin dose (range)	945 mg/m² (440–1510)
Mean bleomycin dose (range)	175 mg/m² (60–250)
Mean etoposide dose (range)	2150 mg/m² (750–4050)
Mean delay between first treatment and PEC + ABMT (range, in months)	14.7 (6–36)
Performance status	
0–1	17
Sites of metastasis	
Liver	7
Lung	6
Abdominal mass	6
Gastric involvement	1
Brain	0
Markers	
HCG positive/negative	13/4
AFP positive/negative	7/10
None	2

PEC = high dose etoposide, cyclophosphamide and cisplatin, ABMT = autologous bone marrow transplantation, HCG = human chorionic gonadotropin, AFP = alphafetoprotein.

were performed in a phase I–phase II trial using high dose etoposide (1750 mg/m²) and a combination of etoposide (1750 mg/m²) and high dose cisplatin (200 mg/m²) [6]. We therefore developed a combination regimen including etoposide, cyclophosphamide and cisplatin (PEC).

PATIENTS AND METHODS

Patients' characteristics

Between April 1984 and May 1985 17 poor risk patients with relapsing or refractory non-seminomatous germ cell tumours were entered in a phase II trial of high dose chemotherapy followed by ABMT as consolidation treatment in salvage chemotherapy. Patients' characteristics are listed in Table 1. The human chorionic gonadotropin (HCG) and alphafetoprotein (AFP) levels were measured by radioimmunoassay. Normal values were HCG 10 mIU/ml (1 ng/l = 6.6 mIU/ml) and AFP 30 ng/ml.

The patient work-up included a complete physical examination, a useful blood chemistry (serum electrolytes, blood urea nitrogen (BUN), serum creatinine, liver enzymes, alkaline phosphatase, glutamyl transferase) and a complete blood count. Metastatic disease was evaluated by plain chest X-ray, chest tomography or computed tomography (CT) of the lungs and CT of the abdomen. All patients have been previously heavily treated with at least one first-line regimen and one or two salvage treatments. First-line regimen included always a conventional dose of cisplatin (100–120 mg/m²). In 13 patients high dose chemotherapy with ABMT was a component of their first salvage chemotherapy which included etoposide with cisplatin at either a conventional or high dose (40 mg/m²/day for 5 days). In 4 patients high dose chemotherapy with ABMT was a component of their second salvage chemotherapy which included high dose etoposide (350 mg/m²/day for 5 days) followed by ABMT in 3 patients and high dose etoposide (same dose), plus bleomycin and high dose cisplatin followed by ABMT in 1 patient.

Table 2. Patients' status before high dose chemotherapy and response

Disease status	Refractory	Maximum PR	CR
Total = 17	11	4	2
Response	NE = 1		
	PD = 2		
	PR = 5		
	CR = 2	CR = 3	CR = 2
	pCR = 1	pCR = 1	
Follow-up	3 relapses	2 relapses	
	2, 4, 8 mo	3–12 mo	
		CR = 74+	CR = 74+
		68+	72+

CR = complete response, pCR = postsurgical complete response, PR = partial response, PEC = high dose etoposide plus cyclophosphamide plus cisplatin, ABMT = autologous bone marrow transplantation, NE = not evaluable.

Treatment

Bone marrow aspiration from the anterior and posterior iliac crests as well as from the sternum was performed, under general anaesthesia, one week before the beginning of the chemotherapy and one month after the last cycle of the conventional regimen. A minimum of 1.10^8 mononuclear cells/kg were obtained. It is worth noting that none of patients had bone marrow involvement [7]. The material was stored under cryopreservation. The chemotherapy regimen was: cisplatin 40 mg/m²/day, days 1–5, etoposide 350 mg/m²/day, days 1–5, cyclophosphamide 1600 mg/m²/day, days 2–5 and mesna 350 mg/m² intravenous push every 4 h, days 2–5. Bone marrow was reinfused on day 8. All patients received vigorous hyperhydration in accordance with Ozols' recommendations [8]. All patients were admitted to the Bone Marrow Transplantation Unit at Institut Gustave-Roussy. Selective decontamination of the gut as well as complete parenteral support were performed. Blood components were irradiated in order to avoid posttransfusional graft versus host (GVH) syndrome.

Assessment of results

Toxicities and responses were coded according to the WHO criteria [9]. Response criteria were as follows. Complete response (CR): disappearance of all evidence of tumour including normalisation of HCG and AFP for at least one month. Partial response (PR): more than 50% reduction in the sum of the products of the largest diameter and its perpendicular for measurable lesions and more than 90% reduction of elevated tumour markers for at least one month with no evidence of progression in any site. Stable and progressive diseases were defined according to classical criteria. When patients achieved a partial response with normal serum markers, the surgical excision of residual disease was indicated. Patients with mature teratoma and/or fibrosis and/or necrosis at histological examination were considered as postsurgical CR (pCR). The response duration was measured from the first day of the high dose chemotherapy to the day of the last follow-up or the day of recurrence. Among the 17 patients two subgroups were defined: refractory patients and responders. The assessment was done after the first or second salvage chemotherapy and during the month preceding the

Table 3. Characteristics of long-survivors

Patient no.	Prior therapy	Cumulative dose (cisplatin)	Status of disease	Extent of disease	Markers AFP/HCG
1	VAB-4, BEhP	950 mg/m²	PR	Liver	N/N
2	VAB-6, EhP	745 mg/m²	PR	0	52/N
3	VAB-4, EP	660 mg/m²	CR	0	N/N
4	VAB-4, BEhP	1000 mg/m²	CR	0	N/N

VAB-4 = cisplatin, bleomycin, doxorubicin, cyclophosphamide and vinblastine, BEhP = bleomycin, etoposide and high dose cisplatin, EhP = etoposide plus high dose cisplatin, EP = etoposide plus cisplatin.
AFP = normal (N) ≤ 30 ng/ml, HCG = normal (N) ≤ 10 mIU/ml.

high dose chemotherapy followed by ABMT. Responders were patients in complete remission or partial response with a 90% decreased marker level. Refractory patients were patients in whom tumour marker levels increased with or without increase of tumour size. There were 11 refractory patients and 6 responders (4 PR and 2 CR).

RESULTS

Response

17 patients were evaluable for toxicity. Only 14 were evaluable for response: 1 died during treatment and 2 responders were in CR before intensive chemotherapy with ABMT. 7 had a complete remission (50%; 95% confidence interval 23–77%) including 2 pCR, 5 a partial remission and 2 patients failed to respond.

3 of the 11 refractory patients achieved a CR and then rapidly relapsed at 2, 4 and 8 months. 4 of the 6 responders are still in sustained CR at 68, 72, 74 and 74 months (Table 2). Detailed characteristics of these long-term survivors are shown in Table 3.

Toxicity

Table 4 shows the major toxicities encountered. Nausea and vomiting were constant. Aplasia was constant and lasted for a mean duration of 17 days (12–26 days). Septicaemia occured in 6 patients, fever of unknown origin in 11. Reversible hyponatraemia revealing Schwartz–Bartter syndrome was observed in 2 patients. A transient increase in the serum creatinine level was noted in 3 patients. Grade 3 and 4 mucositis and stomatitis were observed in all patients and recovered rapidly after the end of aplasia. 1 patient died of toxicity 20 days after ABMT because of severe gastric haemorrhage. This patient had a gastric localisation of his tumour. Potential long-term toxicities in the 4 long-term survivor patients have been evaluated after 5 years and are reported in Table 5.

Table 4. Major toxicities

Toxicity	No. of patients
Nausea and vomiting	17
Myelosupression	
Mean duration of aplasia* (range)	17.8 days (12–26)
Infection	
FUO	11
Staphylococcus	Septicaemia 3
Streptococcus†	Septicaemia 2
Listeria	Septicaemia 1
Candida†	Septicaemia 1
Stomatitis (WHO classification)	
Grade 1–2	1
Grade 3–4	16
Renal function	
Non-modified	14
Reversible rise in serum creatinine level (3 × normal)	3

*Aplasia defined as granulocytes 500/mm³ and/or platelets 20 000/mm³
†One patient with severe stomatitis had documented septicaemia with streptococcus and candida.
FUO = fever of unknown origin.

DISCUSSION

The initial objective of this protocol was to offer to a subgroup of poor risk relapsing or refractory patients an intensification of salvage chemotherapy in order to increase the rate of long-term survival.

In the early 1980s cisplatin, bleomycin, cyclophosphamide, actinomycin D, vinblastine (VAB-6); cisplatin, vinblastine, bleomycin [10]; and PVB [11] were the conventional first-line protocols. Etoposide [12] and high dose cisplatin [13] had already achieved an acceptable remission rate in salvage chemotherapy. A dose effect had also been identified at that time for etoposide [14], cisplatin [13], cyclophosphamide [15] and for some combination chemotherapeutic regimens [16]. The dose of etoposide and cyclophosphamide used in our protocol was inspired by Postmus' studies [17]. He had reported that high dose cyclophosphamide (7 g/m²) can be combined with etoposide (1.5 g/m²) without major extramedullary toxicity. We therefore performed a preliminary phase I trial using high dose etoposide (1750 mg/m²), then a combination of high dose etoposide, bleomycin and high dose cisplatin[6]. The pharmacokinetic results concluded that active metabolites of etoposide were still present 48 h after the last injection of the drug. This prompted us to delay ABMT for 72 h after the end of the treatment [18]. Meanwhile, ifosfamide in combination with etoposide

Table 5. Long-term toxicities

Patient	Age	Professional activity	Neuropathy	Renal function	Audition	Liver function	Sexuality	Other
1	37	No	Grade II	N	Hypoacousia	Grade I	Impotence	Meningitis (listeria)
2	26	Yes	0	N	N	N	N	–
3	33	Yes	0	N	N	N	N	–
4	48	No	Grade II	N	Hypoacousia	Grade III	N	Herpes zoster infection

and conventional cisplatin was being investigated as salvage treatment [19].

Loehrer *et al.* [4] reported 9 relapsing patients out of 16 achieving a CR after VIP (etoposide, ifosfamide, cisplatin) salvage chemotherapy. This prompted us to try to investigate high dose treatment even in patients in CR.

The results of our protocol as salvage treatment, however, did not appear to be superior to the standard VIP regimen [4]. This observation led us to switch to another salvage chemotherapy with etoposide, ifosfamide and high dose cisplatin (VIhP) [20].

Immediate toxicities of high dose chemotherapy with ABMT were acceptable. Major long-term toxicities were only seen in the two patients who had received a very high cumulative dose of cisplatin (1000 mg/m^2) and other drugs. As long-term survivors were only seen among patients with a maximum response, we decided to initiate a protocol with early consolidation of response [21] in poor risk patients [22].

High dose chemotherapy with ABMT was investigated in refractory germ cell tumours during the late 1980s and the results of three trials have been reported. In two of them only short-term complete remissions were observed in 2 out of 7 patients [23] and 3 out of 16 other patients [24]. In the third study 4 CR were observed among 22 refractory patients treated by high dose etoposide and carboplatin, two of whom have non evolutive disease at 10 and 21 months [25]. Other investigators have confirmed that a sustained CR could be obtained in previous responders: respectively 3 out of 4, 6 out of 8 and 7 out of 10 patients [26–28].

As far as a recent randomised trial has shown there is no advantage with the use of a high dose cisplatin regimen in poor risk germ cell tumours [29]. The role of early intensification has to be reappraised. A trial at Memorial in New York is investigating the role of high dose carboplatin, etoposide and cyclophosphamide in refractory patients who failed to respond to the VAB-6 regimen [30]. We are embarked on a randomised trial comparing intensive treatment without ABMT [31] to two cycles of the same regimen followed by early intensification with PEC plus ABMT [21]. In this trial responders and non-responders are submitted to the same regimen.

In conclusion intensive chemotherapy with ABMT cannot be considered as standard salvage treatment. It may be a useful consolidation treatment in salvage only in those patients responding to conventional chemotherapy.

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Serological and Molecular Evidence of Infection by Human T-cell Lymphotropic Virus Type II in Italian Drug Addicts by Use of Synthetic Peptides and Polymerase Chain Reaction

Anita De Rossi, Fabrizio Mammano, Annarosa Del Mistro and Luigi Chieco-Bianchi

Infection with human T lymphotropic virus type I (HTLV-I) is associated with specific forms of tumours and neurological disorders, but the pathogenic activity of HTLV-II is not yet established. Moreover, due to high crossreactivity between the two viruses, differential diagnosis is not readily achieved. To discriminate between HTLV-I and HTLV-II infections, we employed synthetic peptides specific for HTLV-I and HTLV-II env regions, and the polymerase chain reaction (PCR). In a series of 962 intravenous drug addicts (IVDAs) and 50 patients with haematological malignancies, 51 and 2 samples, respectively, were reactive against HTLV-I proteins; among these, HTLV-I infection was confirmed only in 1 patient with adult T-cell lymphoma, while HTLV-II infections were identified in 6 out of 14 PCR-tested IVDAs. These findings provide evidence of HTLV-II infection among Italian IVDAs. The differentiation between HTLV-I and HTLV-II infections may contribute to a better understanding of HTLV-II pathogenicity in man.
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INTRODUCTION

HUMAN T-CELL lymphotropic viruses type I (HTLV-I) and II (HTLV-II) have been associated with specific forms of malignancy in man. HTLV-I, the aetiological agent of adult T-cell leukaemia/lymphoma [1, 2], is also related to neurological disorders, known as tropical spastic paraparesis or HTLV-I-associated myelopathy [3, 4]. HTLV-II has been isolated in rare cases, including subjects with a T-cell variant of hairy cell leukaemia [5], but a conclusive association with this or other diseases has not yet been established.

Besides the endemic areas [6, 7], antibodies reactive to HTLV-I antigens have also been found world-wide in subjects at risk for AIDS, such as intravenous drug addicts (IVDAs), homosexuals and haemophiliacs [8–12]. In this regard, we previously found that 4–5% of HIV-1 seropositive IVDAs living in north-eastern Italy were also seroreactive for HTLV-I [13, 14].

However, antibody crossreactivity between HTLV-I and HTLV-II, due to the high level of genomic and aminoacid sequence homology between the two, makes it difficult to differentiate one infection from the other on the basis of standard serological tests [15]. To this end, molecular methods might be more appropriate, and the polymerase chain reaction (PCR), which specifically amplifies short DNA sequences [16], has recently been applied to discriminate between HTLV-I and HTLV-II infection [17, 18]. This differential diagnosis is important for epidemiological and public health studies, as well as prospective clinical analysis of HTLV-infected persons in order to understand better the natural history and pathogenesis of these infections.

In this study we investigated whether HTLV-II infection could be identified and distinguished from HTLV-I in a series of IVDAs living in the Veneto region of Italy, using the synthetic peptides and the PCR techniques.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 962 IVDAs and from 50 patients with haematological malignancies, consisting of 1 case

Correspondence to A. De Rossi.
The authors are at the Institute of Oncology, University of Padova, Via Gattamelata 64, 35128 Padova, Italy.
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