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Original article

Selection and immunomagnetic purging of peripheral blood CD34+ cells for autologous transplantation in B-cell non-Hodgkin's lymphomas

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Summary

Background: Clonogenic tumor cells in the hematopoietic progenitor cell harvest may contribute to relapse after high dose therapy for B-cell malignancies. Purging of the HPC harvest requires large amounts of anti-B-cell antibodies, whereas CD34-selection enriches self renewing HPC's but malignant cells are still detectable in many CD34+ fractions.

Patients and methods: We examined the feasability and safety of a CD34-selection followed by purging with anti-B-cell antibodies in 11 patients with B-cell non-Hodgkin's lymphomas undergoing high-dose therapy with cyclophosphamide, BCNU and etoposide with retransfusion of autologous HPC's.

Results: A mean number of 340 × 10^8 mononuclear cells was used for CD34-selection and immunomagnetic purging. CD34+ cells were enriched from a mean of 1.7% (range 0.2%–4.5%) to a mean of 68% (range 49%–87%) with a mean recovery of 27% (range 15%–43%). The mean number of retransfused CD34+ cells was 1.2 × 10^6/kg (range 0.6–2.2 × 10^6/kg) body weight with a median of 11 days (range 10–13 days) to neutrophil recovery of 0.5 × 10^9/l and 17 days (range 13–25 days) to platelet recovery of 50 × 10^9/l. Mean number of intravenous antibiotics and inpatient days were 8 (range 0–14) and 22 (range 19–26) respectively. Major toxicity consisted in four septicemias.

Conclusions: CD34-selected and purged HPC's are safe and mediate rapid hematological recovery after high dose therapy for B-cell non-Hodgkin's lymphomas.

Key words: B-cell lymphomas, CD34+ HPC's, immunomagnetic purging

Introduction

Several lines of evidence suggest that contaminating tumor cells in the HPC harvest may contribute to relapse after high dose therapy with autologous transplantation in patients with lymphoid malignancies. Studies at the University of Nebraska have shown that patients who are reinfused with morphologically normal bone marrow containing clonogenic lymphoma cells have an increased incidence of relapse [1]. In addition, patients whose bone marrow contains PCR-detectable lymphoma cells after immunologic purging have an increased incidence of relapse after autologous bone marrow transplantation [2, 3]. Moreover, gene marking studies have demonstrated that marked autologous marrow cells may be detected at the time of relapse, suggesting that reinfused tumor cells after high dose therapy contribute to relapse [4, 5].

Among the methods available to remove residual lymphoma cell from the HPC harvest of patients with B-cell malignancies, immunopurging has the advantage of targeting B cells while sparing normal HPC's. At present, most centers prefer mobilized peripheral HPC harvests where the number of nucleated cells is at least twice as large than in bone marrow harvests. Thus, purging requires large amounts of antibodies and immunomagnetic beads, making the process cumbersome and costly.

The identification of the CD34 antigen, expressed on HPC's but not on lymphoid malignancies, made it possible to remove residual lymphoma cells by positively selecting the population containing the bone marrow repopulating cells. Selection of CD34+ cells from the HPC harvest results in a one to four log depletion of contaminating tumor cells, but malignant cells are still detectable in the CD34-selected fraction of many patients [6–8]. We have recently demonstrated that the majority of patients with t(14;18)+ lymphomas still have a considerable number of contaminating lymphoma cells after selection of CD34+ HPC's [9]. This finding may be due to contaminating lymphoma cells not removed through the CD34-selection or to the co-selection of lymphoma cell progenitors expressing the CD34 antigen [10].

The ability to purify cell fractions enables us to tailor autografts with the aim to enhance their therapeutic benefit and to improve disease-free survival. Therefore, we hypothesized that the combination of a positive selection of CD34+ HPC's followed by a negative selection with anti B-cell antibodies coated immunobeads would result in an optimal depletion of residual lymphoma cells. We thus examined the feasability of such an ap-

proach with regard to safety and hematopoietic recovery in patients with B-cell non-Hodgkin's lymphomas undergoing high-dose therapy with retransfusion of autologous HPC's.

Patients and methods

Patients and clinical samples

Eligibility criteria regarding high-dose therapy with autologous HPC transplantation for patients with B-cell non-Hodgkin's lymphomas were: 1) relapsed chemosensitive follicular lymphoma with or without transformation (n = 6), 2) relapsed chemosensitive large cell lymphoma (n = 1), or 3) first remission of a stage III or IV large cell or Burkitt's lymphoma with elevated LDH and/or tumor bulk at presentation (n = 4) [11]. Chemosensitivity was determined such that the largest nodal mass was less then 5 cm and the bone marrow was infiltrated with less than 20% of the intertrabecular space after induction or salvage therapy. The protocol was approved by the University Hospital ethical committee and informed consent was obtained from each patient.

Collection and processing of mobilized peripheral blood HPC's

Peripheral blood cells were mobilized with cyclophosphamide 1.5 g/m^2 in four patients, 2 g/m^2 in two patients and 3 g/m^2 in five patients. Granulocyte-colony-stimulating factor (G-CSF) was given at 10 µg/kg/day as a subcutaneous injection beginning five days after chemotherapy until the last day of leukapheresis. Patient underwent apheresis using a Fenwal CS3000 Plus blood cell separator (Baxter Fenwal, Deerfield, IL). Three aphereses were performed. Two aphereses were used to select CD34+ cells, whereas an additional apheresis was cryopreserved as unmanipulated backup.

Selection of CD34+ cells

Mononuclear cells were incubated for 25 minutes at room temperature with 20 µg/ml of biotinylated anti-CD34 monoclonal antibodies (moAbs) in 150 ml of phosphate-buffered saline (PBS) containing 0.1% human serum albumin (HSA). Treated cells were washed twice with PBS to remove unbound antibody, diluted in 300 ml PBS/1% HSA and passed over the Ceprate SC Stem Cell Concentrator (Cell Pro, Inc, Bothell, WA), containing a sterile column of avidin-coated polyacrylamide beads. After washing with 300 ml of PBS, CD34+ cells were removed from the beads by mechanical agitation and eluted with 90 ml PBS with 5% HSA. CD34-selected cells were centrifuged at 400 × g at 4 °C and resuspended in 32 ml PBS/1% HSA/0.5% immunoglobulin (Sandoglobulin, Sandoz, Bern).

Immunomagnetic purging of CD34-selected HPC's

Dynal paramagnetic beads (one vial GMP grade Dynabeads M-450 sheep anti-Mouse IgG, Baxter, Maurepas, France) were washed three times with PBS with 1% HSA and 5% citric acid 38 mM, sodium citrate 74.8 mM, glucose 123.6 mM (PBS/1%HSA/5%ACD-A) and resuspended in 9 ml PBS/1%HSA/5%ACD-A. 0.5 mg of each murine anti-human-CD10, -CD19 and -CD20 moAbs (all from Baxter, Unterschleissheim, Germany) were added and incubated for two to six hours at 4 °C under continous mixing. Dynabeads were then washed three times with PBS/1% HSA/5% ACD-A to remove unbound antibodies. CD34-selected cells, resuspended in 32 ml PBS-HSA with 0.5% immunoglobulin, were injected with antibody-coated dynabeads in 10 ml PBS/1% HSA/5% ACD-A in an Isolex 50 magnetic cell separator disposable set (Baxter, Fenwal Division, Deerfield, IL) and incubated at 4 °C for one hour using slow end-over-end rotation. Magnetic separation of rosetted cells was performed using the Isolex 50 Magnetic Cell Separator. Unrosetted cells were drained out of the chamber while the bead/cell fraction (CD10+/CD19+/CD20+ cells) was retained in the Isolex chamber. The rosetted cell fraction was washed once with 50 ml PBS/1% HSA/5% ACD-A to remove non-rosetted cells trapped in the bead/cell fraction. Unrosetted cells were centrifuged at 400 × g for 15 minutes to remove excess antibodies. The pellet was resuspended in PBS with heparin, 0.5% HSA and 10% DMSO at a cell concentration of 20 × 10^6 cell/ml, cryopreserved using a controlled-rate freezing method and stored at −196 °C until further use.

High-dose chemotherapy and retransfusion of CD34-selected and immunomagnetically purged HPC's

High-dose chemotherapy consisted of cyclophosphamide 1500 mg/m^2/day by continous intravenous infusion on days −7 to −4, BCNU 300 mg/m^2 by intravenous bolus infusion on day −7, and etoposide 300 mg/m^2/day in two divided intravenous short term infusions on days −7 to −5. Retransfusion of CD34-selected and immunopurged cells was performed on day 0. G-CSF at 5 µg/kg/day subcutaneously was started on day +1 and administered until the neutrophil count was > 1 × 10^9/l for three days. Supportive care was given as previously published [11].

Results

Positive selection of CD34+ HPC's followed by negative selection of B-cells through immunomagnetic bead depletion with moAbs

CD34+ cells were selected from two pooled aphereses using a CellPro immunoadsorption column. The total number of mononuclear cells loaded on the column, the percentage of CD34+ cells in the HPC harvest and in the CD34-selected and immunomagnetically purged cell fractions, as well as the recovery of CD34+ cells after CD34-selection and immunomagnetic purging are summarized in Table 1. HPC harvests of three patients (Nos. 1, 3 and 4) were purged with anti-CD19 and anti-CD20

Table 1. Purity, recovery and yield of CD34+ cells after CD34-selection and after immunopurging.

PIN	CD34+ cells (%)/total MNC × 10^8		Recovery (%)		CD34+ cells/kg after CD34-selection and purging
	In the progenitor cell harvest	After CD34-selection and purging	After CD34-selection	After purging	
1	2.7/160	84/1.3	45	36	1.3 × 10^6
2	1.8/270	53/1.8	36	18	1.2 × 10^6
3	0.5/710	64/2.4	70	43	2.2 × 10^6
4	0.2/870	49/1.8	50	39	1.0 × 10^6
5	1.1/240	74/0 9	64	25	0.8 × 10^6
6	1.4/340	72/0.5	29	28	0.8 × 10^6
7	4.5/140	87/1.3	19	17	1.5 × 10^6
8	1 3/180	81/1.2	61	43	1.7 × 10^6
9	1.1/440	54/1.5	42	18	1.2 × 10^6
10	1.5/330	81/0.7	31	15	1.0 × 10^6
11	2.8/110	52/0.8	21	15	0.6 × 10^6

Recovery was calculated as 100% × (MNC after CD34-selection or purging × % CD34+ cells)/(MNC before CD34-selection × % CD34+ cells).

moAbs only, because GMP grade anti-CD10 antibodies were not available at the time of their harvest. The mean number of mononuclear cells loaded on the column was 340 × 10^8 (range 110–870 × 10^8) containing a mean of 1.7% (range 0.2%–4.5%) CD34+ cells. The mean number of CD34+ cells recovered after the column separation was 43% (range 19%–70%). CD34-selection and immunomagnetic purging reduced mononuclear cells to a mean of 1.3 × 10^8 (range 0.5–2.4 × 10^8) and enriched CD34+ cells to a mean of 68% (range 49%–87%). The mean number of CD34+ cells recovered after CD34-selection and immunomagnetic purging was 27% (range 15%–43%). The mean number of retransfused CD34+ cells per patient after CD34-selection and immunomagnetic purging was 1.2 × 10^6/kg body weight (range 0.6–2.2 × 10^6/kg body weight).

Safety data

All patients received high-dose chemotherapy with cyclophosphamide, BCNU and etoposide. Three days after completion of chemotherapy, cryopreserved and immunopurged cells fractions were thawed and retransfused. The Kaplan–Meier estimate of the median time to neutrophil recovery to $> 0.5 \times 10^9$/l calculated from the day of retransfusion was 10 days for the eight patients receiving $> 1 \times 10^6$ CD34+ cells/kg body weight and 11 days for the three patients receiving $< 1 \times 10^6$ CD34+ cells/kg body weight as shown in Figure 2A. The Kaplan–Meier estimate of the median time to platelet recovery to $> 50 \times 10^9$/l calculated from the day of retransfusion was 16 days for the eight patients receiving $> 1 \times 10^6$ CD34+ cells/kg body weight and 17 days for the three patients receiving $< 1 \times 10^6$ CD34+ cells/kg body weight as shown in Figure 2B. Patients received a mean number of 2 (range 1–3) platelet transfusions and a mean number of 3 (range 2–8) packed red blood cell transfusions. Mean inpatient days were 22 (range 19–26) and mean days of i.v. antibiotics and antivirals were 8 (range 0–14) and three days (0–8) respectively. Major toxicity consisted in four septicemias (*Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus mitis*). Localized herpes simplex infections were treated with i.v. acyclovir in five patients.

Discussion

In this study we report the feasability and safety of a positive selection of CD34+ cells followed by a negative selection with immunomagnetic purging with anti-B-cell moAbs in 11 patients with B-cell non-Hodgkin's lymphomas undergoing high-dose therapy with retransfusion of autologous HPC's.

Our major concern was that the combination of CD34-selection with immunomagnetic purging would result in an insufficient yield of CD34+ cells impairing hematologic recovery after high dose therapy. The loss of CD34+ cells was considerable, with a mean recovery

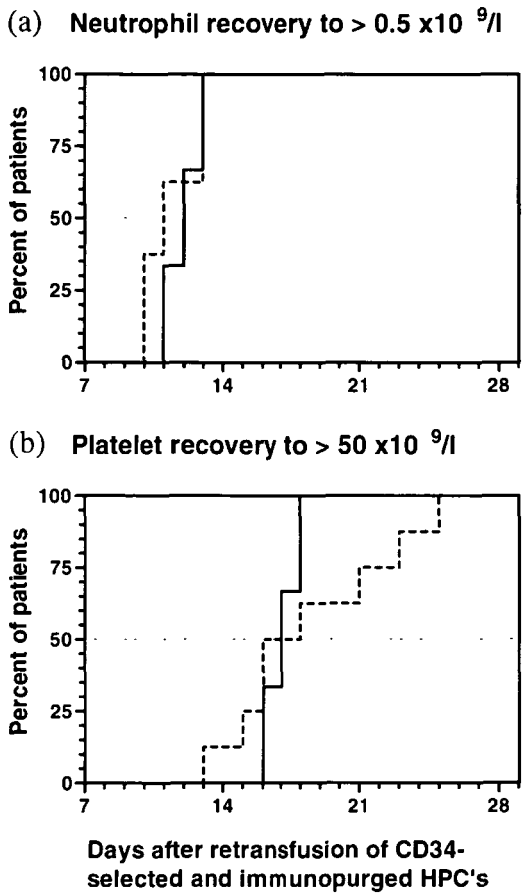

Figure 1 (a) Kaplan–Meier analysis of time to neutrophil count recovery of 0.5 × 10^9/l and (b) Kaplan–Meier analysis of time to platelet count recovery of 50 × 10^9/l. (—), $> 1 \times 10^6$ CD34+ cells/kg body weight, (- - -), $< 1 \times 10^6$ CD34+ cells/kg body weight.

of only 27% (range 19%–70%) after CD34-selection and immunomagnetic purging, resulting in less than 1 × 10^6 CD34+ cells/kg/body weight in three patients. Nevertheless, engraftment was rapid with a median of 11 days to neutrophil recovery of $> 5 \times 10^9$/l and a median of 17 days to platelet recovery of $> 50 \times 10^9$/l; a result comparable to our previous studies using unmanipulated bone marrow [11, 12], or other studies using CD34-selected mobilized peripheral blood HPC's [6–8]. As shown in Figure 2, neutrophil and platelet recovery was not significantly different between patients retransfused with $> 1 \times 10^6$ CD34+ cells/kg body weight and $< 1 \times 10^6$ CD34+ cells/kg body weight. With 82% neutropenic fever and 36% microbiologically documented infections, the rate of complications was comparable to our previous experience with unprocessed bone marrow cells [11, 15]. No late infections have been observed in our short follow up period of 8.3 months (range 1–19 months).

Our mean purity and recovery of 68% (range 49%–87%) and 43% (range 19%–70%) CD34+ cells respectively, after passage over the Ceprate SC Stem Cell Concentrator are comparable to published reports using the same cell separator to enrich CD34+ cells from bone marrow or mobilized peripheral blood HPC harvests [7, 8]. In contrast, other CD34+ cell sorting systems such as

the Baxter Isolex 300 magnetic cell separation system [13] or the Super MACS high gradient magnetic cell sorting system [14] have been reported to achieve mean yields of more than 90% CD34+ cells from mobilized peripheral blood HPC harvests. Therefore, we intend to evaluate the efficacy of the Baxter Isolex 300 magnetic cell separation system for our further studies. Moreover, samples of apheresis products as well as the CD34-selected and immunomagnetically purged cell fractions were collected to assess purging efficacy by PCR analysis for the presence of t(14;18)+ or lymphoma specific IgH CDRIII sequence carrying cells. Results will be reported at a later timepoint.

In conclusion, the retransfusion of CD34-selected and immunomagnetically purged HPC's is safe and mediates rapid hematological recovery after high dose therapy for B-cell lymphomas.

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