Low doses of GM-CSF (molgramostim) and G-CSF (filgrastim) after cyclophosphamide (4 g/m2) enhance the peripheral blood progenitor cell harvest: results of two randomized studies including 120 patients.

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Running Title : G-CSF versus G-CSF plus GM-CSF for mobilization/collection of PBPC

ABSTRACT

The use of a combination of G-CSF and GM-CSF to G-CSF alone, after cyclophosphamide (4g/m²) was compared in 2 randomized phase III studies, including 120 patients. In study A, 60 patients received 5 x2 µg/kg/day of G-CSF and GM-CSF compared to 5 µg/kg/day of G-CSF. In study B, 60 patients received 2.5 x2 µg/kg/day G-CSF and GM-CSF compared to G-CSF alone (5 µg/kg/day). With the aim to collect at least 5 x 10^6/kg CD34 cells in a maximum of 3 large volume leukapherisis (LK), 123 LK were performed in study A, showing significant higher number of patients reaching 10 x 10^6/kg CD34 cells (21/29 in G+GM-CSF arm vs 11/27 in G-CSF arm, *P= .00006)*. In study B, 109 LK were performed, with similar results (10/27 vs 15/26, *P= .003*). In both the study, the total harvest of CD34 cells/kg was 2-fold higher in G-CSF plus GM-CSF group (18.3 x 10^6 in study A and 15.85 x 10^6 in study B) than in G-CSF group (9 x 10^6 in study A and 8.1 x 10^6 in study B), a difference particularly seen in multiple myeloma, with no significant difference in terms of mobilized myeloma cells between G-CSF and GM-CSF groups.

Key words: G-CSF, GM-CSF, hematopoïetic stem cells.

INTRODUCTION

Autologous peripheral blood progenitor cells (PBPC) provide a rapid and sustained hematopoïetic recovery after the administration of high dose therapy in patients with haematological malignancies and certain solid tumors (1-5). The mobilization of PBPC is usually achieved with the use of haematopoietic growth factors (HGFs) alone or in combination with chemotherapy, in which case a higher yield of CD34+ cells can be reached and collected (6). It is generally recognized that granulocyte-colony-stimulting factor (G-CSF) as a single agent mobilizes more CD34+ cells than does granulocyte-macrophage-colony stimulating factor (GM-CSF) (7,8 and for review, 9). Other HGFs, such as Flt3 ligand (10), interleukin-3 (11), stem cell factor (12-13) and erythropoietin (14) or antagonists of SDF-1-CXCR4 (15) have been tested generally in combination for mobilizing PBPC. The recommended dose of G-CSF after chemotherapy is 5µg/kg/d (16). Increasing the dose of G-CSF superior to 5µg/kg/d (17, 18) or changing the administration schedule (19) has not been proven to improve PBPC mobilization. More recently, the use of a single dose of pegfilgrastion was demonstrated as equivalent to a daily administration of filgrastim for mobilizing PBPC (20). It has been shown the therapeutic relevance of high dose of CD34 cells in autologous PBPC transplantation. The infusion of $5x10^6$ CD34/kg minimum results in rapid engraftment, reduces the transfusion need and may have a clinical influence (21-23). The synergistic effect of co-administration of HGFs like G-CSF and GM-CSF on PBPC mobilization has been suggested in phase I/II study (24), in order to improve the harvest of CD34 cells (25). Several non-randomized or randomized clinical trials have been performed, showing a little or no benefit for sequential administration of standard doses (5-10 µg/kg/d) of GM-CSF and G-CSF (26-37). Neverthelesss, the use of these HGFs was not well explored and particularly the minimal efficient dose for their concomitant administration, following high dose cyclophosphamide (CY). We report here two randomized studies in order to

determine first, whether the concomitant administration of GM-CSF and G-CSF could improve the PBPC mobilization and collection to achieve a target yield of 5 x 10^6 CD34 cells/kg, and secondly what is the minimal dose for this combination of HGFs.

MATERIALS AND METHODS

Study design.

As shown on Fig.1, this was a randomized, open-label, unicenter study, including two parts (study A and study B). The primary objective of this study was to achieve $10x10^6$ CD34 cells/kg in a minimal number of leukapheresis. Calculation of the number of subjects was based on the fact that the percentage of the subjects reaching more than $10x10^6$ CD34 cells/kg in the combination arm will be twice than that observed in the single-cytokine arm. With a power of 90% and a 5% risk, the number of patients is 25 per arm. For that reason, we decide to include 30 patients per arm due to the possibility of unevaluable patients. There was no stratification. The secondary objectives of this study include the tolerance, the factors influencing the mobilization of progenitor cells, the percentage of myeloma cells mobilized in PB, and the difference of mobilization at the level of $5x10^6$ CD34 cells/kg between the 2 arms. In study A, 60 patients were randomized to receive after CY (4 g/m^2) the co-administration of 5 µg/kg/day (d) of rHu-GM-CSF (Molgramostim, Schering-Plough, Kenilworth, NJ, USA) and 5 µg/kg/d of rHu-G-CSF (Filgrastim, Amgen, Thousand Oaks, CA) (G + GM-CSF, total HGF dose = 10 µg/kg/d) compared to 5 µg/kg/d of G-CSF (Filgrastim). In study B, 60 subsequent patients have received the same design of treatment with 2.5 µg/kg/d of GM-CSF and 2.5 µg/kg/d of G-CSF (G + GM-CSF, total dose of HGF = 5 µg/kg/d) compared to 5 µg/kg/d of G-CSF. The 4 g/m^2 CY was administred by intravenous (IV) route to all the patients followed 24 hours later by the HGF administration subcutaneously until the last day of leukapheresis (LK). For patients receiving the 2 HGFs, they were injected in separate sites.

Patient Eligibility

Written informed consent was obtained from all patients enrolled in the study. Patients with histologically confirmed history of cancer and requiring a high dose myeloablative

chemotherapy with PBPC rescue were eligible. Disease status and demographic characteristics are detailed in Table I. Inclusion criteria were: age between 18 and 65 years, performance status $\leq$ 2, half-life expectancy of at least 6 months, normal organ function as defined by serum creatinine, transaminase <2 times normal range, cardiac ejection fraction within the institution's normal range, and normal blood count (ANC > 1.5 x 10^9/L, platelets > 100 x 10^9/L) at the day of mobilization chemotherapy. Exclusion criteria were: patients who had received HGF within 2 weeks before study entry, patients with other malignancies within 5 past years, HIV1 or 2, HTLV 1 or 2, hepatitis C sero-positivity, hepatitis B positive virological status, patients with psychiatric, addictive or any disorder which compromised their ability to give truly informed consent.

Stem cell collection.

White blood count (WBC) were assessed daily after the beginning of chemotherapy. When it reaches 0.5 10^9/L, the percentage of circulating CD34 cells was monitored daily according to the ISHAGE protocol, by using a phycoerythrin-conjugated (PE) antibody to CD34 (Q-Bend 10, Immunotech, Marseille, France) (38). For each labelled sample, 30,000 events were recorded on forward-versus-side scatter dot-plot using a FACScan (Becton-Dickinson, San-Jose, CA, USA). The estimated number of CD34 cells/kg that can be collected in one LK was calculated by the central laboratory according to the following formula: (%CD34 x WBC x 8/kg body weight (8 being a coefficient corresponding approximately to 2 blood volumes in L). Then, a LK was started if the estimation of harvestable CD34 number was $\geq$ 10^6 CD34/kg. If the estimation of harvestable CD34 cell number was below 10^6 CD34/kg the collection was delayed. A maximum of three LK was attempted to reach a minimum target of 5 x 10^6 CD34/kg. For MM patients, at this time, we performed a CD34 selection in order to decrease contaminating tumoral cells. For that reason, due to the loss of cells during this procedure, we attempt to collect 10 x 10^6 CD34/kg. Large volume LK was performed through a dual

peripheral venous puncture using a Cobe Spectra separator version 4 (CS 3000; Baxter Healthcare Corp Lakewood, Co). Median time of processing was 5 hours and the mean volume of processed blood ranged between 12 to 16 L.

Analysis of product of leukapheresis.

The determination of CD34 cell content in the LK was carried out according to the following procedure. Incubation of 10^6 cells with 30% AB serum (100 µL) for 10 mn, two washings with PBS, suspension in a final volume of 100 µL, incubation with 20 µL of monoclonal antibody CD34 conjugated with PE, or 20 µL of murine IgG recognizing no human antigen and conjugated with PE as a negative control, or 5 µL anti-CD 45 conjugated with PE as a positive control for 30 mn at 4°C, cell lysis followed by two washings and resuspension in 200 µL PBS. Cells are analysed with a FACSCAN cytometer. At least 30,000 events were acquired for each sample.

In patients with Multiple Myeloma (MM), myeloma cells were enumerated by FACS analysis using the FITC-conjugated MI15 (anti-CD138 mAb (39). 10^6 cells were incubated with 1 µg $MI15^{FITC}$ (10 µL) or to 1 µg IgG^{FITC} negative control (20µL) and fluorescence was analysed with FACSCAN cytofluorometer. No assesment of CD34 cells was performed on the LK product.

Autologous stem cell transplantation (ASCT).

After a resting time of a maximum of 8 weeks, patients received high dose chemotherapy (HDC) followed by autologous PBPC transplant. MM patients were scheduled to receive two subsequent HDC and PBPC transplants. The first HDC was 140 mg/m^2 Melphalan. At least three months later, patients received the second HDC consisting of 200 mg/m^2 melphalan or 140mg/m^2 melphalan and total body irradiation delivering 12 Gy followed by 5 µg/kg/d G-CSF support administrated from day 2 after HDC and until ANC > 1.5 x 10^9/L on two consecutive counts. Patients having malignant lymphoma received a BEAM regimen

consisting on 300 mg/m^2 BCNU, Etoposide 200 mg/m^2/d for four days, Cytarabine 200 mg/m^2/d for four days and Melphalan 140 mg/m^2 without post-transplant G-CSF support. Breast cancer, testis and ovarian cancer patients were scheduled to receive one or two HDC and PBPC transplant. The following parameters of haemotologic recovery were analyzed: day of ANC $\geq$ 0.5 x 10^9/L, day of platelet count $\geq$ 50 x 10^9/L, cost estimation of the whole transfusion procedure and duration of hospitalization.

Statistical analysis.

An intent-to-treat analysis was done taking into account all patients who received CY and HGFs. A Wilcoxon non-parametric test was carried out to assess the comparability of group at randomization. The comparison of number of LK per group were evaluated by an exact Fischer's test. The median values of CD34 cells harvested in the different groups were compared by a Mann-Withney non-parametric test. The comparison of distribution of patients with 1 to 3 LK to reach the pre-defined threshold was done by the Chi 2 test. If none LK was performed it is considered as mobilization failure and not evaluated. Data were analysed using SAS (Statistical Analysis System) software version 6.08.

RESULTS

Patient population (Table I)

One hundred twenty consecutive patients were enrolled and randomized in the department. All patients had a disease requiring high-dose chemotherapy and PBPC transplantation as salvage therapy. They were: 58 (48%) MM patients (stage II or III), 32 (27%) non-Hodgkin Lymphoma (NHL) (large cell type with IPI≥3 or sensitive relapse), 8 (7%) Hodgkin disease (HD) (sensitive relapse or refractory), and 4 (3%) chronic lymphocytic leukemia (CLL) (Binet stage B and C), and 18 (15%) solid tumors (ST) including 13 metastatic (or high-risk) breast cancer, 4 metastatic testis cancer, 1 metastatic ovarian cancer. All patients were newly diagnosed and received a range of 3 to 16 courses of chemotherapy before PBSC mobilization lasting less than one year.

Sixty patients were included in study A (5 µg/kg/d G-CSF plus 5 µg/kg/d GM-CSF versus 5 µg/kg/d G-CSF): 26 MM, 12 NHL, 6 HD, 2 CLL, 14 ST.

Sixty subsequent patients were included in study B (2.5 µg/kg/d G-CSF plus 2.5 µg/kg/d GM-CSF versus 5 µg/kg/d G-CSF): 32 MM, 20 NHL, 2 HD, 2 CLL, 4 ST.

4 were not evaluable for the following events at time of chemotherapy: infection at time of chemotherapy (1 case), disease progression or relapses (2 cases), death before mobilization (1 case). Patients characteristics were comparable, particularly age, sex ratio, body weight and blood cell count before mobilization.

Kinetics of mobilization of circulating CD34 PBPC

In study A, the circulating CD34 cells appear in the peripheral blood (PB) respectively between day 9 to 12 in the G-CSF group and day 9 to 13 in G-CSF plus GM-CSF group. In study B, the circulating CD34 cells appear between day 9 to 11 in the G-CSF group and day 9 to 13 in G-CSF plus GM-CSF group (data not shown).

Collection of PB CD34 cell

There was a good correlation between the estimated number of CD34 cells and the collected number of CD34 cells measured (Fig. 2). Parameters of LK procedures are shown in Table II. A total of 232 cytaphereses were performed: 123 in study A, and 109 in study B.

One to three large volume LK were performed to harvest the required number of CD34 cells. The following parameters of LK were analysed: number of LK processed per patient (LK/patient), the day of first LK after CY, WBC at the first LK, and the median number of blood volumes treated per LK (BV/LK). None of these parameters were statistically different between the randomized groups.

Number of patients achieving a harvest of 5 x 10^6 and 10 x 10^6 CD34 cells/kg

In study A, 123 LK were performed (61 in the G-CSF group, 62 in the G + GM-CSF group). The distribution of patients undergoing one, two or three LK were identical in both groups. Among the patients who have undergone LK, the proportion of patients reaching a minimum target of 5 x 10^6 CD34 cells/kg was not statistically different between the G-CSF arm (21/27, 77.8%) and the G-CSF plus GM-CSF arm (25/29, 86.2%). When the objective of CD34 collection was 10 x 10^6 cells/kg, the proportion of patients reaching this target was significantly higher in the G-CSF plus GM-CSF arm (21/29, 72.4%) compared to the G-CSF arm (11/27, 40.7%: *P= .00006*).

In study B, a total of 109 LK were performed (56 in G-CSF arm and 53 in G-CSF plus GM-CSF arm). The distribution of patient undergoing one, two or three LK are identical in both groups. Among the patients who have undergone LK, the proportion of patients reaching a minimum target of 5 x 10^6 CD34 cells/kg was not statistically different between the G-CSF arm (20/27, 74.1%) and the G-CSF plus GM-CSF arm (20/26, 76.9%). When the objective of CD34 collection was 10 x 10^6 cells/kg, the proportion of patients reaching this target was significantly higher in the G-CSF plus GM-CSF (arm 15/26, 57.7%) compared to the G-CSF arm (10/27, 37%: *P= .003*) (Fig 3).

CD34 cell harvest (Table III).

In study A, the median number of CD34 cells x 10^6 /kg collected in LK1, LK2 or LK3 in the G-CSF and G + GM-CSF groups is respectively 3.1 (range 0.9-44.7), 3.7 (1.2-13.1), 2.7 (1.6-7.6) versus 7.3 (1-30.9), 6.28 (0.8-59), 5.72 (0.8-12.5). The total number of CD34 cells x10^6 /kg harvested is 2 fold higher in the G + GM-CSF group 18.3 (3-90) compared to 9 (3-45) in the G-CSF group (P= .09).

In study B, the median number of CD34 cells x 10^6 /kg collected at LK1, LK2, LK3 in the G-CSF and G + GM-CSF groups is respectively 3.7 (range 0.6-30.1), 3.0 (0.6-14.3), 3.3 (2.9-7) versus 7.07 (0.9-24.5), 8.02 (0.7-29.8), 6.71 (0.6-9.3). The total number of CD34 x 10^6 /kg harvested is about 2 fold higher in the G + GM-CSF group 15.85 (2.5-34.9) compared to 8.1 (1.6-33.9) in the G-CSF group (P= .09).

To avoid a bias due to the variable number of LK, and due to the variable blood volume treated during one LK, we have evaluated the efficacy of CD34 PBPC collection by calculating the number of CD34 cells x 10^6 /kg collected during the first LK according to the number of blood volume processed (CD34 LK_1/BV). In study A, this parameter was again increased about 2 fold in the G-CSF plus GM-CSF group 2.4 (0.3-13) compared to 1.1 (0.3-12) in the G-CSF group (P= .02). In study B, the same observation could be done in the G + GM-CSF group: 2.52 (0.3-10.2) versus 1.37 (0.2-11.6) in the G-CSF group (P= 0.04).

Patients with MM

In the study A : 14 MM patients received G-CSF and 12 received G-CSF plus GM-CSF (11 are evaluable). The total CD34 cells x 10^6 /kg harvested is 2.2 fold higher in the G-CSF plus GM-CSF group compared to the G-CSF group respectively 23.8 (11.1-89.9) versus 11.02 (3.2-29.2) (P= .005). The CD34 LK_1/BV is 2.4 fold higher in the G-CSF plus GM-CSF group 2.81 (0.9-13.3) versus 1.17 (0.3-8.1) in the G-CSF group (p=0.02). In the MM patients, we recommended to collect a minimum target of 10 x 10^6 CD34 cells/kg before the CD34

positive selection. In these conditions, 11/11 (100%) of patients receiving the G-CSF plus GM-CSF combination reach this target compared to only 8/14 (57%) in the G-CSF group (P = .02) (Fig 4). The same observation were done in patients of study B, 17 MM patients received G-CSF (15 are evaluable) and 15 received G-CSF plus GM-CSF (14 are evaluable). The total CD34 cells x 10^6 /kg harvested is about 2.5 fold higher in the G-CSF plus GM-CSF group (24.6 , range: 2.5-34.9) compared to the G-CSF group (9.26, range: 2.8-33.9) (P= .03). The CD34 LK_1/BV is 2.2 fold higher in the G-CSF plus GM-CSF group 2.95 x 10^6/kg (0.4-10.2) compared to 1.35 x 10^6/kg (0.1-11.6) (P= .014). 12/14 (86%) of patients receiving the G-CSF plus GM-CSF combination reach the defined target of 10 x 10^6 CD34+ cells/kg compared to only 7/15 (44%) in the G-CSF group (P= .03) (Fig. 4).

Patients with other lymphoid malignancies (NHL, HD and CLL)

Mobilization by CY and G-CSF plus GM-CSF did not significantly improve the amount of collected CD34 cells compared to the CY and G-CSF mobilization especially the CD34 LK_1/BV was not significantly increased (Table III). The comparison between myeloma and lymphoma patients is detailed in Table IV. MM patients received less chemotherapy courses (median 3, range: 2-6) while lymphoma patients received a median of 6 courses (median 2-16) chemotherapy courses (P = .02). As shown on Fig 5, no differences were seen between the two arms (G-CSF and G+GM-CSF) in the group of patients with more than one year of chemotherapy before mobilization.

In study A, among patients receiving G-CSF, the CD34 LK_1/BV is not significantly higher in MM 1.2 x 10^6 (0.3-8) than in Lymphoma 0.9 x 10^6 (0.4-12) (P= .12) while among patients receiving G-CSF plus GM-CSF, the CD34 LK_1/BV is 1.5 higher in MM (2.8 x 10^6 , range :1-13) than in Lymphoma (1.9 x 10^6 , range: 0.3-7) (P= .047). In study B, same observations was made, the CD34 LK_1/BV is not significantly higher in MM than in lymphoma (P= .8) while among patients receiving G-CSF plus GM-CSF, the CD34 LK_1/BV

is 2.1 higher in MM (2.95 x 10^6 , range: 0.4-10) than in Lymphoma (1.4 x 10^6 , range: 0.3-5) (P= .01).

Patients with solid tumors

Due to of low number of patients with solids tumors and their heterogenicity, none comparison was made between the two groups of treatment.

Mobilization failure

The percentage of patient that failed to mobilize sufficient PBSC was similar in study A (4/56, 7%) and in study B (3/53, 6%). None difference has been observed according to the HGF treatment group (Table V).

Adverse events

No serious adverse events were associated with the administration of cyclophosphamide at 4 g/m^2. Grade 1 and 2 nausea and vomitis were easily controlled by systematic administration of andosetron. In study A, three patients with full dose of G + GM-CSF experienced grade 2 symptoms (chill, bone and muscle pains, tachycardia) within the first two days. These patients stopped themself the GM-CSF. No patient experienced grade 1 and 2 toxicities during HGF treatment in the study B.

Effects of HGF on residual plasma cells (PC) mobilization in MM patients

In study A, an estimation of residual PC in product of LK was possible in 19 patients. The number of PC was 4.6 x 10^6/kg in the G-CSF group and 5.39 x 10^6/kg in the G-CSF plus GM-CSF group (P = NS).

In study B, the numeration of residual PC in LK was done in 21 patients with MM. The number of PC was 2.58 x 10^6/kg in the G-CSF group and 6.19 x 10^6/kg in the G + GM-CSF group (P = NS).

When comparing the total PC collected to the total CD34 cells collected in each patient, the PC/CD34 ratio is in study A 1 for 2.4 in G-CSF group and 1 for 4.42 in the G-CSF plus

GM-CSF group (P = NS). In study B, the PC/CD34 ratio is 1 for 3.6 in G-CSF group and 1 for 3.97 in the G-CSF plus GM-CSF group (P = NS) showing no enhancement of PC mobilization by G-CSF plus GM-CSF regimen.

Hematologic recovery after myeloablative treatment

97 patients were transplanted: 49 patients in Study A, 48 in study B. All MM patients were scheduled to receive a transplantation with a graft purged by CD34 positive selection. In study A, the CD34 positive selection was done in only 9/14 (64.3%) MM patients receiving G-CSF and 11/11 (100%) in patients receiving G-CSF plus GM-CSF (P= .03). In study B, this regimen was done in only 8/15 (53.3%) patients receiving G-CSF and 13/14 (92.8%) patients receiving G-CSF plus GM-CSF (P= .02).

Because of purification and double HDC in MM, the median number of CD34 cells infused was similar in all group of treatment and ranged between 3.78 to 4.32 x 10^6/kg. As expected, all patients had an engraftment, the median times to reach an ANC of $\geq$ 0.5 x 10^9/L and platelet $\geq$ 50 x 10^9/L were respectively 11 days (range 9 to 17) and 15 days (8-34). The transfusion cost is estimated at 1,619 $ (265-8,752). The median duration of hospitalisation was 24 days (16-94).

DISCUSSION

These two randomized studies demonstrate two major points:

-the combination of GM-CSF plus G-CSF following CY results in a 2 fold enhancement of the number of PBPC, in a safe and efficient manner for an homogenous group of newly diagnosed patients;

-the concomitant administration of these two HGFs, had additive effects, as observed by the superiority of the combination at equivalent doses (5µg/kg vs 2.5+2.5µg/kg).

Most of the patients included in these studies, achieved a minimal target of 5 x 10^6 CD34/kg, but more patients receiving G-CSF plus GM-CSF achieved a target of 10 x 10^6 CD34/kg. This result was obtained with a median of 2 LK. No adverse effects related to LK procedures have been reported. Although CD34 cell dose is currently considered to be the prefered indicator of satisfactory engraftment, the optimal CD34 cell dose for autologous transplantation remains to be defined. A collection of a threshold value of at least 3 to 5 x 10^6 CD34/kg is recommended to insure rapid and successful engraftment and long term hematological recovery. A dose-response relationship is evident between the number of CD34 cells per kilogram infused and neutrophil and platelet engraftment kinetics, decrease hospital stay and cost (40-42). Infusion of very high dose superior to 15 x 10^6 CD34/kg was shown to be associated with a shorter time for platelet recovery compared to that observed for patients receiving between 2.5 and 15 x 10^6 CD34/kg (43). However, Dercksen et al found no difference for neutrophil recovery when the number of CD34+ cells were superior to 6 x 10^6 cells/kg (44). Recently, different groups observed a linkage between the number of CD34 harvested and reinfused, and survival or duration of the response in different diseases including breast cancer and multiple myeloma (45, 46). The mecanism of such effect remains uncertain, but may be linked to a high number of immunocompetent cells collected in addition to the stem cells, as suggested by Porrata et col. who observed a correlation between

progression-free survival and the number of lymphocytes readministered (46). In addition, GM-CSF had several immune activities, including differentiation of dendritic cells from monocytes and it has been associated to different strategies of immune therapy including anti-tumoral vaccination (46, 47). These observations may represent a rationale for re-discovering the role of GM-CSF for mobilizing both haematopoietic stem cells and immune cell effectors (48,49). The response we made through this study appears important to define the minimal efficient dose for a combination of G-CSF and GM-CSF, prior to add other compounds for mobilization such as selective CXCR4 antagonists (15).

We observed that the efficacy of dual HGFs was more evident in patients having MM, where 2.5 fold higher PBSC are collected with the combination of HGFs. However, no difference have been observed in the group of lymphoma's patients. The impressive results observed with MM compared to the lymphoma group, are probably due to prior chemotherapy compared to the lymphoma group. Some investigations have highlighted the importance of prior exposure to the specific stem cell toxicity of chemotherapy containing alkylating agent and/or anthracycline and their impact on mobilization efficacy (50). Haas R and col. suggest the same findings showing a loss of 0.2 x 10^6 CD34+ cells/kg per chemotherapy course including alkylating agent (51). In that way, it seems that 6 courses of chemotherapy appears as a significant critical threshold. This feature is probably not corrected by the HGF treatment, including the co-administration of G-CSF and GM-CSF. The dexamethasone administred preferentielly to MM patients should also stimulate the hematopoïesis in synergy to HGFs. The role of glucocorticoids, espacially dexamethasone, on proliferation of haemotopoïetic progenitors has been suggested *in vitro* (52, 53). This particular benefit observed in MM patients and the fact that we use a concomitant administration, may represent the differences observed between the literature and our results.

The timing of harvest is one of the major parameter of successfull yield of CD34+ PBSC. The criteria used to define the optimal harvesting day was the peripheral WBC or mononuclear cells. But, these criterias are associated with a weak correlation to the CD34 cells amount in the product of leukapheresis (54). At the present time, the absolute number of circulating CD34 cells is correlated closely with the CD34 cell yield of the corresponding leukapheresis product. The usual critical threshold of circulating CD34 cells as indicator of onset of cytapheresis is 40 CD34 cells per µL (55). But, this method doesn't provide an estimation of CD34 cells collection. We use an estimation of the CD34 PBSC yield determined just before each cytapheresis to give directly an estimation of the final collect of CD34+ PBSCs.

In addition to PBSC mobilization, the mobilization of tumoral cells remains likely. The role of these residual tumoral cells in the graft for the relapse after transplantation is still controversial (56, 57). In this study, we evaluate the tumors cells co-mobilization in MM patients. We have shown no trend to mobilize more tumoral cells with G-CSF plus GM-CSF regimen than that observed with G-CSF alone. All patients recovered after myeloablative treatment.

As expected, no clear benefit appears in terms of engraftment, transfusion cost according to HGF regimen. The median number of CD34 cells infused to the patients in all treatment group is comparable (3.8 to 4.32 x 10^6 CD34+/kg). The majority of the patients having MM or breast cancer were scheduled to receive a double intensive chemotherapy followed by a CD34 immunoselected graft rescue. This schedule was possible more frequently in the G-CSF plus GM-CSF mobilization regimen (93 to 100% of patients) than in G-CSF group (53 to 64% of patients).

Future insight of graft manipulation as high level tumor cell purging, genetic manipulation, monocytes mobilization in view of anti-tumoral vaccination by dendritic cells, *ex-vivo*

expansion will invariably result in loss of a substantial fraction of hematopoietic progenitor cells harvested, a situation that needs to collect higher number of hematopoietic stem cells. The combination of cytokines, including GM-CSF may represent a particular interesting combination, in addition to the effects of GM-CSF on the immune system, as demonstrated in vaccination programs.

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Figure and Table legends.

Table I. Demographic and disease status of patients at diagnosis.

Table II. Parameters of leukapheresis (LK) procedures

Table III. Study A and B: Collection of CD34+ PBSC (x 10^6/kg) for harvested patients (n) in each group.

Table IV: Differences between MM and Lymphoma

Table V. Mobilization failure in patients receiving the hematopoietic growth factor

Figure 1. Design of the study: mobilization and collection of CD34 cells.

Figure 2. Correlation between the estimated number of CD34 cells (%CD34 x WBC x 8)/kg body weight (8 being a coefficient corresponding approximately to 2 blood volumes in L) and the number of collected CD34 cells.

Figure 3. Percentage of patients reaching 5 or 10x 10^6 CD34 cells/kg in study B.

Figure 4. Percentage of patients with multiple myeloma and reaching 10x 10^6 CD34 cells/kg.

Figure 5. Kinetic of circulating CD34 cells in patients with less (A) and more (B) than one year of prior chemotherapy.

Table I.

	Study A		Study B	
HGF	**G-CSF**	**G+GM-CSF**	**G-CSF**	**G+GM-CSF**
dose (µg/kg/d)	*5*	*5 + 5*	*5*	*2.5 + 2.5*
N	**29**	**31**	**30**	**30**
Age (Yr)				
Median	**54**	**51***	**53**	**57***
(range)	*(22-65)*	*(32-70)*	*(23-69)*	*(35-74)*
Weight (kg)				
Median	**62.5**	**68.5***	**65**	**67.5***
(range)	*(44-114)*	*(47-110)*	*(43-104)*	*(40-100)*
Sex				
Male	16 *(55 %)*	17 *(55 %)**	16 *(53 %)*	14 *(47 %)**
Female	13 *(45 %)*	14 *(47 %)**	14 *(47 %)*	15 *(53 %)**
MM	**14**	**12**	**17**	**15**
Stage II	3	-	3	3
III	11	12	14	12
NHL	**5**	**7**	**8**	**12**
Fol. SR	2	1	1	3
Dif.	1	4	6	9
MZ	1	2	1	-
Cut.	1	-	-	-
HD	**4**	**2**	**2**	**-**
SR	3	1	1	-
Ref.	1	1	1	-
CLL (St. B)	**1**	**1**	**1**	**1**
Solid Tumors	**5**	**9**	**2**	**2**
Breast HR	1	5	-	1
M1	1	3	1	1
Testis M1	2	1	1	-
Ovarian M1	1	-	-	-

MM= Multiple Myeloma, stage according to Salmon-Durie classification. NHL= Non-Hodgkin's Lymphoma, Fol.= follicular, Dif.= diffuse, MZ= mantle zone, Cut.= cutaneous, SR= sensitive relapse, Ref.= refractory. HDK = Hodgkin disease, (all NHL and HDK are stage IV according to Ann Harbor classification). CLL= Chronic Lymphocytic Leukemia, stage according to Binet Classification. Breast, HR= high risk according to Scarff-Bloom/Richardson score $\geq$ 6 and/or more than 6 loco-regional metastatic nodes, M1 presence of long-distance metastase according to UICC-TNM classification. Ovarian= Ovarian cancer.

* not significantly different from data of the G-CSF group using a Wilcoxon non parametric test.

Table II.

	Study A		Study B	
HGF	**G-CSF**	**G+GM-CSF**	**G-CSF**	**G+GM-CSF**
dose (µg/kg/d)	*5*	*5 + 5*	*5*	*2.5 + 2.5*
	Median values and *(range)*			
Leukapheresis N=232	**61**	**62***	**56**	**53***
LK/patient #	**2** *(0-3)*	**2*** *(0-3)*	**2** *(0-3)*	**2*** *(0-3)*
Day of the 1st LK	**11** *(9-13)*	**10*** *(8-17)*	**11** *(8-16)*	**10*** *(7-12)*
WBC at 1st LK	**6** *(0.7-39)*	**8.8*** *(0.8-46.2)*	**7.2** *(0.9-51.2)*	**4*** *(1-25.9)*
BV/LK °	**3.01** *(1.5-4.3)*	**2.55*** *(1.8-4)*	**2.9** *(1.8-5.1)*	**2.8*** *(1.7-3.5)*

LK/patient# = Leukapheresis undergone by patient. WBC= White Blood Count x 10^9/L. BV/LK ° = number of Blood Volume processed during a leukapheresis.
* not significantly different from data of the G-CSF group using a Wilcoxon non parametric test.

Table III.

	Study A			Study B		
HGF	**G-CSF**	**G+GM-CSF**		**G-CSF**	**G+GM-CSF**	
dose (µg/kg/d)	*5*	*5 + 5*		*5*	*2.5 + 2.5*	
	Median values and *(range)*					
Global results						
(n)	**27**	**29**	*P*	**27**	**26**	*P*
LK_1	3.1 *(1-45)*	7.3 *(1-31)*	*.03*	3.7 *(0.6-30)*	7.07 *(1-24.5)*	*.04*
LK_2	3.7 *(1-13)*	6.3 *(1-59)*	*.04*	3 *(0.6-14)*	8.02 *(1-30)*	*.02*
LK_3	2.7 *(2-8)*	5.7 *(1-12)*	*.04*	3.32 *(2.9-7)*	6.71 *(1-9)*	*.2*
Total CD34/kg	9 *(3-45)*	18.3 *(3-90)*	*.09*	8.1 *(2-34)*	15.85 *(2-35)*	*.09*
CD34 LK_1 /BV	1.1 *(0.3-12)*	2.4 *(0.3-13)*	*.02*	1.48 *(0.1-12)*	2.41 *(0.3-10)*	*.04*
MM						
(n)	**14**	**11**		**15**	**14**	
LK_1	3.2 *(1-18)*	9.3 *(3-31)*	*.03*	3.7 *(1-30)*	8.8 *(1-24)*	*.01*
LK_2	5.7 *(1.2-13)*	10.4 *(4-59)*	*.04*	5.6 *(1.5-14)*	10.5 *(1-30)*	*.02*
LK_3	2.9 *(2.3-8)*	4.5 *(3-12)*	*.10*	3.4 *(3-7)*	7.3 *(1-9)*	*.10*
Total CD34/kg	11 *(3-29)*	23.8 *(11-90)*	*.005*	9.26 *(3-34)*	24.6 *(2-35)*	*.03*
CD34 LK_1 /BV	1.17 *(0.3-8)*	2.81 *(1-13)*	*.02*	1.35 *(0.1-12)*	2.95 *(0.4-10)*	*.01*
NHL/HD/CLL						
(n)	**9**	**9**		**10**	**10**	
LK_1	2.6 *(1-35)*	4.2 *(1-22)*	*NS*	3.36 *(1-14)*	3.61 *(1-10)*	*NS*
LK_2	2.6 *(1-4)*	3.8 *(2-5)*	*NS*	2.37 *(1-3)*	2.4 *(1-4)*	*NS*
LK_3	2.6 *(2-3.5)*	0.8	*/*	*/*	1.8	*/*
Total CD34/kg	6.4 *(3-35)*	5.4 *(3-26)*	*NS*	5.4 *(2-14)*	5.2 *(3-10)*	*NS*
CD34 LK_1 /BV	0.9 *(0.4-12)*	1.9 *(0.3-7)*	*NS*	1.6 *(0.3-6.5)*	1.4 *(0.3-5)*	*NS*

CD34+ PBSC are harvested during one or two or three maximum large volume leukapheresis (LK). LK_1 = 1 st Leukapheresis, LK_2 = 2 nd Leukapheresis, LK_3 = 3 rd Leukapheresis. CD34 LK1/BV= CD34+ cells harvest in the 1 st LK in function of number of blood volume. P values were determined with a Wilcoxon test.
Datas of Solids Tumors are not detailed.

Table IV.

	Study A		Study B	
HGF	**G-CSF**	**G+GM-CSF**	**G-CSF**	**G+GM-CSF**
dose (µg/kg/d)	*5*	*5 + 5*	*5*	*2.5 + 2.5*
	Median values and *(range)*			
Number of administred courses				
MM	**3** *(2-6)*		**3** *(3-4)*	
NHL/HD/CLL	**5** *(2-16)*		**6** *(3-20)*	
	P = .02		*P = .005*	
CD34 LK_1 /BV				
MM	**1.2** *(0.3-8)*	**2.80** *(1-13)*	**1.35** *(0.1-12)*	**2.95** *(0.4-10)*
NHL/HD/CLL	**0.9** *(0.4-12)*	**1.9** *(0.3-7)*	**1.6** (*0.3-6.5)*	**1.4** *(0.3-5)*
	P = .12	*P = .047*	*P = .8*	*P = .01*

Table V.

	Study A			Study B		
						P
Number of failure *(%)*	**4/56** *(7.14 %)*			**3/53** *(5.66 %)*		*NS*
HGF	**G-CSF**	**G+GM-CSF**		**G-CSF**	**G+GM-CSF**	
dose (µg/kg/d)	*5*	*5 + 5*		*5*	*2.5 + 2.5*	
	2/27 *(7.4 %)*	**2/29** *(6.9%)*	NS	**2/27** *(7.4 %)*	**1/26** *(3.8 %)*	*NS*

Number and percentage of patients who received the HGF and impaired the mobilization
p = Fisher's exact test

Figure 1.

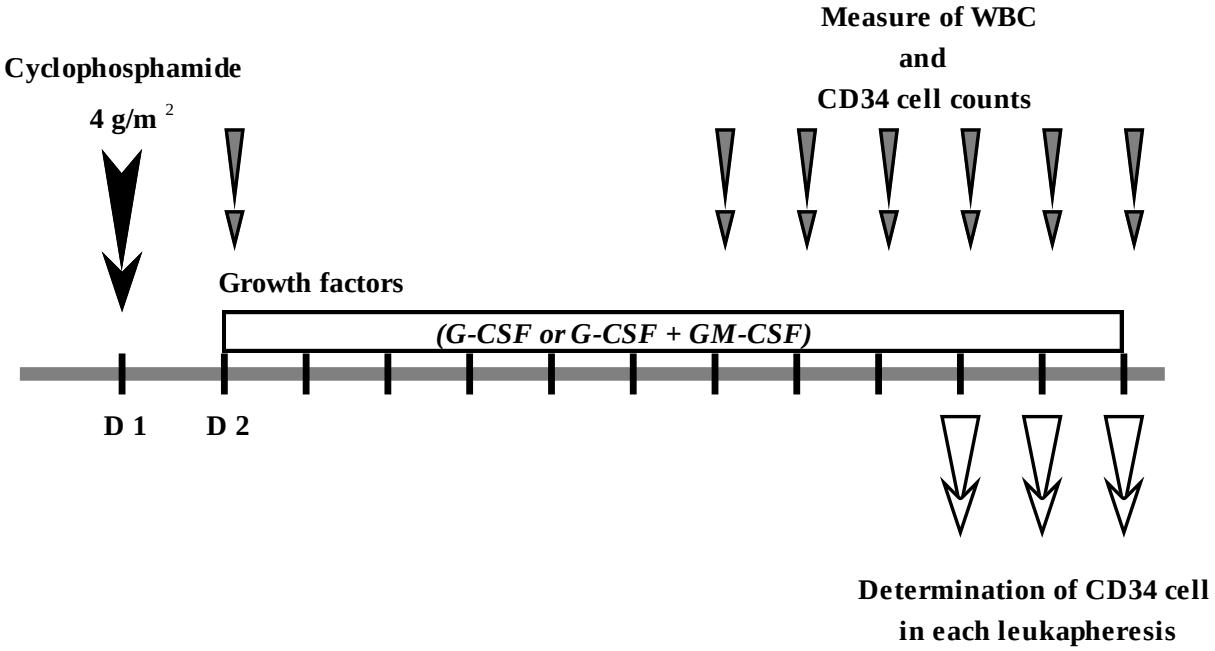
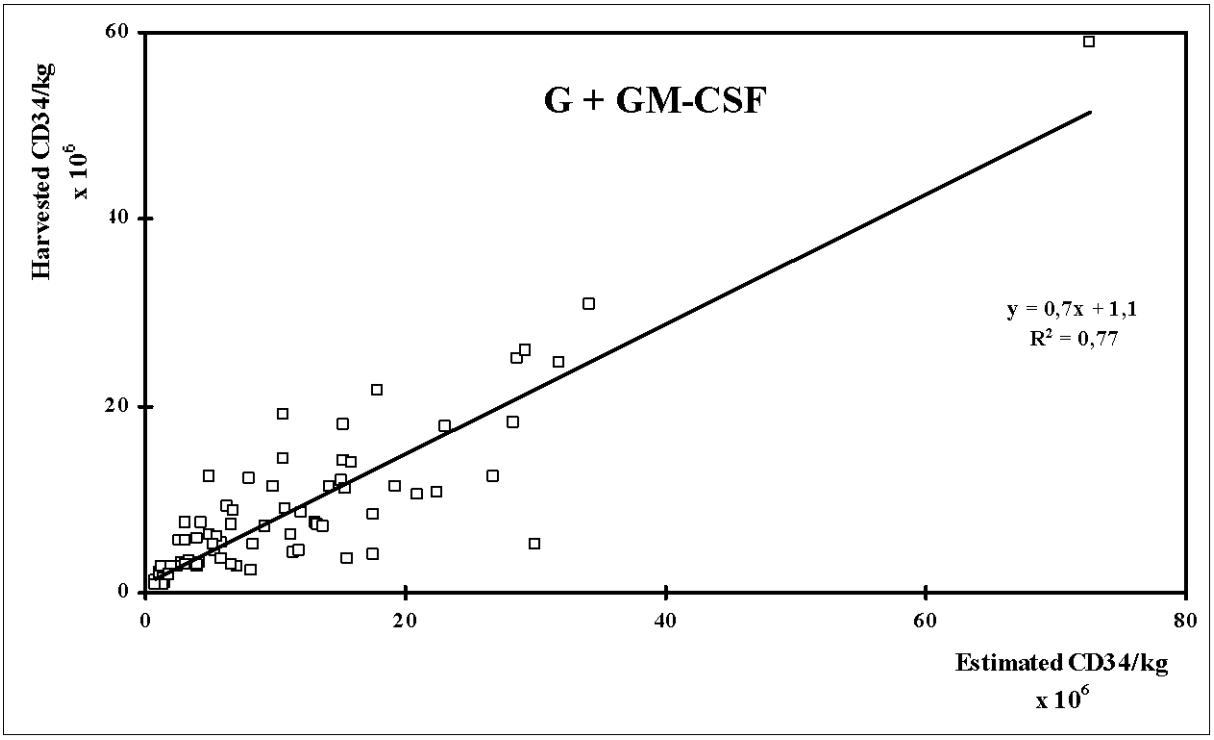

Measure of WBC
and
CD34 cell counts
Cyclophosphamide
4 g/m 2
Growth factors
(G-CSF or G-CSF + GM-CSF)
D 1
D 2
Determination of CD34 cell
in each leukapheresis

Figure 2.


G + GM-CSF
Harvested CD34/kg x 10^6
Estimated CD34/kg x 10^6
y = 0,7x + 1,1
R² = 0,77
0
20
40
60
80

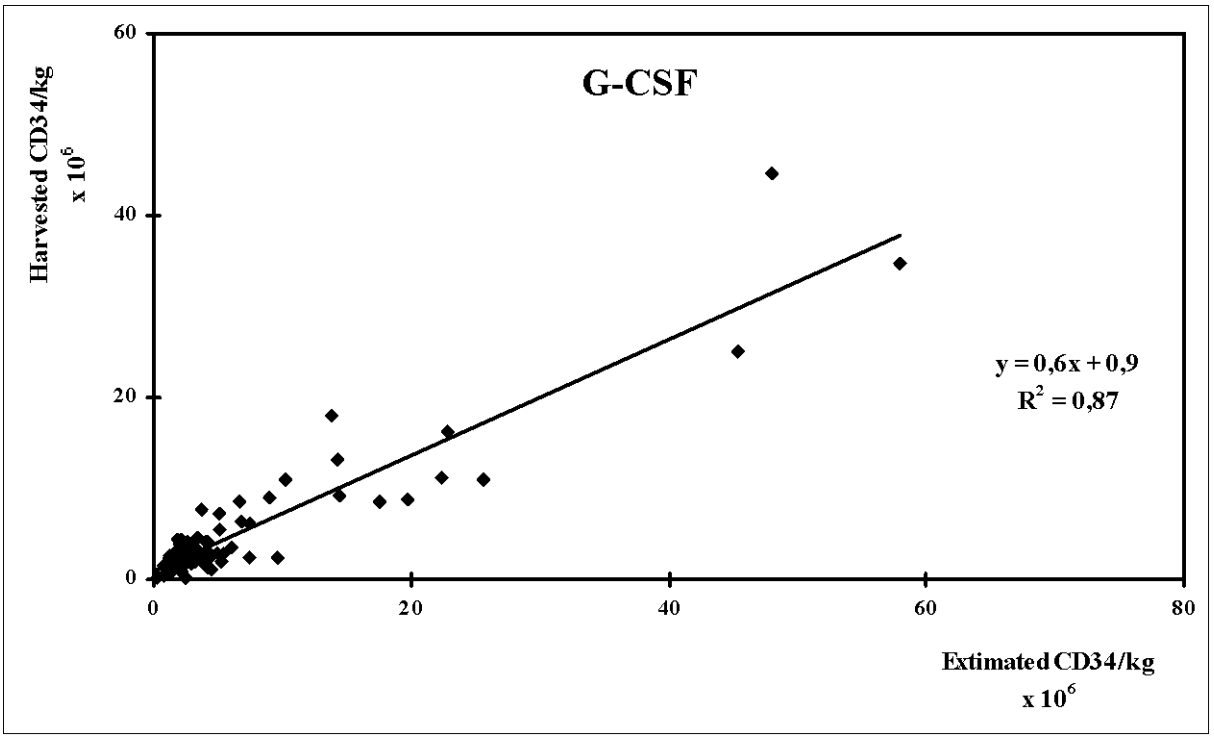

G-CSF
Harvested CD34/kg x 10^6
Extimated CD34/kg x 10^6
y = 0,6x + 0,9
R² = 0,87
0
20
40
60
80

Figure 3.

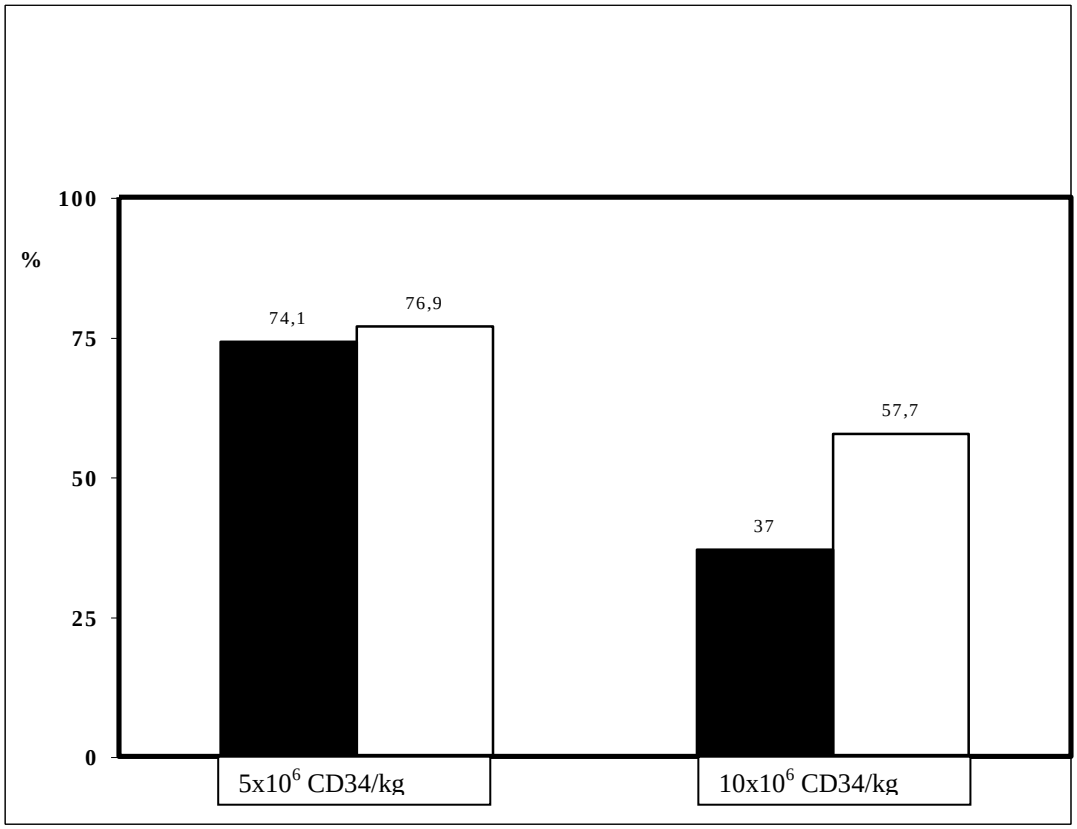


Figure 4.

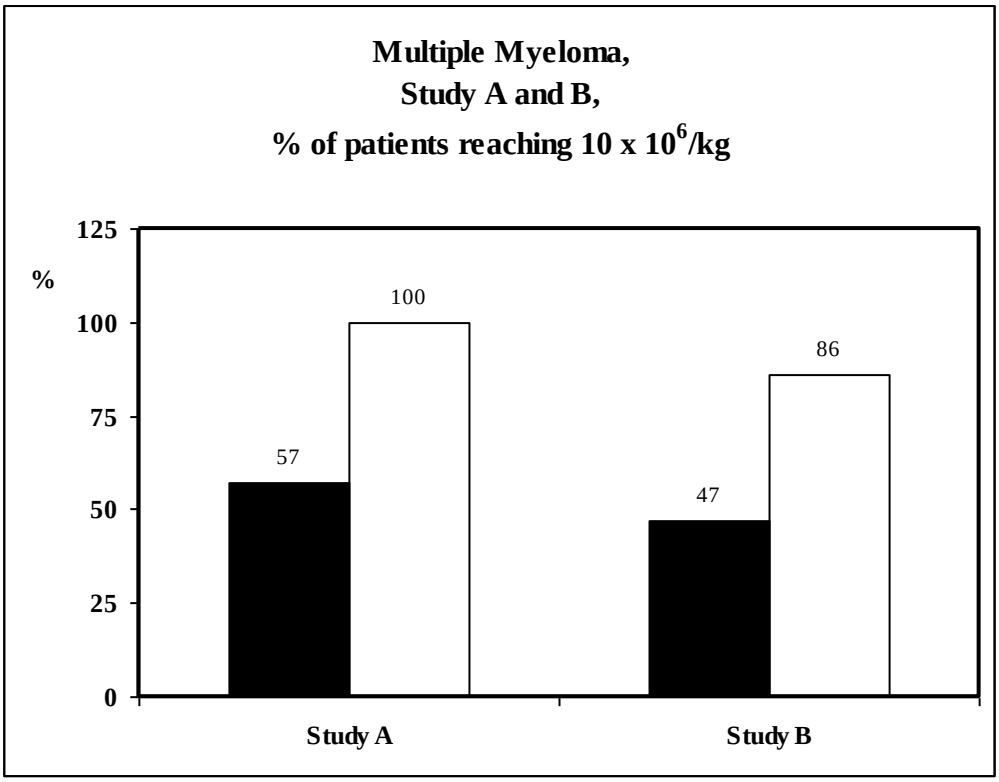
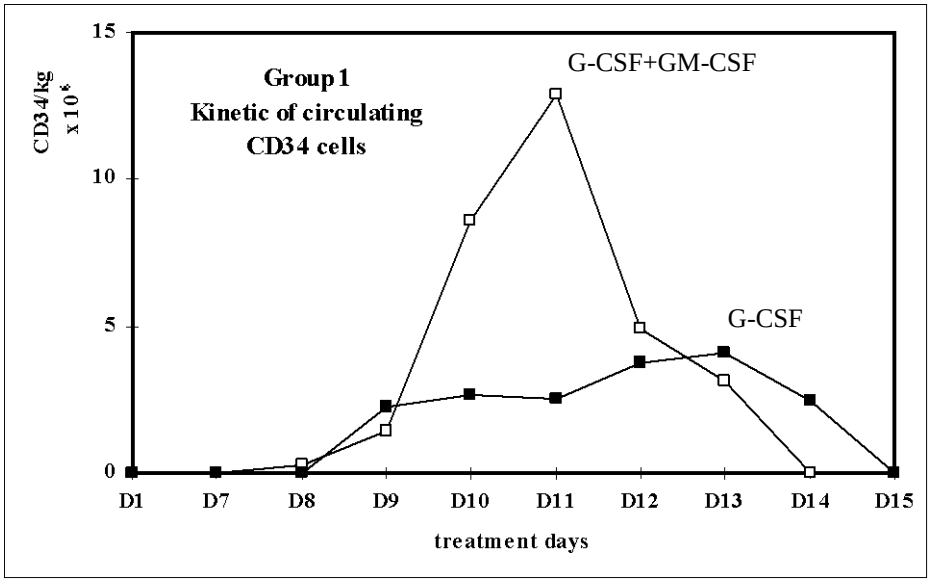
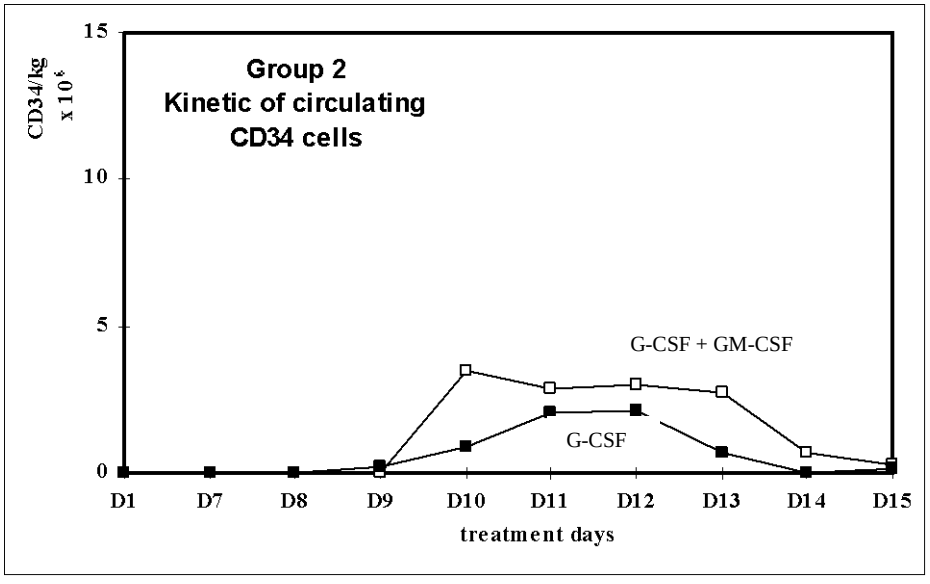

Multiple Myeloma,
Study A and B,
% of patients reaching 10 x 10^6/kg
%
125
100
75
50
25
0
57
100
47
86
Study A
Study B

Figure 5.


Group 1
Kinetic of circulating
CD34 cells
CD34/kg
x 10⁶
G-CSF+GM-CSF
G-CSF
15
10
5
0
D1
D7
D8
D9
D10
D11
D12
D13
D14
D15
treatment days

A

B


Group 2
Kinetic of circulating
CD34 cells
CD34/kg
x 10⁶
G-CSF + GM-CSF
G-CSF
15
10
5
0
D1
D7
D8
D9
D10
D11
D12
D13
D14
D15
treatment days