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MOBILIZATION OF CIRCULATING PROGENITOR CELLS IN MULTIPLE MYELOMA DURING VCAD THERAPY WITH OR WITHOUT rhG-CSF

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ABSTRACT

Background. **Circulating progenitor cells (CPC), when infused in large numbers, rapidly repopulate the marrow after myeloablation with high-dose therapy. In multiple myeloma (MM), as in other disorders, different chemotherapy regimens, including single-as well as multiple-agent chemotherapy, with or without hemopoietic growth factors, have been proposed to mobilize these progenitor cells into the blood. Here we report our experience with a drug combination called VCAD and compare the results to those obtained by adding rhG-CSF to the same combination.**

Methods. **Fourteen MM patients were given one course of VCAD, a chemotherapy association of vincristine 2 mg, cyclophosphamide 4×0.5 g/m², adriamycin 2×50 mg/m² and dexamethasone 4×40 mg, before undergoing apheresis to collect CPC for autografting. Seven also received rhG-CSF (filgrastim) 5 mcg/kg/day over the period of apheresis. These latter were allocated to rhG-CSF treatment sequentially from the time the drug became available for clinical use.**

Results. **Following VCAD-induced pancytopenia, CFU-GM peaked at a median of 853/mL (range 96-4352; 7.6 times basal level). RhG-CSF administration increased CFU-GM levels but not significantly. With rhG-CSF the CFU-GM peak was reached sooner, toxicity was reduced and granulocytopenia less protracted. Fewer aphereses were run in the rhG-CSF group, there were higher yields per single run, and patients began and completed their collection program more quickly.**

Conclusions. **The VCAD association is able to mobilize CPC in patients with MM, and rhG-CSF is recommended as a fundamental part of the priming schedule.**

Key words: multiple myeloma, autologous transplantation, circulating progenitor cells, apheresis, recombinant human granulocyte colony-stimulating factor (rhG-CSF)

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Efforts to improve response rate and survival in multiple myeloma (MM) include the use of marrow-ablation regimens followed by autologous transplantation.[1,2] However, transplantation carries an extra risk in these patients since they are often elderly and in poor clinical condition.[3]

Circulating progenitor cells (CPC) can hasten hemopoietic reconstitution after high-dose therapy,[4] particularly if there is a large number of committed progenitors in the grafted sample.[5] The CPC level is very low in the steady phase but shows a dramatic increase contemporaneously with the rise in leukocytes after the post-chemotherapy nadir.[6] CPC samples can then be consistently obtained with a few apheretic procedures. They can also be mobilized with the hemopoietic growth factors rhGM-CSF and rhG-CSF,[7,8] or following cytotoxic treatment.[9-12] The combination of both chemotherapy and rhGM-CSF or rhG-CSF is even more effective.

In the present study of 14 patients with MM, we examined the mobilizing effect of a combi-

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nation of 4 drugs: vincristine, cyclophosphamide, adriamycin and dexamethasone (VCAD). We also tested whether the addition of rhG-CSF to chemotherapy would reduce its toxicity and improve the collection yield, resulting in the need for fewer apheresis procedures.

Materials and Methods

Patients

Fourteen patients with MM were recruited between May 1989 and January 1993. Their details are given in Table 1. There were 6 men and 8 women, median age 53 years (range 40 to 61), 9 were classified as IgG, 3 as IgA, 1 as BJ and 1 had cytologically proven IgM myeloma. Initially all had a high tumor mass[13] and the median interval from diagnosis to mobilization chemotherapy was 12.5 months (range 3 to 72).

Before CPC mobilization was begun, 8 patients had achieved a partial remission (#1, 2, 3, 5, 9, 10, 12 and 14), defined as a reduction of at least 50% in marrow plasmacytosis and monoclonal component, as measured by electrophoresis of serum and/or urine. Another (#4) was in complete remission (reduction of 95% in marrow plasmacytosis and no detectable monoclonal component in serum or urine). One (#13) had relapsed after receiving the standard melphalan-prednisone combination but was still responsive to cyclophosphamide and dexamethasone. The remaining 4 subjects (#6, 7, 8 and 11) were primarily resistant to two or more chemotherapy combinations. The median plasma cell infiltration was 16% (range 4-90) when patients were evaluated before starting VCAD therapy. The antitumor efficacy of VCAD was evaluated by the variation in the serum monoclonal component.

Table 1. Patient characteristics at diagnosis and chemotherapy prior to VCAD.

Pt.	*Age sex*	*Stage at diagnosis*	*Paraprotein*	*Therapy prior to mobilization*	*Time from diagnosis to mobilization (months)*
1	50/M	III B	IgG λ	RT/CY+PDN	8
2	40/F	III A	IgG κ	CY+PDN	10
3	54/M	II A	IgG λ	RT/CY+PDN	11
4	43/M	III A	IgM κ	MP/VAD/CY+DEXA	72
5	53/F	III A	IgG κ	CY+PDN	15
6	60/M	III A	IgA κ	RT/VAD/CY+PDN/CED	3
7	61/M	III A	IgG κ	CY+PDN or +DEXA	17
8	54/F	III A	IgG κ	MP/CHOP	24
9	56/F	II A	IgG κ	VAD/CY+PDN/CED	14
10	41/F	II A	IgG λ	MP	9
11	55/F	III A	IgG λ	VAD/CY+PDN/CED/CY+DEXA	9
12	52/M	III A	IgA κ	MP/VAD/CY+PDN/CED	15
13	44/F	III A	BJ κ	MP/CY+DEXA	22
14	52/F	III A	IgA κ	CY+DEXA	6

CED: cisplatinum 60 mg/m^2, etoposide 100 mg/m^2, dexamethasone 40 mg/d x 4: (median 1 cycle, range 1-2). CHOP: cyclophosphamide 750 mg/m^2, vincristine 1.4 mg/m^2, adriamycin 50 mg/m^2 and prednisone 100 mg/d x 4 (3 cycles). CY+DEXA or CY+PDN: cyclophoshamide 1.2 g/m^2/d x 2 and dexamethasone 40 mg/d x 3, or prednisone 250-125 mg/d x 4: (median 6 cycles, range 1-11 cycles). MP: melphalan 6 mg/m^2/d x 7 and prednisone 60 mg/m^2/d x 7: (median 6 cycles, range 4-9). VAD: vincristine 1 mg, adriamycin 50 mg/m^2, dexamethasone 40 mg/d x 4: (median 1 cycles, range 1-3). RT: local radiation therapy on bone lesions.

Mobilization and collection of CPC

All patients received a single course of VCAD chemotherapy intravenously. Vincristine 2 mg was given on day 1 only, cyclophosphamide 500 mg/m^2/day on days 1 to 4, adriamycin 50 mg/m^2/day on days 1 and 2, and dexamethasone 40 mg/day on days 1 to 4. This combination, which had already been used for mobilization,[23] employs drugs reported to be effective in MM, and here there were delivered in amounts that exceeded the conventional dosages. The patients also received oral fungal, bacterial and viral prophylaxis.

Recombinant human G-CSF (filgrastim) became available during the study and patients 8-14 were sequentially treated with it. Administration was by continuous iv infusion at 5 mcg/kg/day, starting 24 hours after the last dose of dexamethasone (day 5) and continuing until the last apheretic procedure or until the WBC count rose above 40×10^9/L.

At the beginning of the study (patients #1-7), the criteria for starting apheresis were rises in WBC above 1.0×10^9/L with monocytosis and increases in platelets above 50.0×10^9/L following the chemotherapy nadir. As soon as the CD34 assay was made available to our laboratory (patients #8-14), apheretic collections were started the day after the appearance of detectable CD34$^+$ cells in the peripheral blood.

Apheresis was performed on consecutive or alternate days using 9000 mL of blood sampled

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vein-to-vein or, when needed, via a femoral venous catheter (VASCATH). In our experience the femoral vein is easier to catheterize than the subclavian and carries a lower risk of immediate complications. Continuous-flow cell separators (Baxter CS-3000 or Fresenius AS 104) were programmed to collect mononuclear cells. No sedimenting agents were used. Following the apheretic procedure, cells were suspended in 10% DMS0 in culture medium (DF-700 Gambro bags) and frozen at a controlled rate of –1°C/min to –40°C (Planer R203) before being stored in liquid nitrogen.

The number of apheretic procedures was determined empirically.

In the first group of patients (#1-7), a standard of 6-7 procedures was originally planned on the basis of our previous experience with lymphoma patients.[12] However, in two patients (#6, 7) the collections were stopped earlier due to poor performance and the appearance of fever and infection (see the section *Non-hematologic response to VCAD*). In patients monitored by the $CD34^+$ cell assay (#8-14), the apheretic procedures were stopped as soon as the $CD34^+$ cell level declined following a peak value. In three patients (#8, 11, 12), however, the $CD34^+$ cell level failed to increase substantially and the apheresis procedures were stopped as consequence of documented poor yields.

CFU-GM assay

Analysis of colony-forming units granulocyte-macrophage (CFU-GM) was performed on peripheral blood and apheresis samples by the two-layer agar-gel technique:[14] 1 mL samples containing 10^5 mononuclear cells suspended in IMDM-15% FCS and 0.3% agar were plated on top of the feeder layer. They were gelled at +4°C, then incubated at 37°C in a humidified atmosphere with 5% CO_2 for 14 days. Aggregates of ≥ 40 cells were scored as colonies. With this method the normal reference range for our laboratory is 6.7-1260, median 436/mL circulating CFU-GM.

Flow cytometry progenitor cell assay

The CD34 assay was also performed on peripheral blood and apheresis samples after immunofluorescent labelling using standard protocols. Red blood cells were eliminated by incubation with 2 mL ammonium chloride (Ortho-mune Lysing Reagent) for 5-10 minutes. Mononuclear cells were then washed twice and $0.5\text{-}1 \times 10^5$ cells were incubated with 10 μL CD34 anti-HPCA-2 MoAb (Becton & Dickinson) for 30 min at 4°C. For each reading 10^4 cells were collected and analyzed by flow cytometry using a FACScan (Becton & Dickinson). To reduce signal overlap, the Ig isotype control was set separately for the lymphocyte and monocyte regions using large contiguous gates on mononuclear cells.[15] Nevertheless, we analyzed all events with lympho- and monocyte features so as to reduce the risk of missing cells because of arbitrarily defined gates. Granulocytes were not analyzed. With this method the normal reference range for our laboratory is 0-14.6 (median 5.6)$\times 10^6$/L $CD34^+$ circulating cells.

Autograft and hematologic reconstitution

Eleven patients were autografted. Three others died of disease progression before the autograft could be performed (#7, 11 and 12).

For the autograft patients were nursed in sterile, positive-pressure or laminar-flow single rooms with oral fungal, bacterial and viral prophylaxis. They were treated according to the high-dose transplant regimen with a combination of drugs administered intravenously (BEM protocol), which included BCNU 200 mg/m^2/day on days –8 to –7, etoposide 250 mg/m^2/day on days –8 to –6, and melphalan 140 mg/m^2 on day –2.

A stem cell infusion was either given on day 0, or spread over two days if the volume of the apheresis products exceeded 500 mL. Seven patients (#1, 3, 4, 9, 10, 13 and 14) received these alone, while 4 (#2, 5, 6 and 8) were also given autologous bone marrow cells. The reason for infusing bone marrow was a poor CFU-GM collection ($< 10 \times 10^4$/kg) in 3 patients (#2, 5, 6) and a previous episode of transient ischemic attack in another (#8). The Gambro bags containing the peripheral and bone marrow cells were placed in a 37°C waterbath to thaw them rapidly, then immediately reinfused into the patient without further manipulation.

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Blood counts and differentials were performed each day after the graft. The time to hematological reconstitution was expressed as the number of days post graft until readings were steady and unsupported for at least three days. Systemic antibiotics were administered if fever (>38°C) persisted beyond 2 hours, and standard criteria were used when changing or stopping antibiotics.

Statistical methods

Correlations were derived by the least-squares linear regression technique, and numerical data were compared with the Student's t-test. Probability curves were compared using the Peto-Wilcoxon test.

Results

Non-hematologic response to VCAD

Nine patients developed fever (>38°C) of short duration (median 3 days, range 1-4) which was empirically treated with systemic antibiotics. Mild or moderate nausea and vomiting occurred in 9 patients. Mild mucositis was observed in 7 and mild hemorrhagic cystitis in 2. There were no toxic deaths.

There were no marked toxic reactions to rhG-CSF and it was never necessary to withdraw it. VCAD toxicity was also milder in the patients who received it: there was a significant reduction in the duration of fever (median 1 vs 3 days, $p < .01$) and of systemic antibiotic therapy (3 vs 10 days, $p < .001$). The two documented episodes of infection (one gram$^+$ sepsis and one clinically diagnosed bronchopneumonia) were in the untreated group (#6 and 7), though both responded rapidly to antibiotic therapy. In these patients the collections were stopped earlier.

Hematologic recovery after VCAD

Severe granulocytopenia (below 0.5×10^9/L) developed in 12 patients over a period of 6 to 10 days (median 7) after VCAD. It lasted from 1 to 11 days (median 3) and subsequent recovery took 11 to 21 days (median 14). Recovery was quicker in the rhG-CSF-treated group (median 12 days, range 11-14 vs. 16 days, range 15-21; $p < .001$). Nine patients experienced mild thrombocytopenia (between 20 and 50×10^9/L) lasting from 1 to 6 days (median 1). Recovery to over 50×10^9/L occurred within 11 to 16 days (median 14), and only 2 patients required platelet transfusions. Recovery was not affected by rhG-CSF treatment.

Effect of VCAD on tumor cell population

Thirteen subjects had a measurable tumor mass at the onset of VCAD administration and 6 (46%) showed beneficial effects. Of these, 4 (#1, 5, 9 and 10) were in partial remission and had reductions of ≥30% in the monoclonal component. Two more patients (#6 and 8) were in a progressive phase and showed greater improvement (≥50%).

Timing and yields of progenitor cell collections

The two groups, those treated with rhG-CSF and those not, were very similar with regard to age, chemotherapy resistance and duration of treatment before VCAD mobilization (Table 1, #1-7 and 8-14).

Post-VCAD values are shown in Table 2. The peak values of CFU-GM ranged from 96 to 4352/mL (median 853), a 7.6-fold increase over basal values (range 1.7 to 1125). These peak values occurred earlier in the rhG-CSF treated group (13 days vs. 20, $p < .004$) and were higher, though not significantly, in the treated than in the untreated group.

The rhG-CSF-treated patients began apheresis earlier than the others (12 days, range 9-14 vs. 14, range 13-21; $p < .03$) and completed it sooner (15 days, range 10 to 17, vs. 23 days, range 20 to 30, $p < .0005$). The yield of CFU-GM per procedure was also higher in the rhG-CSF-treated group (4.9×10^4/kg vs. 1.2×10^4/kg, Table 3), but this difference was not significant.

Non-hematologic response to autograft

Transplant was well tolerated and there were no deaths. Several patients experienced fever (median 2 days, range 0-8) and received systemic antibiotics (median 11 days, range 0-16). Transfusion needs were modest: 2 packed cell units (range 0-4) and 2 single-donor concentrates (range 1-4). There were no significant dif-

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Table 2. Peak values of CPC cells compared with rhG-CSF administration, WBC level at day of first apheresis, peak value of $CD34^+$, day when CPC and $CD34^+$ values peaked . The table also shows the number of aphereses and the day of first and last apheresis.

Pts	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*
rhG-CSF	no	no	no	no	no	no	no	yes	yes	yes	yes	yes	yes	yes
WBC level x10^9/L at day of 1st APH	1.1	1.4	1.2	1.4	0.8	0.8	0.8	4.1	2.1	2.8	3.5	2.0	4.8	4.0
CPC peak value*	1953	622	4352	1262	555	123	96	ND	ND	893	114	813	1125	1166
CD34 peak value°	ND	ND	ND	ND	ND	ND	ND	8.2	56	22	2.6	10.7	35.5	64.8
Day CPC peaked	17	30	19	20	21	17	28	ND	ND	9	15	17	13	13
Day CD34 peaked	ND	ND	ND	ND	ND	ND	ND	15	13	10	14	16	13	13
Day of 1st APH	15	17	14	13	14	13	21	12	11	9	14	13	12	13
No. of APH	7	6	6	6	6	4	3	3	2	2	2	2	3	3
Day of last APH	25	30	21	21	23	20	28	15	13	10	15	17	14	16

ND: measurement not made. APH: apheresis. *Peak values of CPC expressed as CFU-GM/mL; °CD34 x 10^6/L. The CFU-GM and $CD34^+$ basal levels were, respectively, 0-320/mL (median 56) and 0-15.4x10^6/L (median 6.4).

ferences between the responses of the rhG-CSF-treated and untreated groups.

Hemopoietic reconstitution after autograft

The autograft provided an infusion of 3.2×10^8/kg circulating mononuclear cells (range 1.1-5.8) and 12.1×10^4/kg CFU-GM (range 1.0-33.2). The granulocyte level recovered rapidly in all cases, reaching 0.5×10^9/L in 13 days (range 11-17) and 1.0×10^9/L in 16 days (range 12-21).

Corresponding figures for platelet recovery were 13 days to 50.0×10^9/L (range 10-56) and 29 days (range 12-350) to 100.0×10^9/L. Two patients took more than 120 days to reach this second level. The time to reach a count of $>0.5\times10^9$/L granulocytes and $>50.0\times10^9$/L platelets was different, although not significantly, between rhG-CSF-treated and untreated subjects (Table 3). No difference in granulocyte and platelet recovery was seen between CPC and CPC+BM-cell autografted patients.

We were unable to demonstrate a correlation between the number of circulating CFU-GM and the time to hemotopoietic recovery since subjects with the lowest levels engrafted as quickly as the rest.

However, there was a significant inverse relationship between the time taken to reach the 50×10^9/L platelet level and the number of $CD34^+$ cells infused ($r=-0.92$, $p<.02$).

Discussion

Patients receiving CPC show more rapid hemopoietic recovery after autologous transplantation, with a smaller demand for blood derivative products and a shorter time in hospital.[4] This effect is due in part to the number of CPC infused, itself a product of mobilization therapy.

In MM, mobilization of CPC has used single-drug[16-22] as well as multiple-drug schedules.[23] But although some regimens are very effective, particularly those using high-dose drugs,[17,19,21,24] there is still concern over the variation in the resulting levels of progenitor cells,[25] a fact that may reflect the degree of involvement of bone marrow by the myeloma cell population.[20]

In our present investigation we show that the combination of vincristine, cyclophosphamide, adriamycin and dexamethasone (VCAD) is effective in mobilizing CPC in a good portion of myeloma patients, especially when rhG-CSF is given following chemotherapy. Although this study was not randomized, the groups were well matched, showing that rhG-CSF administration is associated with a reduction in the extent and duration of cytopenia following VCAD. It also strongly suggests there is an increase in CFU-GM yield in treated patients. We also confirm broad patient-to-patient variation in MM in the level of circulating progenitors after mobilization.

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Table 3. Yield of mononucleated cells, CFU-GM and CD34+ cells on apheresis, expressed per kg body weight, and for CFU-GM also as single apheresis yield. Number of patients undergoing autograft and hematologic recovery after graft is also shown.

Pts.	*MNC x 10^8/kg*	*CPC x 10^4/kg*	*CD34 x 10^8/kg*	*CPC x 10^4/kg per single APH*	*BMT*	*days to PMN >0.5/1.0 x10^9/L*	*days to PLT >50/100 x10^9/L*
1	8.6	63.8	ND	9.1	yes	12/15	15/37
2	8.1	2.6	ND	0.4	yes*	16/17	15/44
3	6.7	62.6	ND	10.4	yes	16/18	11/12
4	6.3	13.9	ND	2.3	yes	14/18	14/30
5	3.3	7.2	ND	1.2	yes*	13/14	13/29
6	3.4	1.8	ND	0.4	yes*	17/21	32/145
7	2.1	1.6	ND	0.5	no	–	–
8	4.9	23.0	0.5	7.7	yes*	15/16	56/350
9	1.6	34.2	4.6	17.2	yes	12/15	12/14
10	4.1	9.9	7.9	4.9	yes	13/15	10/15
11	0.5	5.2	0.3	1.6	no	–	–
12	1.9	6.7	0.6	2.2	no	–	–
13	3.9	10.3	6.6	3.4	yes	13/16	12/13
14	5.5	51.8	7.0	17.2	yes	11/12	12/20
Median	4	10.1	4.6	2.8		13/16	13/29
Range	0.5-8.6	1.6-63.8	0.3-7.9	0.4-17.2		11-17	10-56
						12-21	12-350

MNC: mononucleated cells; CPC: CFU-GM; APH: apheresis.
*CPC+BM cell infusion at graft.

We were unable to assess precisely the efficacy of VCAD as an antitumor treatment in our selected group of patients, although half had a measurable response. But since the combination of VCAD and rhG-CSF demonstrated acceptable toxicity, it seems possible to repeat the course in the same patient.

Previous studies on solid tumors and hematological neoplasms involving the bone marrow[26-28] have shown that engraftment is further accelerated by *priming* the CPC with the hematopoietic growth factors rhG-CSF and rhGM-CSF. We have made similar observations in MM, showing distinct advantages for rhG-CSF priming after chemotherapy. Apheretic sampling is more reliable, demonstrating the need for fewer procedures with a higher yield each session and a shorter overall collection program. The 3 patients in our study with total CFU-GM yields below 5×10^4/kg were all in the rhG-CSF-untreated group.

However, the increase in CPC after rhG-CSF priming was inferior to that observed in other conditions.[8] This may be due to a limited mobilizing capacity of VCAD. In fact, this drug combination contains adriamycin, which may not be a stem cell-sparing agent. Nevertheless, progenitor cell release in MM may also be influenced by other factors such as the extent of marrow plasmocytosis,[25] prior use of alkylating agents[17] and previous prolonged cytotoxic drug exposure.[21] It is impossible to evaluate all these factors with the limited number of patients available in one center, and a cooperative study would be necessary for that.

In the present study rhG-CSF priming did not seem to affect the speed of hematological recovery after autograft. This finding has already been reported[29] but is in contrast with other studies conducted on different categories of patients.[30] Although the unprimed group required more apheretic collections, it ultimately received a similar number of cells and recovered granulocytes in a similarly short time following autograft. All our subjects maintain a stable hemopoiesis following initial engraftment. This is further evidence of the long-term safety of CPC.

We conclude that even a strongly myelosuppressive association like VCAD allows collection of a sufficient number of CPC for safe autologous transplantation. This especially holds true when rhG-CSF is added to the mobilizing regimen.

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