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Monoclonal Gammopathies

High-dose therapy in multiple myeloma: effect of positive selection of CD34+ peripheral blood stem cells on hematologic engraftment and clinical outcome

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ABSTRACT

Background and Objectives. **Positive selection of peripheral blood stem cells (PBSC) has been investigated in multiple myeloma (MM) with the aims of reducing plasma cell (PC) contamination of the leukaphereses and improving clinical outcome of autografted patients.**

Design and Methods. **In our center 39 untreated patients with stage II and III MM, younger than 65 years, started high-dose therapy consisting of 4 VAD cycles, collection of PBSC mobilized by 7 g/m² cyclophosphamide + G-CSF, and myeloablative treatment with 12 mg/kg busulfan plus 120 mg/m² melphalan. The leukaphereses from 23/39 patients (59%) were processed for positive selection of CD34+ cells using an avidin-biotin immunoaffinity device.**

Results. **A reduction of PC contamination of as much as 2 log was found in the post-selection products by a flow-cytometric technique using the monoclonal antibody CD 138 alternatively coupled with CD38 and cytoplasmatic κ or λ light chains in separate samples. Hematologic reconstitution and clinical outcome of the 23 patients reinfused with selected CD34+ cells (SEL group) were compared with those of the 16 patients reinfused with unselected cells (UNSEL group). No significant differences were observed between the 2 groups with regards to the median duration of neutropenia and thrombocytopenia, the hematologic support required, the incidence of febrile episodes and bacteremias. At a median follow-up of 18 months (range 5-34) after ASCT, there were 7/23 (32%) continuous complete remissions (CR) in the SEL group and 4/16 (25%) in the UNSEL group; there were 10/23 (44%) continuous partial remissions (PR) and 5/16 (31%) in the SEL and UNSEL groups, respectively. Two patients in the UNSEL group and one patient in the SEL group died of progressive disease.**

Interpretation and Conclusions. **Our data show that positive selection allows rapid engraftment of hematopoiesis and low morbidity. Although no significant difference was detected between the two groups in the frequency of CR and PR 3 and 18 months after ASCT, a longer follow-up is needed to evaluate definitively the effect of CD34+ selection on the clinical outcome after ASCT.**

Key words: myeloma, autologous transplantation, positive selection, CD34+ cells.

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Multiple myeloma (MM) is an incurable disease with a median overall survival of 3-4 years and a 5-year survival of only 25%.[1] The conventional treatment is commonly based on the association of melphalan and prednisone; no other drug combination has been definitively proven to be superior to this standard therapy despite several clinical trials.[2] Escalation of the dose of intravenous melphalan and other alkylating agents can produce an increase in response rate and overcome drug resistance;[3] complications of prolonged myelodepression can be circumvented by transplantation of allogeneic or autologous hematopoietic stem cells. While allogeneic bone marrow transplantation (BMT) is limited by availability of donors and by the recipient's age, autotransplantation has enjoyed wider use, having been shown to induce long-term responses with acceptable toxicity.[4-6] In 1996 one large randomized prospective study demonstrated that autologous bone marrow transplantation is superior to conventional chemotherapy in terms of event-free and overall survival.[7] Nevertheless, relapse remains a problem also in autografted patients. The progression of the disease can be explained by reinfusion of the clonal plasma cells together with the hematopoietic progenitors and/or by the lack of efficacy of the myeloablative treatment. Consequently, new clinical trials have proposed increasing tumor cytoreduction (tandem transplantation)[8,9] or purging leukaphereses or both. In 1994 in our center we started treating patients with advanced myeloma younger than 65 years with high-dose therapy; two years later we added positive selection of CD34+ cells of the leukaphereses products to this plan with the aim of reducing neoplastic contamination of the graft. In the present study we examine the effect of positive selection on the reduction of plasma cell contamination, on hematologic reconstitution and clinical outcome

after autologous stem cell transplantation (ASCT), comparing the clinical results of the patients reinfused with selected CD34+ cells with those of the patients treated with the same myeloablative regimen but reinfused with unselected cells.

Design and Methods

Patients

Between July 1995 and December 1998 a total of 39 patients with MM started on high-dose therapy. Criteria of inclusion were: age ≤ 65 years, advanced disease (stage II and III, stage I with marrow plasmacytosis > 50%), patient's informed consent.

Diagnosis was made according to the guidelines of the Chronic Leukemia Myeloma Task Force[10] and stage classification was based on Durie and Salmon's criteria.[11] Nineteen of the patients were male and 20 female with a median age of 54 years (range 31 to 62). At diagnosis, 11 patients had stage I MM with a marrow plasmacytosis greater than 50%, while 3 had stage II MM and 25 had stage III MM. All the patients received 4 cycles of induction chemotherapy; VID (vincristine, idarubicin, dexamethasone) in 17 cases and VAD (vincristine, doxorubicin, dexamethasone) was used in 22 patients. Twenty-five of the 39 patients (62%) were considered to have responded to the chemotherapy by the time of peripheral blood stem cell (PBSC) collection, as the M component has decreased to under 50% of the initial value; 14 patients did not respond to chemotherapy.

Collection of stem cells

Collection of PBSC was performed at a median time of 7 months (range 6-14) after the beginning of induction therapy. Cyclophosphamide 7 g/m^2 plus recombinant human granulocyte colony-stimulating factor (G-CSF) at the dose of 5 µg/kg/die s.c. were administered; leukapheresis were started when the number of CD34+ cells in the peripheral blood was higher than 20/µL; a median of 2 leukapheresis were needed to collect a median of 9.0×10^6/kg CD34+ cells. Procedures were performed using the Fenwal CS 3000 (Baxter) or Cobe Spectra cell separator. In 23/39 patients (59%) one or two leukapheresis products were processed to positively select CD34+ cells, using an avidin-biotin immunoaffinity device (CEPRATE, Cell Pro); an additional apheresis was cryopreserved as unmanipulated back-up. Since the minimum amount of CD34+ cells to be reinfused after a single myeloablative procedure is 2×10^6/kg and the efficacy of the selection procedure varies between 40% and 70%, only leukapheresis containing $\geq7\times10^6$/kg CD34+ cells were processed. Of the 16 patients who did not undergo positive selection, 9 were mobilized before this technique was available in our center and the remaining 7 cases did not mobilize a sufficient number of CD34+ cells after cyclophosphamide.

Plasma cell contamination

CD34+ cells (1×10^6) were incubated simultaneously with the phycoerythrin conjugated (PE) monoclonal antibody anti-HPCA-2 (CD34, Becton Dickinson) and with the fluorescein conjugated (FITC) monoclonal antibody antiHLE-1 (CD45, BD). At the end of the incubation red blood cells were lysed using FACS-lysing solution and analyzed using a FACSalibur flow cytometer. According to the ISHAGE protocol[12] forty-five thousand CD45+ events or at least one hundred CD34+ events were acquired using a cumulative gating strategy to identify true CD34+ cells and minimize the number of non-specifically stained events. Plasma cell contamination pre- and post-selection was evaluated according to the method described by van Zaanen *et al.*[13] with some modification. A total of 10^6 cells, pre-treated with Facs-lysing solution, were incubated with the monoclonal antibody CD138[14] alternatively coupled with CD38 and intracellular κ and λ light chains in 100 µL of a PBS saponine solution (0.02%) for 15', in separate samples. At least one hundred CD138/38 events were acquired and the intracellular light chain pattern of CD138+ cells was subsequently analyzed, considering only plasma cells with the same isotype as that of the plasma cells present at diagnosis in each patient.

Myeloablative therapy

Median time between diagnosis and transplantation was 9 months (range 6-19). The conditioning regimen consisted of busulfan 1.0 mg/kg orally every 6 hours for 12 doses on days –5, –4, –3 and melphalan 120 mg/m^2 in 100 mL normal saline over 1 hour on day -2. A median number of 2.7×10^6 /kg (range 1.4-5) CD34+ cells were reinfused on day 0. Antibiotic prophylaxis included daily oral ciprofloxacin and itraconazole. G-CSF was started at the dose of 5 µg/kg s.c. on day +4 and was continued until granulocyte count exceeded 2×10^9/L for 3 consecutive days. A second ASCT has been performed, so far, in 6 patients: in these cases myeloablative therapy consisted of melphalan 200 mg/m^2 in 100 mL normal saline over 1 hour on day -2 and the cryopreserved PBSC from the previous mobilization were reinfused on day 0. Maintenance treatment with interferon 3 MU three times a week was started as soon as hematologic recovery (neutrophil count > 1.5×10^9/L and platelet count > 100×10^9/L) had been reached in the patients who obtained a partial or complete response.

Response criteria after induction therapy and ASCT

Complete response (CR) was defined as the disappearance of the monoclonal component (evaluated by immunofixation) from serum and urine together with less than 5% of plasma cells in the bone marrow biopsy (which had to be polyclonal with Ig light chain immunostaining).

Partial response (PR) was considered as a greater than 50% decrease in measurable paraprotein and bone marrow infiltration, which had to last for at least 3 months.

Progressive disease (PD) or relapse was defined as follows: reappearance of M component in serum and/or urine for patients in CR; a 25% increase in serum paraprotein or a 90% increase of Bence-Jones proteinuria or new lytic lesions in other patients.

The category of no response (NR) included all patients not satisfying PR criteria.

Results

Detection of plasma cell contamination in leukaphereses

Leukaphereses started on day 12 (median, range 10-19) after high-dose cyclophasphamide. On the day of the first procedure the median number of peripheral blood $CD34^+$ cells was 94 (range 30-189). A median of 2 procedures/patient (range 1-3) were performed. Samples of the leukapheresis products contained a median percentage of 0.25 (range 0.003-1.6) plasma cells, that is a median number of 8.3 plasma cells $\times 10^5$/kg (range 0.09-78). In 23/39 patients (59%) one or two leukapheresis products, with a total median number of 8.8×10^6/kg cells (range 4.7-16)/patient were processed to positively select CD34 cells. A median percentage of 0.1 (range 0.0004-0.93) plasma cells, which corresponded to a median number of 8.0 plasma cells $\times 10^3$/kg, was detected in the post-selection products, demonstrating a reduction of plasma cell contamination of as much as 2 log, as evaluated by this cytometric method.

Response to transplantation

The outcome after ASCT and the transplant-related complications of the 23 patients reinfused with positively selected $CD34^+$ cells (SEL group) were compared with those of the 16 patients who received non-selected hematopoietic progenitors (UNSEL group). Both groups were well balanced according to main clinical features such as age, stage, M protein, incidence of renal impairment and marrow plasma cell infiltration (Table 1). Moreover, they received the same induction and conditioning treatment. Table 2 describes the outcome after the subsequent steps of high-dose treatment. CRs were occasional in both groups after induction therapy (0% in the SEL group vs.6% in the UNSEL group) and rose to 18% in SEL patients and to 25% in UNSEL patients after a single myeloablative procedure. A higher frequency of patients with resistant or progressive myeloma after the first ASCT was detected in the UNSEL group, although the difference observed between the two groups was not significant. Three out 4 patients in the SEL group and one out 2 patients in the UNSEL group, all in PR after the first ASCT, gained a CR after a second transplant.

Table 1. Clinical features of the patients.

	SEL group	*UNSEL group*
Sex		
Male/Female	11/12	8/8
Age (years) median (range)	54 (41-63)	55 (31-62)
Stage		
I	6/23 (26%)	5/16 (31%)
II	2/23 (9%)	1/16 (6%)
III A	15/23 (65%)	8/16 (50%)
III B	–	2/16 (13%)
M protein		
IgG	12/23 (53%)	9/16 (56%)
IgA	5/23 (22%)	4/16 (25%)
BJ	4/23 (17%)	2/16 (13%)
IgD	1/23 (4%)	–
Non secretor	1/23 (4%)	1/16 (6%)
Marrow PC*		
Median (range)	55 (10-100)	50 (5-90)
Prior chemotherapy		
VID	7/23 (30%)	10/16 (62%)
VAD	16/23 (70%)	6/16 (38%)

**PC=percentage of marrow plasma cell infiltration.*

Median marrow plasma cell infiltration progressively decreased during high-dose treatment in both groups: it was 30% (10-50) after VAD in both groups, then reduced to 10% (0-50%) in the UNSEL group and 20% (10-50%) in the SEL group after cyclophosphamide and was < 5% in 9/16 (56%) and 14/23 (61%) patients respectively after ASCT. Marrow plasma cells were polyclonal as demonstrated by Ig light chain staining in 5/15 (33%) patients in the UNSEL group and in 6/19 (31%) of those in the SEL group.

Follow-up and survival

The median follow-up of our patients was 18 months (5-34) from ASCT without difference between the 2 groups. Of the patients in the UNSEL group, 2

Table 2. Response to transplantation in evaluable patients, related to disease status pre-transplant.

Response	*Response to induction*		*Response to first ASCT*		*Response to second ASCT*		*Disease status at the last follow-up*	
	SEL	UNSEL	SEL	UNSEL	SEL	UNSEL	SEL	UNSEL
CR	0	1 (6%)	4 (18%)	4 (25%)	3 (75%)	1 (50%)	7 (32%)	4 (25%)
PR	14 (61%)	10 (63%)	15 (68%)	7 (44%)	1 (25%)	1 (50%)	10 (44%)	5 (31%)
NR	7 (30%)	4 (25%)	3 (14%)	3 (19%)			3 (14%)	2 (13%)
PD	2 (9%)	1 (6%)	0	2 (12%)			2 (10%)	5 (31%)
NE	0	0	1	0			1	0
Total	23	16	23	16	4	2	23	16

CR: complete response; PR: partial response; NR: no response; PD: progressive disease; NE: not evaluable.

relapsed after 14 and 15 months from ASCT and 2 died of disease progression 34 and 39 months after diagnosis. In the SEL group, 2 patients relapsed 12 and 22 months after ASCT and one of these died soon after of clinical progression of disease. One of the relapses in both groups occurred after double ASCT. One patient in the SEL group, transplanted with resistant disease, did not achieve hematologic recovery and died 46 days after ASCT of a cerebral hemorrhage.

Hematologic recovery and adverse effects

The median duration of neutropenia (<0.5×10⁹/L granulocytes) was 12 days in both groups. The median duration of thrombocytopenia (<50×10⁹/L platelets) was longer in the SEL group (21 vs. 16 days), but the difference was not significant (Figure 1). Four patients had a mild but prolonged thrombocytopenia (platelets <100×10⁹/L 3 months after ASCT). Three of them had received positively selected CD34⁺ cells; only one of them (belonging to the SEL group) had received ≤ 2×10⁶/kg CD34⁺ cells after myeloablative treatment. There was no difference in the hematologic support in the aplasia period between the two groups. Fifteen out of 23 SEL patients (65%) and 10/16 UNSEL patients (75%) developed fever after ASCT; febrile episodes were generally short-lasting with a median duration of 3 days in both groups. Bacteriemias were detected in 1/23 (4%) patients in the SEL group and in 5/16 (31%) patients in the UNSEL group. Severe stomatitis (WHO grade III or IV) occurred in 16/23 (70%) of patients in the SEL group and in 8/16 (50%) in the UNSEL group.

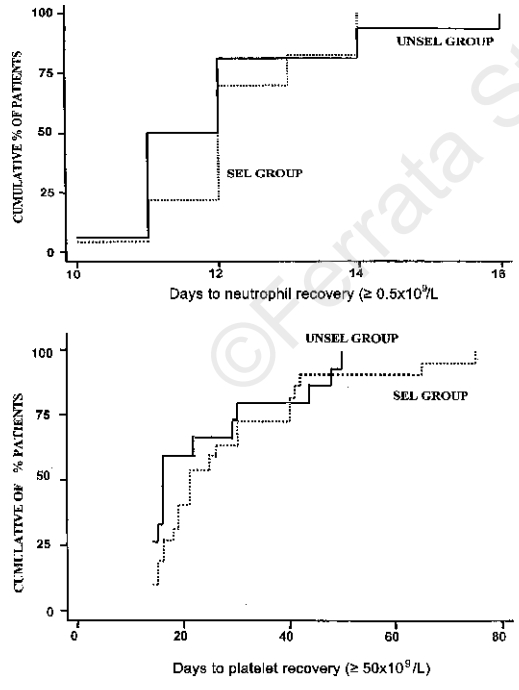

Figure 1. Hematopoietic engraftment after high-dose therapy followed by reinfusion of purified CD34⁺ cells (SEL group) or unmanipulated PBSC (UNSEL group). Data are presented as the cumulative percentage of patients who achieved neutrophil (≥0.5×10⁹ absolute neutrophil count/L) or platelet engraftment (≥50×10⁹ platelets/L). Differences between the 2 curves are not significant.

Discussion

Several publications in the literature report the persistence of myeloma cells in grafts used for autotransplantation. Immunophenotyping and molecular analyses show that the majority of PBSC collections, if not all, are contamined by myeloma cells, which represent up to 10% of PB mononuclear cells.[15-18]

Mobilizing regimens, high-dose cyclophosphamide as well as growth factors, are believed to enhance the contamination of PBSC harvests, influencing the expression of adhesion molecules associated with the myeloma cell membrane. This hypothesis is confirmed by the observation that the maximum peak of neoplastic cells is concomitant with that of circulating CD34⁺ cells.[19]

Polymerase chain reaction (PCR) techniques using an IgM chain gene fingerprinting or a patient-specific sequence derived from the rearranged variable region (VDJ) of immunoglobulin heavy-chain genes (IgH) have the greatest sensitivity and specificity to evaluate minimal residual disease; they can detect 1 clonal cell in 10^4-10^5 normal cells and can be used to perform quantitative analyses by generating titration curves of tumor cells. These studies are generally time-consuming and concern only small numbers of cases.[17-19]

Immunophenotyping techniques, which evaluate the expression of monoclonal cytoplasmatic light chains together with peculiar plasma cell antigens, are generally believed to be less accurate for evaluating neoplastic contamination. While the majority of studies use the CD38 Mab that is also expressed on subpopulations of T and B cells and on early hematopoietic progenitor cells,[20,21] in our study the specificity was enhanced by the addition of CD138. This Mab[14,22] is directed towards syndecan 1 expressed on and actively shed from the surface of plasma cells and myeloma cells, representing a central molecule in the regulation of myeloma growth and osteclastic activity. The sensitivity of this flow cytometric method can be enhanced by increasing the total number of cells analyzed. In our study up to 100 CD38/CD138 events were acquired, all the leukaphereses were positive and contained a median of 0.25% PC before selection.

The biological and prognostic significance of cancer cells present in autologous grafts is still unknown, but it is possible to hypothesize that reseeding of reinfused malignant cells contributes to relapse. Gertz, infact, found that increased numbers of monoclonal PC in the stem cell harvest were associated with a shortened relapse-free survival.[23] Therefore, we attempted to remove myeloma cells from PBSC autografts by positive selection of CD34⁺ cells using an avidin-biotin immunoabsorption technique. A few molecular studies have demonstrated that this technique removes 2 to 4 logs of clonal plasma cells.[17,19] In our study the flow cytometric re-analysis of the post-selection leuka-

phereses confirmed a 2-3 log reduction of the amount of plasma cell contamination.

Our study reports the clinical data of 23 patients reinfused with selected CD34+ cells; these results were compared with those obtained in a group of 16 patients with similar clinical features at diagnosis, treated with the same myeloablative regimen but reinfused with unselected PBSC. Our study confirmed no significant difference in hematologic reconstitution and no increase in toxicity or hematologic support requirement, as reported previously for small groups of patients.[19,24] A large phase III trial reported that the median time to platelet engraftment was slightly prolonged in a subgroup of patients receiving a CD34-selected transplant with less than 2×10^6 CD34+ cells/kg.[25] In our series, although the short-term megakaryocytic reconstitution was similar in both groups, in the SEL group we noted a higher frequency of mild prolonged thrombocytopenia that was not correlated with a threshold of infused CD34 cells. A greater incidence of Gram positive septicemias in the latter group might not be significant since all febrile episodes were short lasting and responded promptly to antimicrobials.

The issue of a possible better outcome after reinfusion of selected CD34+ cells was not addressed by previous studies and is difficult to do so with this one considering the small number of cases, the retrospective analysis and the short-lasting follow-up. A larger proportion of patients had a response after the first ASCT in the SEL group than in the UNSEL one (86% vs. 69%); no advantage in terms of CR was detected. These results were similar to the preliminary data reported by Vescio *et al.* in a randomized trial, in which no significant difference was reported between the two arms in the outcome 1 year after a single ASCT.[25] However in our study, in the few patients who underwent tandem transplantation, CRs were more frequent after reinfusion of selected blood progenitors suggesting a possible benefit from increasing tumor cytoreduction. Plasma cell infiltration progressively decreased during the successive stages of the treatment and became undetectable by immunohistochemical evaluation of the marrow in about one third of the patients, without differences between the two groups. All the studies agree on the persistence of residual plasma cell clone in the marrow of the majority of the patients after ASCT: CRs that vary from 20 to 40% on the basis of classical criteria (negative immunoelectrophoresis and negative marrow histology)[7,26-29] decreased to 7% after a PCR-based approach using patient-specific tumor markers.[30] The lack of myeloma eradication can explain the relapses after one and even double ASCT occurring in both our series of patients. However, a longer follow-up is needed to evaluate whether the amount of the residual plasma cell contamination after purging is related to the duration of the relapse-free survival.

In conclusion, our study suggests that the 2-log reduction of plasma cell contamination of the leukaphereses obtained by positive selection permitted rapid hematopoietic engraftment after ASCT but did not produce a significant enhancement of the frequency of CR in comparison with the frequency in patients receiving unselected cell transplantation after a median follow-up of 18 months, although longer observation is needed to draw definitive conclusions.

A greater reduction of tumor contamination of leukaphereses (up to 4-5 log) achieved by new purging systems and an increase of tumor cytoreduction through double transplantation should be considered in designing the future intensive treatment plans for myeloma patients.[31,32]

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Contributions and Acknowledgments

FP, DD and RF designed the study, FP was responsible for data management and prepared the manuscript. DD and RF contributed to paper writing. DD and SF performed the cytometric analyses and interpretated the data. AG, MC, FZ and FS collaborated in patient care and data analysis. AS contributed to the execution of the study and the statistical analysis. MB participated in the study design and critically revised the final version of the manuscript. The criteria for the authors' name order are: 1st name: principal investigator and writer; 2nd and 3rd name: contribution in study design and paper writing; 4th name: cytometric analysis; 5 to 9th name: clinical work; 10th name: head of the Department in which the study was performed.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

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Potential implications for clinical practice

- Positive selection of PBSC can be safely applied to reduce PC contamination of leukaphereses because it allows rapid engraftment of hematopoiesis and mild toxicity after myeloablative treatment.
- A flow-cytometric technique using the Mab CD138 alternatively coupled with CD38 and cytoplasmic light chains can be used for quantitative evaluation of the PC contamination of leukaphereses and the efficacy of purging.
- In our study no statistical difference was reported in clinical outcome at a median follow-up of 18 months between patients reinfused with selected CD34+ cells and a historical control group receiving unmanipulated PBSC. However, the issue of a possible better clinical outcome after reinfusion of selected CD34+ cells is worthy of investigation by randomized studies and patients need to be followed-up for longer.

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