Oncology

Oncology 2003;65(suppl 2):94–98
DOI: 10.1159/000073368

Blood Stem Cell Collections after Mobilization with Combination Chemotherapy Containing Ifosfamide Followed by G-CSF in Multiple Myeloma

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Key Words
Peripheral blood stem cells · Mobilization · Stem cell collection · Multiple myeloma · G-CSF · Ifosfamide

Abstract
High-dose chemotherapy with autologous peripheral blood stem cell transplantation is the standard treatment of patients with multiple myeloma today. In this study we used a combination mobilizing chemotherapy containing ifosfamide with G-CSF before stem cell collection. The chemotherapy regimen consisted of ifosfamide (2,500 mg/m^2 days 1–3), epirubicin (100 mg/m^2 day 1) and etoposide (150 mg/m^2 days 1–3) followed by G-CSF (5 μg/kg from day 5). In 30 younger patients (median age 51 years; range 41–60 years) who received the IEV regimen in 100% dosage, a median of 11.15 × 10^6 CD34$^+$ cells/kg (range 0–44.60 × 10^6 CD34$^+$ cells/kg) was collected. In 22 elder patients (median age 64 years; range 59–72 years) similar collection results were obtained with a median of 10.82 × 10^6 CD34$^+$ cells/kg (range 0.99–42.22 × 10^6 CD34$^+$ cells/kg) after the IEV regimen in 75% dosage. The pretreatment chemotherapy cycles before mobilization were fewer in elder patients with a median of 0 cycles (range 0–7 cycles) compared with younger patients with a median of 4 cycles (range 0–7 cycles). These collection results were favorable and allowed to support a tandem transplantation procedure in younger and elder patients in 97 and 95%, respectively. In the majority of patients, the hematological toxicity of IEV was of WHO grade 3/4. The extramedullary toxicity was mild to moderate and there were only few cases (5–10%) of relevant nephrotoxicity or neurotoxicity associated with the application of ifosfamide.

Introduction

High-dose therapy followed by autologous peripheral blood stem cell transplantation was found to be superior to conventional chemotherapy in patients with multiple myeloma [1–4]. Gianni et al. [5, 6] pioneered the use of chemotherapy with hematopoietic growth factors to mobilize and collect peripheral blood stem cells in lymphoma and multiple myeloma. The standard mobilization regimen in multiple myeloma in the 1990s was high-dose cyclophosphamide (4–7 g/m^2) followed by GM-CSF or G-CSF [7–9]. Clinical studies which aim at improving the yield of stem cell collections with combination chemo-

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therapy as compared to single-agent cyclophosphamide are ongoing. To optimize stem cell collections is important because nowadays frequently tandem transplantation programs [2] are offered to patients with multiple myeloma which were found to improve survival over single high-dose chemotherapy in a prospective randomized trial [10].

The median age at diagnosis in patients with multiple myeloma is between 65 and 70 years. Today, high-dose chemotherapy with melphalan and autologous blood stem cell transplantation is increasingly being offered to elder patients. Age-adapted dosing of melphalan in high-dose protocols appears to be favorable [11, 12]. The outcome of elder patients with multiple myeloma treated with high-dose chemotherapy does not seem to be inferior to the results obtained for younger patients [11, 13, 14].

In this investigation we used a combination of ifosfamide (2,500 mg/m^2 days 1–3), epirubicin (100 mg/m^2 day 1) and etoposide (150 mg/m^2 days 1–3) followed by G-CSF (5 μg/kg) for stem cell mobilization and collection in multiple myeloma. There is experience with this combination for stem cell mobilization in lymphoma patients [15]. The combination of ifosfamide, epirubicin and etoposide with G-CSF was found to produce better collection results than high-dose cyclophosphamide alone with G-CSF. Here we report our experience with this mobilization regimen in multiple myeloma. For patients above the age of 60, generally a dose reduction to 75% was carried out.

Table 1. Patient characteristics

	Younger patients	Elder patients
Number	30	22
Age		
Median	51	64
Range	41–60	59–72
Gender		
Male	17 (57%)	11 (50%)
Female	13 (43%)	11 (50%)
Salmon-Durie stage		
I	1 (3%)	–
II	12 (40%)	5 (23%)
III	17 (57%)	17 (77%)
M-protein type		
G	20 (67%)	15 (68%)
A	2 (7%)	2 (9%)
M	1 (3%)	–
Bence-Jones	5 (17%)	5 (23%)
Anaplastic	1 (3%)	–
Non-secretory	1 (3%)	–
Creatinine		
>2 mg%	2 (7%)	2 (9%)
<2 mg%	28 (93%)	20 (91%)
β_2-Microglobulin, mg/l		
Median	3.3	3.4
Range	1.0–11.5	1.9–9.2
CRP, mg/dl		
Median	0.4	0.5
Range	0.1–11.0	0.1–4.6

Patients and Methods

Patients

Two cohorts of consecutive multiple myeloma patients were treated in age-adapted high-dose chemotherapy protocols of the German Study Group on multiple myeloma (DSMM) at two centers. The characteristics of the patients are shown in table 1.

IEV Chemotherapy and G-CSF

The IEV chemotherapy regimen was given for blood stem cell mobilization. The IEV regimen consists of ifosfamide 2,500 mg/m^2, i.v., days 1–3, epirubicin 100 mg/m^2, i.v., day 1, and etoposide 100 mg/m^2, i.v., days 1–3, followed by G-CSF (filgrastim; Amgen, Thousand Oaks, Calif., USA) at a dose of 5 μg/kg, s.c., daily from day 5 until the completion of blood stem cell harvesting. The younger patients up to an age of 60 years received the IEV regimen in a 100% dosage. Apart from individual dose reductions, patients with an age of 60 years or above generally received the IEV regimen in a 75% dosage.

Blood Stem Cell Collection and Cryopreservation

Autologous blood stem cells were harvested when the post-nadir, G-CSF-stimulated leukocyte count rose up to 5,000–10,000/μl or above using a Cobe Spectra (Cobe, Heimstetten, Germany) or an AS104 (Fresenius, St. Wendel, Germany) cell separator and standard programs. Approximately 10 l of blood were processed at a flow rate of 50 ml/min. The harvested blood stem cells were mixed with an equal volume of a freezing solution which was prepared with 5% HSA and 100% DMSO (Cryoserv, Tera Pharmaceuticals, Midvale, Utah, USA) (4:1). The final DMSO concentration was 10%. After computerized controlled-rate freezing, the bags containing the blood stem cells were stored in the vapor phase of liquid nitrogen.

CD34$^+$ Cell Enumeration of Autologous Blood Stem Cells

The determination of CD34$^+$ cells was carried out according to the guidelines of ISHAGE [16] using a FACScan (BD, Mountain View, Calif., USA) or an EPICS XL-MCL (Electronics, Miami, Fla., USA) flow cytometer equipped with an argon laser. Whole blood was incubated for 30 min at 4°C in the dark with the PE-conjugated monoclonal anti-CD34 antibody and the FITC-conjugated monoclonal anti-CD45 antibody followed by a wash and red blood cell lysis (BD). Seventy-five thousand cells were analyzed. To exclude cell debris, platelets, remaining red cells and all CD45-negative cells, a forward scatter versus CD45 fluorescence dot plot was used. The double-positive CD34$^+$/CD45$^+$ cell population was then defined and backgated for low CD45 expression and low side scatter properties. The percentage of the so defined CD34$^+$ cells was multiplied with the total nucleated cell content of the apheresis product to obtain the absolute number of CD34$^+$ cells harvested. The nucleated cell con-

Table 2. Treatment characteristics and collection results

	Younger patients	Elder patients
Number	30	22
Chemotherapy cycles		
Median	4	0
Range	0–7	0–7
Radiation therapy		
Yes	13 (43%)	6 (28%)
No	17 (57%)	16 (72%)
IEV dose		
50%	–	1 (5%)
66%	1 (3%)	–
75%	2 (7%)	21 (95%)
100%	27 (90%)	–
Leukaphereses		
Median	2	2
Range	0–5	1–6
$CD34^+$ cells collected, $\times 10^6$/kg		
Median	11.15	10.82
Range	0–44.60	0.99–42.22
Proportion with $>4 \times 10^6$/kg		
$CD34^+$ cells	97%	95%

Table 3. Toxicity of IEV chemotherapy

	Younger patients	Elder patients
Number	30	22
Hematological		
WHO grade 1	1 (3%)	1 (5%)
WHO grade 2	7 (23%)	1 (5%)
WHO grade 3	5 (17%)	7 (31%)
WHO grade 4	17 (57%)	11 (50%)
Undetermined	–	2 (9%)
Infection		
WHO grade 1/2	3 (10%)	2 (9%)
WHO grade 3	0	1 (5%)
Gastrointestinal		
WHO grade 3/4	3 (10%)	4 (18%)
Neurological		
WHO grade 1/2	1 (3%)	2 (9%)
WHO grade 3/4	1 (3%)	0
Nephrological		
WHO grade 1/2	1 (3%)	1 (5%)
WHO grade 3/4	1 (3%)	1 (5%)

tent was determined by automated cell counting using a Coulter STKS (Coulter, Miami, Fla., USA). The anti-CD34 antibody (HPCA-2), the anti-CD45 antibody (2D1) and the isotype controls used were from BD.

High-Dose Treatment Protocols

The IEV regimen was part of a sequential treatment plan. Up to an age of 60 years, patients first received induction chemotherapy with 3–4 cycles of ID or VAD. Then the IEV regimen was applied. After adequate stem cell collections, the patients proceeded either to tandem high-dose melphalan at a dose of 200 mg/m^2 or to a single high-dose treatment with an intensified conditioning regimen consisting of total marrow irradiation with shielding of the liver and the lungs ($3 \times 2 \times 1.5$ Gy), busulfan 9 mg/kg, and cyclophosphamide 2×60 mg/kg. At an age of 60 years and above, the patients were either treated with dexamethasone alone or with 3–4 cycles of ID or VAD before IEV. When stem cell collections were adequate, the patients proceeded to tandem high-dose melphalan at a dose of 100–140 mg/m^2.

Results

Younger (median age 51 years; range 41–60 years) and elder (median age 64 years; range 59–72 years) multiple myeloma patients were treated with a combination of ifosfamide, epirubicin and etoposide (IEV) followed by G-CSF before blood stem cell collections. The patient characteristics for both cohorts of patients are shown in table 1. All patients were in stage II or III of their disease with the exception of 1 stage I patient. Less than 10% in both cohorts had a creatinine level >2 mg%. The levels of β_2-microglobulin and C-reactive protein were also similar in both groups. Pretreatment with chemotherapy, however, had been carried out with more cycles in the younger patient group (table 2). Dexamethasone alone was the treatment before stem cell mobilization in 45% of elder patients but only in 3% of younger patients. Pretreatment with radiotherapy again was similar for both groups.

The younger patients usually were to receive a 100% dosage of the IEV regimen, what was actually carried out in 90% of younger patients. In 2 patients, the dose was reduced to 75% because of renal insufficiency and in 1 patient IEV could not be completed because of neurological toxicity of ifosfamide, resulting in a reduced dose of ifosfamide and etoposide of 66%. The elder patients usually were to receive a 75% dosage of IEV, what was possible in 95% of patients. In 1 patient the dose was further reduced to 50% because of renal insufficiency.

The number of leukapheresis harvests carried out in both patient groups was similar with a median of two leukaphereses (table 2). Also the number of $CD34^+$ cells collected was similar between younger patients with a median of 11.15×10^6 $CD34^+$ cells/kg and the elder patient cohort with a median of 10.82×10^6 $CD34^+$ cells/

kg. In 1 younger patient, stem cell collection was not possible because of chemorefractory disease and persistence of hyperviscosity after IEV. Since the patients were candidates for a tandem high-dose chemotherapy with melphalan, 4×10^6 $CD34^+$ cells/kg were required for both transplants. This was achieved in the younger patients in 97% and in the elder patients in 95%.

The toxicity of IEV in the younger and elder patients (with dose reduced IEV) was similar (table 3). The majority of patients developed WHO grade 3/4 hematological toxicity. There was one WHO grade 3 infectious complication in an elder patient. The gastrointestinal toxicity WHO grade 3/4 was somewhat higher in elder patients with 18% versus 10% in the younger patients. An ifosfamide-related encephalopathy which required the application of methylene blue occurred in 1 of the younger (3%) and in 1 of the elder (5%) patients. There were singular cases of a WHO grade 3/4 nephrotoxicity among younger and elder patients. No treatment-related mortality was observed in both groups.

Discussion

Effective blood stem cell collections could be carried out after combination chemotherapy with ifosfamide, epirubicin and etoposide followed by G-CSF in patients with multiple myeloma. Both in younger and elder patients, similar collection results with a median of around 10×10^6 $CD34^+$ cells/kg after a median of two leukapheresis were obtained. In the younger patients, the IEV regimen was applied in a 100% dosage. The younger patients had received more previous chemotherapy with a median of 4 cycles. In elder patients, the IEV dosage was generally reduced to 75% because of concerns regarding toxicity. Elder patients had received less previous chemotherapy with a median of 0 cycles due to a strategy of early stem cell harvesting. In patients with no conventional chemotherapy before stem cell harvesting, dexamethasone alone had been given for symptom control and stability of disease during the harvesting period. These results demonstrate that a strategy of early stem cell collection in elder patients with dose-reduced mobilizing chemotherapy can result in a similar high collection efficiency than in younger patients being treated according to the conventional strategy of stem cell mobilization after 3–4 cycles of induction chemotherapy.

The stem collections in our patients aimed at a minimum dose of 4×10^6 $CD34^+$ cells/kg to support a tandem transplantation procedure. This target dose was achieved in $\geq 95\%$ of both younger and elder patients. When compared to the collection results obtained after high-dose cyclophosphamide (4–7 g/m^2) with G-CSF in multiple myeloma, collection results appear to be superior with IEV [17, 18]. Indeed, in lymphoma patients, it was found that a mobilization with IEV and G-CSF gave superior collection results than high-dose cyclophosphamide with G-CSF [15]. In a study in lymphoma and multiple myeloma, high-dose ifosfamide alone with GM-CSF was compared with high-dose cyclophosphamide followed by GM-CSF regarding stem cell collection results [19]. Blood stem cell collections yielded slightly less $CD34^+$ cells after high-dose ifosfamide but without statistical significance. The toxicity profile, however, after high-dose ifosfamide was favorable. This shows that ifosfamide can make an important contribution to stem cell mobilization within combination chemotherapy regimes.

In order to avoid the substantial hematological and non-hematological toxicity after high-dose cyclophosphamide, lower doses of cyclophosphamide (1.5 g/m^2) were investigated for stem cell mobilization in lymphoma and multiple myeloma [20]. A target dose of 2.5×10^6 $CD34^+$ cells/kg to support a single transplant, however, could be harvested in only 64% of patients. This shows that reducing myelosuppression also reduces the following stem cell mobilization and collection during the post-nadir hematological rebound.

Blood stem cell mobilization with G-CSF alone at a higher dose of 10 μg/kg or above has the advantage of a low toxicity and the days of collection can be precisely planned. Yet, the collection results frequently are inferior to what can be obtained with the combination of chemotherapy and hematopoietic growth factors for mobilization [21, 22]. This, however, does not necessarily lead to an inferior engraftment [22].

Several studies have shown that pretreatment with melphalan decreases stem cell mobilization and collection results significantly [9, 17]. In our study, only 1 younger patient (3%) and 1 elder patient (5%) had received previous melphalan treatment.

In order to ameliorate toxicity, ifosfamide was given over several hours and a glucose infusion was co-administered. In around 10% of cases, neurotoxicity developed in our patients which presented as an encephalopathy and required a therapy with methylene blue in 1 younger (3%) and 1 elder (5%) patient. Severe WHO grade 3/4 nephrotoxicity occurred in $\leq 5\%$ of cases. The majority of younger and elder patients receiving IEV with G-CSF developed a hematological toxicity WHO grade 3/4. It therefore is required to perform regular blood tests and a clini-

cal evaluation of the patient, for example three times weekly after discharge of the patient from the hospital following the administration of IEV chemotherapy. Platelet transfusions may be necessary in some patients. There is a potential risk of neutropenic fever around day 10 and a potential risk of bacterial sepsis has to be taken into consideration. Altogether, the toxicity profile appears to be adequate when put into relation with the high efficiency of this regimen in stem cell mobilization and collection.

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