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Original Paper

High-dose Melphalan with Re-infusion of Unprocessed, G-CSF-primed Whole Blood is Effective and Non-toxic Therapy in Multiple Myeloma

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In order to shorten the pancytopenic period following high-dose melphalan 140 mg/m^2 (HDM) treatment of multiple myeloma patients, we studied the effects of re-infusing granulocyte colony stimulating factor (G-CSF) [Filgrastim, Neupogen®]-primed unprocessed whole blood. 30 patients with multiple myeloma were treated with HDM. One litre of blood after 5 or 6 days stimulation with G-CSF (10 μg/kg) was drawn, kept unprocessed for 1 day and re-infused 24 h after chemotherapy. Time to granulocyte recovery (>0.5 × 10^9/l) and platelet recovery (>20 × 10^9/l) were assessed as well as length of hospital stay, number of transfusions and antibiotic use. These 30 patients were compared with 20 historical control patients who were similarly treated but without stem cell support. The response rate was 75% (21/28) including a complete remission (CR) rate of 29% (8/28). Two early deaths due to *Aspergillus* pneumonia were observed. The median overall survival after HDM has not been reached after a median follow-up of 14 months. 10 patients showed progression at a median of 7 months. Currently, 23 patients are alive with a median follow-up time of 14 months. Haematological recovery was significantly faster in the study group as compared to the historical control group. The neutrophil count reached 0.5 × 10^9/l at a median of 14 days after infusion of 1 litre of unprocessed whole blood compared with 38 days in the historical control group. A platelet count of 20 × 10^9/l was reached at a median of 26 days compared with 36 days in the historical control group. Length of hospital stay decreased from a median of 43 to 18.5 days. The number of days with antibiotics was reduced from a median of 21 to 6 days. HDM is effective therapy for multiple myeloma. Toxicity of the regimen is considerably reduced by the use of G-CSF-stimulated unprocessed whole blood, an easy to perform and cheap technique to mobilise and collect stem cells.

Key words: high-dose melphalan, stem cell transplantation, multiple myeloma, whole blood

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INTRODUCTION

STANDARD CHEMOTHERAPY in multiple myeloma consists of various combinations. Among these combinations, the most often used is melphalan and prednisone, given orally in short courses at monthly intervals producing remissions in approximately 40% of patients [1]. In patients treated with these conventional chemotherapeutic regimens, complete remissions are rare (<10%) and not very longlasting, while median survival does not exceed 2.5 years. This brief duration of response has led to the investigation of dose intensification. McElwain and Selby were the first to evaluate toxicity and efficacy of a single high dose of intravenously administered melphalan (140 mg/m^2) [HDM] without stem cell support [2]. Since then, several studies on the use of HDM have shown a complete remission (CR) rate of about 25% in untreated and 10% in resistant patients with a me-

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dian time to relapse of 20 months in chemotherapy naive and less than 5 months in resistant patients [3–5]. However, without stem cell support, toxicity of HDM is considerable and mortality ranges between 8 and 20% depending on the selection of patients [3–6]. This mortality is mainly due to extended duration of granulocytopenia following HDM. Recently, in a preliminary study in 6 patients, we showed that recovery of granulocytes after HDM can be improved markedly by re-infusion of G-CSF-primed unprocessed whole blood after HDM [7]. We have extended this study to 30 patients and show that HDM supported in this way is effective and safe. In addition, it is an easy way to perform rescue and results in a considerable cost reduction, mainly due to shortening of the hospital stay.

PATIENTS AND METHODS

Patients

Between July 1992 and September 1994, 30 patients previously treated for multiple myeloma were entered into a study in which HDM (140 mg/m^2) was administered followed by re-infusion of G-CSF-stimulated unprocessed whole blood. Patient characteristics are listed in Table 1. The majority of the patients were heavily pretreated, and 17 had a disease history of more than 1 year. Refractory disease to at least a melphalan-containing regimen was present in 11 patients, stable disease in 8, while 11 patients were on treatment with and sensitive to conventional chemotherapy (melphalan/prednisone (MP) or vincristine, doxorubicin, dexamethasone (VAD)).

Table 1. Patient characteristics

	Study group	Historical control group
Number of patients	30	20
Age (years)		
Median	53	49
Range	39–61	37–56
Sex		
Female	11	6
Male	19	14
Performance status (WHO)		
0–1	26	16
2–3	4	4
M-Protein		
IgG	21	12
IgA	8	5
Non-secreting	1	1
Bence Jones only	0	2
Previous chemotherapy		
MP	3	2
MP, VAD	17	12
MP, VMCP, VAD	4	1
MP, VMCP	2	2
CMVP, VAD	4	2
VAD, CHOP	0	1
Time between diagnosis and HDM		
0–6 months	5	2
7–12 months	8	5
>12 months	17	13
Disease status at HDM		
Chemosensitive	11	6
Stable	8	5
Refractory	11	9

HDM, high-dose melphalan; MP, melphalan, prednisone; VAD, vincristine, doxorubicin, dexamethasone; VMCP, vincristine, carmustine, cyclophosphamide, prednisone; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone.

Mobilisation and stem cell harvest

To mobilise stem cells, G-CSF (Filgrastim, Neupogen$^{(R)}$, Amgen, Thousand Oaks, California, U.S.A.) at a dosage of 10 μg/kg was administered subcutaneously daily starting on day −6 or −5 for 6 or 5 days, in an outpatient setting. Patients were admitted to the hospital on day −1, and a subclavian vein catheter inserted. Just prior to administration of HDM on day 0, two phlebotomies of 500 ml each were performed via an antecubital vein. During this procedure, the patients were monitored carefully for blood pressure and heart rate; hypotension was corrected by infusion of colloids. The blood was collected in two 2,3-ethyl, hexyl-phthalate plasticised, polyvinylchloride bags with 70 ml CPD as an anticoagulant (Nederlands Productie Laboratorium voor Bloedtransfusieapparatuur en Infusievloeistoffen B.V., Emmer-Compascuum [NPBI], The Netherlands). The two bags of whole blood were kept at room temperature for 24 h for the first 20 patients and at 4°C for the last 10 patients without any further processing. Immediately after blood collection, HDM (140 mg/m^2) was infused in 10 minutes. Twenty-four hours after HDM, the two bags of blood were re-infused over 4 h. G-CSF was started again at day 2 at a fixed dosage of 300 μg/day by subcutaneous injection until granulocytes were $>0.5 \times 10^9$/l.

Clinical status was assessed daily and complete blood counts were performed three times a week until recovery. Patients received antibiotics for selective decontamination of the gastrointestinal tract (Ciprofloxacin 1000 mg and Fluconazole 50 mg daily), and parenteral antibiotics or antifungal agents were started if needed, as described elsewhere [8]. Red blood cell and platelet transfusions were performed according to standard criteria.

In a number of cases, immediately after phlebotomy and just before infusion, the committed colony-forming units granulocyte-macrophage (CFU-GM) in the stored blood were counted in methylcellulose after incubation for 10 days using placenta conditioned medium as a source of colony stimulating factor. The total number of progenitors present in the one litre of blood was calculated. The total number of CD34$^+$ cells was calculated after determination of the percentage CD34$^+$ cells using flow cytometry.

Historical control group

The results of the study patients were compared with a group of 20 patients with multiple myeloma who met the same entry criteria and whose pretreatment characteristics were equivalent. In this previous consecutive study, patients 1–12 received no G-CSF, patients 13–16 received G-CSF starting at day +28 and patients 17–20 started G-CSF at day +3 after chemotherapy. Melphalan dosage, antibiotic regimen and supportive care were the same as described for the study patients. Despite the addition of G-CSF in 8 patients, there were no differences with regard to neutrophil and platelet recovery, days with fever, number of transfusions or hospital stay between these 8 patients and the other 12 patients in the control group. We, therefore, regarded

Table 2. Cause of death

	Study group	Historical control group
Early death (infectious)	2	3
Infectious	1	2
Progressive disease	4	10

the control group of 20 patients as homogeneous and pooled the data.

Assessment of response

Response was measured starting 2 months after HDM. A complete response was determined as disappearance of myeloma proteins and less than 5% of plasma cells in one normocellular bone marrow aspirate. A partial response (PR) was defined as a 50% or greater reduction in serum and urine myeloma protein for at least 8 weeks. Refractory disease was defined as no CR or PR or when progression occurred during or immediately after treatment. Early death (ED) was defined as death within the first 2 months after treatment with HDM.

Statistical analysis

Comparisons between the two groups were made using either the Wilcoxon rank sum test or the Wilcoxon–Gehan rank sum test for censored observations. All analyses tested two sided. P values < 0.05 were considered significant. For estimation of duration of remission and survival curves the Kaplan–Meier method was used.

RESULTS

In the HDM group of 30 patients with whole blood support, 2 patients were not evaluable for response because of early death due to *Aspergillus* pneumonia. The overall response rate was 75% (21/28), with a CR rate of 29% (8/28). 10 patients showed progression after HDM at a median of 7 months (range 2–18 months). Currently, 23 patients are alive and 7 are dead with a median follow-up time of 14 months (Table 2). The median overall survival after high-dose melphalan has not yet been reached. The projected overall survival at 24 months is 70% (Figure 1). The median duration of response in the 28 evaluable patients was 17 months (Figure 2). The projected overall survival taken from the time of diagnosis is shown in Figure 3; median survival has not yet been reached with a follow-up varying between 1 and 85 months.

In the historical control group of 20 patients, the overall response rate was 65% and CR rate 15%. Early death occurred in 15% of the patients.

The acute toxicity of the HDM regimen consisted of mild to moderate anorexia, and in more than 75% mucositis <grade 2 was observed. No patient had > grade 3 mucositis. The median duration of granulocytopenia ($< 0.5 \times 10^9$/l) was 14 days (range 10–35 days) and of thrombocytopenia ($< 20 \times 10^9$/l) 26 days (range 13–78 days) (Table 3). To reach a platelet count $>100 \times 10^9$/l, a median of 52 days was required. The median duration of hospital stay was 18.5 days (range 14–39 days). As shown in Table 3, in comparison to the historical control group, a dramatic shortening of the pancytopenic period and hospital stay was noted. A decrease in antibiotic use was also found.

After 5 or 6 days of G-CSF treatment for stem cell mobilisation, the WBC rose from a median value of 4.2×10^9/l (range 2.5–8.8) to 23×10^9/l (range 8.6–65). The single observed side-effect in only a few patients was bone pain.

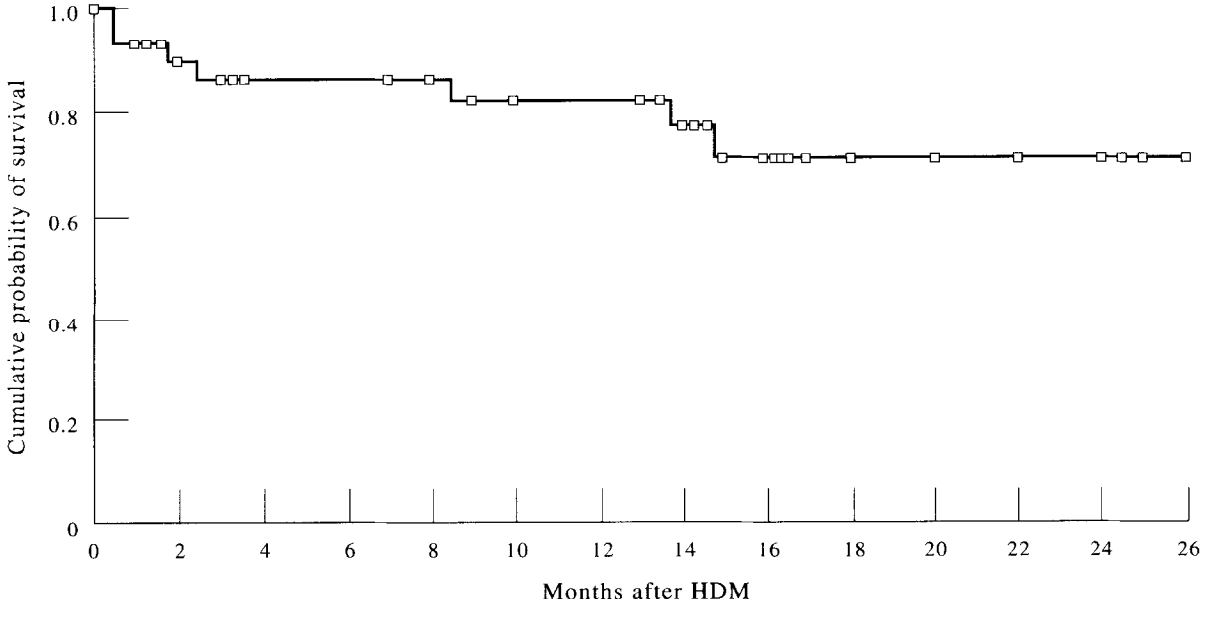

Figure 1. Overall survival of the 30 patients after high-dose melphalan (140 mg/m^2) supported by re-infusion of 1 litre G-CSF-primed unprocessed whole blood.

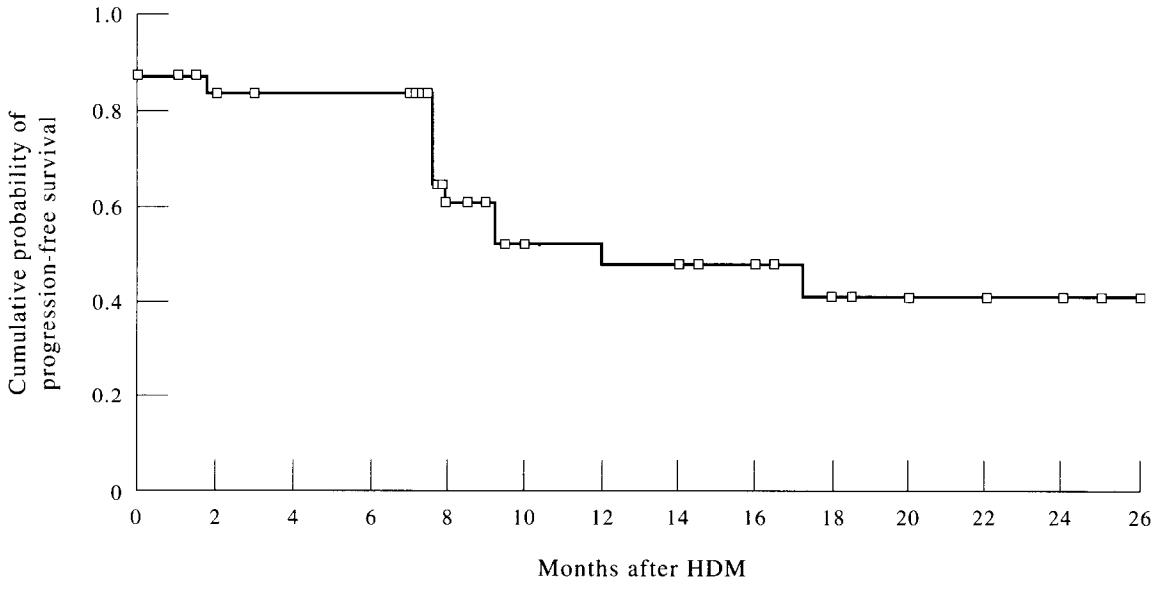

Figure 2. Time to progression after HDM (140 mg/m^2) in 28 evaluable patients.

Phlebotomy led to hypotension in 4 patients, rapidly responding to fluid infusion. One litre of blood could be obtained in all patients. The re-infusion of whole blood was without side-effects with only the first patient developing fever. In the one litre of whole blood after G-CSF priming, the median number of CD34+ cells was 0.3×10^6/kg (range 0.1–1.75), while the median number of CFU-GM was 9.6×10^4/kg (range 0.1–64.2).

For the patients for whom data were available, the number of days to platelet and neutrophil recovery has been plotted as a function of the total number of transfused CD34+ cells/kg body weight (Figure 4) or as a function of CFU-GM/kg body weight in one litre of whole blood (Figure 5). There was a trend that higher numbers of re-infused CD34+ cells and CFU-GM was paralleled by a faster haematological recovery. However, as may also be observed from these data, recovery can be very fast even with very low numbers of re-infused CD34+ cells.

DISCUSSION

High-dose melphalan is valuable therapy in patients with multiple myeloma. When used as frontline therapy, high response rates can be reached including approximately 25% CRs [3]. In this study of unselected patients responding and

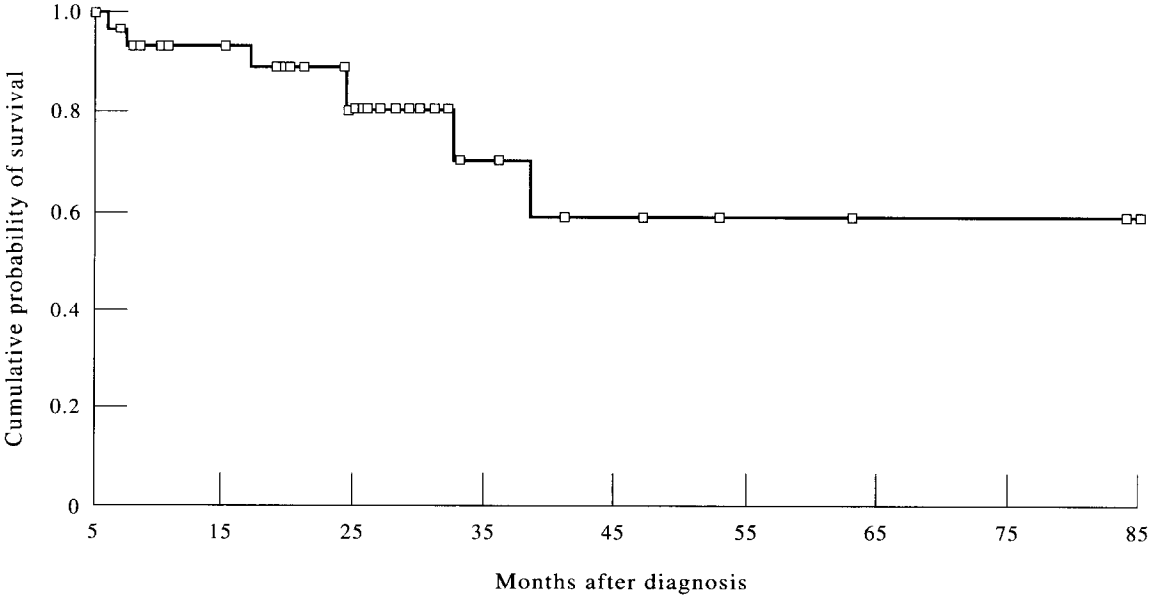

Figure 3. Overall survival from diagnosis of the 30 patients treated with high-dose melphalan (140 mg/m^2) supported by re-infusion of 1 litre G-CSF-primed unprocessed whole blood.

Table 3. Clinical data comparison between study and control group

	Study group ($n = 30$)*	Historical control group ($n = 20$)*	P value
Granulocytes < 0.5×10^9/l	14 (10–35)	38 (24–65)	0.002
Platelets < 20×10^9/l	26 (13–78)	36 (25–172)	0.02
Hospital stay (days)	18.5 (14–39)	43 (33–71)	0.0003
Antibiotic use (days)	6 (0–36)	21 (0–48)	0.008
Platelet transfusions†	24 (12–102)	54 (30–228)	0.02
Red cell transfusions†	9 (3–39)	12 (3–66)	ns

* Data are given as median (range). †Donor units transfused within 12 weeks from HDM. ns, not significant.

non-responding to conventional therapy, a complete response rate of 29% and a total response rate of 75% was noted. These data are in concordance with other series of patients treated with high-dose melphalan [4, 5, 9]. Recently, Cunningham and associates showed, in 63 previously untreated patients with a median follow-up of 74 months, an overall response rate of 82% with 32% of the patients being complete responders [3]. 23 patients are alive with a median survival duration of 47 months and 35% of patients are expected to be alive at 9 years [3]. Unfortunately, most remissions after high-dose melphalan are not durable. Long-term follow-up data of patients treated in this way show a disease-free survival (DFS) of less than 5% at 70 months. To prolong DFS and overall survival, several groups are exploring multiple courses of high-dose chemotherapy followed by autologous stem cell support [10–12]. HDM is usually one of the constituents of these sequential high-dose regimens. Introduction of Interferon after reaching a state of minimal residual disease could possibly prolong response and survival in multiple myeloma [13].

The myelosuppression induced by HDM, given without any stem cell support, leads to a considerable number of toxic deaths, ranging from 8–20%. The median duration of neutropenia (>0.5×10^9/l) in a number of studies has been reported to be 28, 36, 42 and 29.5 days [14, 9, 6, 11], respectively and are well in line with the data derived from our historical control group. In one study, because of toxicity from the first course of HDM in 25% of patients with advanced multiple myeloma and 51% of newly diagnosed patients, it was not possible to proceed with a second planned course of HDM [11]. In our study, we shortened dramatically the pancytopenic period following HDM by infusion of growth factor-mobilised stem cells without the necessity of leucapheresis and cryopreservation. Compared with the historical control group, the median duration of neutropenia could be reduced by 24 days, median duration of thrombocytopenia by 10 days and median length of hospital stay by 24 days. This was reflected by less need for platelet and red cell transfusions and reduced antibiotic use. Toxic death was observed in 2 patients (7%) due to *Aspergillus* pneumonia. In our historical control group, G-CSF administration starting 3 days after high-dose melphalan did not hasten marrow recovery compared with the other control patients. Moreover, in another study, GM-CSF shortened neutrophil recovery only to a limited extent, i.e. to 0.5×10^9/l in patients < 50 years of age, and < 12 months from initial diagnosis to a median of 21 days receiving an intermediate dose of melphalan (100 mg/mg^2); in the remaining patients, neutrophil recovery was reached at a median of 31 days [15].

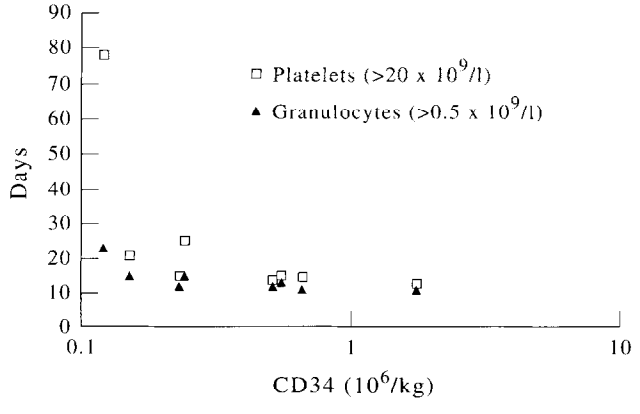

Figure 4. Relation between total number of CD34+ cells reinfused after HDM and recovery of granulocytes (> 0.5×10^9/l) (▲) or platelets (> 20×10^9/l) (□). Data were available for only the last 8 patients enrolled in the study due to earlier problems with standardisation of the CD34 analysis.

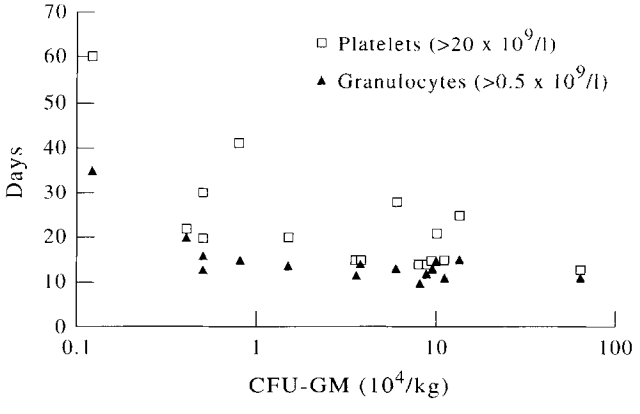

Figure 5. Relation between total number of CFU-GM reinfused after HDM and recovery of granulocytes (> 0.5×10^9/l) (▲) or platelets (> 20×10^9/l) (□). Data were available for only the last 16 patients enrolled in the study due to earlier problems with standardisation of the assay procedure.

This introduction of stem cell support with G-CSF-primed unprocessed whole blood is very attractive from a pharmaco-economic point of view. Besides reducing toxicity, a cost saving of approximately 50% was calculated [16].

Although the number of re-infused CD34+ cells was relatively low, neutropenic recovery was fast and comparable to the expected recovery time found after infusion of the number of CD34+ cells commonly used as a threshold in peripheral blood progenitor cell support (PBPC) [17]. The low number of infused CD34+ cells was reflected by a prolonged thrombocyte recovery time as compared to the recovery time that is observed after re-infusion of more than 2×10^6/kg CD34+ cells. Nevertheless, even after re-infusion of as few as 0.5×10^6/kg CD34+ cells, a rather rapid platelet recovery was still observed (Figure 4).

We analysed WBC recovery from the day of infusion, but, when the true neutropenic period was measured, an even more pronounced difference was observed. It took a median of 5 days after HDM before granulocytes were below 0.5×10^9/l, so the real neutropenic period lasted only a median of 9.5 days. This might be due to the presence of more mature committed myeloid precursors such as CFU-GM, promyelocytes and myelocytes in the re-infused unprocessed whole blood.

From observations in our laboratory and also from data shown by Pettengell and colleagues [18], it is now clear that G-CSF-primed unprocessed whole blood can be kept for 72 h with viability and CFU-GM content preserved. This is important for multiple myeloma patients with renal insufficiency in whom clearance of HDM is not predictable and probably takes longer than 24 h. Furthermore, this offers an opportunity to deliver chemotherapy for more than one day, which we are now exploring in resistant lymphoma patients who are conditioned with a modified BEAM regimen before re-infusion of unprocessed whole blood kept at 4°C for 72 h [19]

In conclusion, the use of G-CSF-stimulated unprocessed whole blood as a stem cell source reduces toxicity of HDM considerably and changes HDM from a very toxic regimen to a good manageable chemotherapeutic scheme retaining its high anti-plasma cell activity. Because the response to HDM is not durable, we are now exploring the addition after HDM of a second conditioning regimen (Busulphan/Cyclophosphamide) supported by PBPC harvested by leucapheresis.

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