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Phase I/II trial of cure-oriented high-dose chemoradiotherapy with transplantation of CD34+ peripheral blood stem cells purified by the immunomagnetic bead method for refractory hematological malignancies

Abstract A multicenter phase I/II clinical trial was conducted to evaluate the safety of a device (Isolex System; Baxter Health Corporation, Irvine, Calif., USA) using the immunomagnetic bead method to purify $CD34^+$ stem cells from peripheral blood and to assess the efficacy and toxicity of high-dose chemoradiotherapy with peripheral blood stem-cell transplantation (PBSCT) using purified $CD34^+$ stem cells in patients with refractory hematological malignancies. Patients eligible for the study included those who had T-cell acute lymphoblastic leukemia (T-ALL), lymphoblastic lymphoma (LBL), mantle-cell lymphoma (MCL), high-risk aggressive non-Hodgkin's lymphoma (NHL), and adult T-cell leukemia/lymphoma (ATLL) in first complete remission (CR) and those who had standard-risk aggressive NHL, indolent lymphoma, Hodgkin's disease, or acute promyelocytic leukemia (APL) in second CR or first partial remission (PR) after the completion of first-line chemotherapy and were chemosensitive to salvage chemotherapy, in whom tumor contamination of harvested peripheral blood stem cells (PBSCs) was possible due to bone marrow or peripheral blood involvement. Lack of CD34 expression by tumor cells was an important selection factor. Eight patients with hematological malignancies (six NHL patients, one ATLL patient, and one APL patient) were enrolled; their median age was 41 years (range 26–49 years). After consolidation and mobilization chemotherapy, two or three courses of apheresis were performed in each patient. After high-dose chemo(radio)therapy, in each patient a median of 1.8×10^6 cells/kg (range $8.2\times10^5-5.1\times10^6$ cells/kg) purified $CD34^+$ PBSCs were infused; granulocyte colony-stimulating factor was given from day 1. Median times to hematopoietic recovery were as follows: WBC of $\geq$1,000/μl, day 11; platelet count of $\geq$50,000/μl, day 19; and reticulocyte count of $\geq$10‰, day 15. Two NHL patients relapsed at 23 and 9 months after PBSCT, respectively; the remaining six patients are alive and in CR. No severe toxicity was observed in any patient. Tumor contamination as measured using a polymerase chain reaction-mediated RNase protection assay at the 10^{-4} level was detected in the $CD34^+$-purified fractions of 2 of the 5 samples analyzed; however, a reduction in contaminating lymphoma cells from the autograft of at least 1,000 to 10,000 orders of magnitude was achieved by $CD34^+$ selection using the immunomagnetic bead method. High-dose chemoradiotherapy with transplantation of $CD34^+$ PBSCs purified by the immunomagnetic bead method was thus shown to be an active and safe therapy for refractory hematological malignancies with bone marrow or peripheral blood involvement. However, it is too early for evaluation of the long-term survival benefit.

Key words $CD34^+$ selection · High-dose chemoradiotherapy · Immunomagnetic beads · PBSCT · Non-Hodgkin's lymphoma

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Introduction

It is well known that refractory hematological malignancies, including high-risk non-Hodgkin's lymphoma (NHL),

adult T-cell leukemia/lymphoma (ATLL), acute T-cell leukemia (ATL), and mantle-cell lymphoma (MCL), have very poor prognosis, even if an initial complete remission (CR) is achieved [10, 18, 28, 32]. Similarly, postchemotherapy-relapsed Hodgkin's disease, NHL, and acute myeloid leukemias also have a poor prognosis [7]. High-dose intensification followed by autologous bone marrow (ABMT) or peripheral blood stem-cell transplantation (PBSCT) is currently used to consolidate or salvage patients with these diseases, particularly those with malignant lymphomas. This approach has been shown to improve prognosis in patients with intermediate- or high-grade NHL or multiple myeloma when used in chemosensitive relapses or in first remission [1, 16, 24, 27, 31]. Similarly, several ongoing studies are investigating the impact of high-dose intensification with stem-cell rescue in early remission of low-grade lymphoma [12, 13, 26].

Infusion of stem cells into NHL patients carries the intrinsic risk of also infusing tumor cells, even when tumor contamination of the graft has not been detected histologically. Effective purging of the bone marrow autograft has been shown to correlate with a lower relapse rate [14]. Current methods of purging lymphoma cells in NHL consist of lysis using monoclonal antibodies (mAbs) and complement [31] and incubation with a cyclophosphamide derivative, mafosfamide [9, 11]. However, these techniques are cumbersome and potentially harmful to normal progenitor cells. Recent studies have shown that bone marrow and peripheral blood progenitor cells bearing the CD34 antigen can be isolated and concentrated using a biotin-avidin immunoadsorption method and can reconstitute hematopoiesis in animals [3, 4] and in neuroblastoma, breast cancer, NHL, and multiple myeloma patients [5, 14, 27, 30]. Lymphoma tumor cells [6] and some acute promyelocytic leukemia (APL), T-cell acute lymphocytic leukemia (T-ALL), and ATL cells do not usually bear the CD34 antigen; therefore, positive selection may be of benefit in NHL and other $CD34^-$ refractory lymphoma/leukemia patients, with potential benefits including a reduction in tumor contamination of the graft. We report on the preparation of $CD34^+$ peripheral blood stem-cell (PBSC) concentrates using the immunomagnetic bead method, engraftment data obtained after high-dose chemoradiotherapy, and minimal residual disease data as the conclusion of a phase I/II multicenter clinical trial conducted by the Nagoya $CD34^+$ PBSCT Study Group.

Patients and methods

Patients

Eight patients aged 26–49 years (median 44 years) and bearing refractory hematological malignancies defined using the protocol's eligibility criteria were enrolled between August 1994 and March 1996. The study design, the primary and secondary end points of which were recognition of hematopoietic reconstitution after $CD34^+$ PBSCT and assessment of therapeutic effects, respectively, was approved by the institutional review boards of all participating institutions. All patients gave written informed consent. Purified $CD34^+$ fractions were prepared from peripheral blood harvested from eight NHL and APL patients in preparation for PBSCT. All patients underwent PBSCT.

Eligibility criteria

Eligibility criteria included the following:

1. Patients with T-ALL, lymphoblastic lymphoma (LBL), MCL, high-risk aggressive NHL, and ATLL in first partial remission (PR) or CR.
2. Patients with standard-risk aggressive NHL, indolent lymphoma, Hodgkin's disease, and APL in second CR or first PR (after the completion of first-line chemotherapy; chemosensitive to salvage chemotherapy).
3. The possibility of tumor contamination of harvested PBSCs due to bone marrow or peripheral blood involvement, including patients with relapsed or refractory NHL histologically diagnosed to be of low, intermediate, or high grade according to the Working Formulation [22] and those with histologically proven APL, T-ALL, and ATL. Involvement of bone marrow or peripheral blood was critical, but patients with tumor involvement of $>5\%$ of bone marrow cells or peripheral blood cells were excluded.
4. The absence of a donor for allogeneic bone marrow transplantation.
5. Tumor cells negative for CD34 antigen.
6. Age was restricted to the range of 15–54 years.
7. The performance status had to be 0 or 1.
8. Renal (serum creatinine values of <1.5 mg/dl and creatinine clearance of >60 ml/min) and hepatic (aspartate transaminase and alanine transaminase levels of <3 times normal and serum levels of total bilirubin of <2.0 mg/dl) function had to be adequate, as did the left ventricular ejection fraction ($>50\%$).
9. Written informed consent had to be obtained.

Induction treatment

Induction treatment for NHL patients was CHOP-like chemotherapy. For patient 2, VEPA-L [600 mg/m^2 cyclophosphamide (CPA) given on days 1 and 15, 40 mg/m^2 doxorubicin (ADR) given on days 8 and 22, 1.4 mg/m^2 vincristine (VCR), (to a maximum of 2 mg) given weekly for 4 weeks, 60 mg prednisolone (PSL) given for 28 days, 6,000 U/m^2 L-asparaginase given from day 25 to day 34, and 10 mg intrathecal (i.t.) methotrexate (MTX) and 20 mg/m^2 PSL given on day 15] therapy was carried out; for patient 1, the VEPA regimen was used; and for patients 3, 4, and 6–8, biweekly treatment with the CHOP [750 mg/m^2 CPA given on day 1, 50 mg/m^2 ADR given on day 1, 1.4 mg/m^2 VCR (maximum of 2 mg total) given on day 1, 100 mg PSL given on days 1–5, and granulocyte colony-stimulating factor (G-CSF) given from day 3 to day 13] regimen was carried out. For the APL patient (patient 5) the second induction treatment consisted of 70 mg all-*trans* retinoic acid (Bethanoid; Nihon Roche, Tokyo, Japan) given for 10 weeks and the DCV [40 mg/m^2 daunorubicin (DNR) given on days 1–3, 200 mg/m^2 cytarabine (Ara-C) given on days 1–5, and 2 mg/m^2 vindesine (VDS) given on day 1] regimen.

Mobilization chemotherapy

Patients with a wide variety of hematological malignancies were eligible for this trial; thus a strict mobilizing chemotherapy regimen was not defined in the protocol. However, to enhance the mobilizing effect, several kinds of combination chemotherapy, all including Ara-C, CPA, or etoposide (VP-16) and recombinant human G-CSF, were used. These included mitoxantrone (MIT)/Ara-C (7 mg/m^2 MIT given on days 1 and 2, 200 mg/m^2 Ara-C given on days 1–5, 60 mg/m^2 PSL given on days 1–5, and 250 μg lenograstim given s.c. daily after chemotherapy; used in the follicular mixed-cell lymphoma patient), CHASE (1,200 mg/m^2 CPA given on day 1, 2 g/m^2 Ara-C given on days 2 and 3, 100 mg/m^2 VP-16 given on days 1–3, 40 mg dexamethasone given on days 1–3, and G-CSF given from day 5 until recovery of the WBC to $>10{,}000/\mu l$; used in the follicular small

cleaved-cell lymphoma patient), or biweekly CHOP (used in MCL and ATLL patients). The mobilizing effect of biweekly CHOP with lenograstim has been confirmed and reported elsewhere [23]. For two patients with LBL in first CR and one patient (patient 5) with APL in second CR, consolidation regimens MEVP (6 mg/m^2 MIT given on day 1, 100 mg/m^2 VP-16 given from day 1 to day 5, 1.4 mg/m^2 VDS given on day 1, and 40 mg/m^2 PSL given from day 1 to day 5) and DCVP (40 mg/m^2 DNR given from day 1 to day 3, 80 mg/m^2 Ara-C given from day 1 to day 6, and 2.4 mg/m^2 VDS given from day 1 to day 6) or behenoyl Ara-C (BHAC)/DNR (200 mg/m^2 BHAC given from day 1 to day 5 and 40 mg/m^2 DNR given from day 1 to day 3, with G-CSF) and BHAC/MIT with G-CSF (250 mg/m^2 BHAC given from day 1 to day 5 and 7.5 mg/m^2 MIT given on days 1 and day 2), respectively, were used to mobilize PBSCs.

PBSC harvesting, CD34$^+$ selection, and cryopreservation

Autologous blood progenitor cells were obtained after mobilizing chemotherapy with G-CSF as described above. Blood progenitor-cell apheresis was begun at 11–14 days after the beginning of chemotherapy. Continuous-flow leukapheresis was performed using AS104 (Fresenius AG, Bad Homburg, Germany). The blood volume processed per run was 10 l at a flow rate of 50 ml/min.

After this procedure, mononuclear cells were collected and CD34$^+$ cells were isolated using an immunomagnetic bead method (Isolex 50 Magnetic Cell Separator; Baxter Health Corporation, Irvine, Calif., USA). This concentration technique includes the following major steps:

1. Harvested cells were treated with Ficoll-Paque (Pharmacia Biotech AB, Uppsala, Sweden) for 30 min at 1500 rpm at room temperature.
2. Target cells were sensitized using a mouse anti-human CD34 antibody and unbound antibody was removed by centrifugation.
3. Sensitized cells were rosetted using Dynal (Dynal AS, Oslo, Norway) paramagnetic microspheres coated with sheep anti-mouse antibodies.
4. CD34$^+$ cells were then captured using a magnet.
5. Paramagnetic beads were released from the cells by treatment with chymopapain and, as of January 1996, PR34$^+$ stem cell-releasing-agent. Released CD34$^+$ cells were concentrated by centrifugation.

CD34$^+$ cells were cryopreserved with 25% human serum albumin and extracellular cryoprotectant solution (CP-1; Kyokuto Seiyaku Kogyo Co. Ltd., Tokyo, Japan) with final concentrations of 4% albumin, 5% dimethylsulfoxide (DMSO), and 6% hydroxyethyl starch (HES) and were stored frozen at –135 °C. A nonpurged backup peripheral-blood harvest sample was cryopreserved for all patients.

Transplantation conditioning regimen

Two conditioning regimens were planned. Regimen 1, designated CEM, comprised 60 mg/kg CPA given daily and 300 mg/m^2 VP-16 given daily in two divided doses for 3 days (day –4 to day –2) and 130 mg/m^2 melphalan (L-PAM) given on day –1. Regimen 2, designated L-PAM/TBI, comprised 60 mg/m^2 L-PAM given for 3 days (days –5 to –3) and total body irradiation (TBI) applied at 3 Gy twice daily for 2 days (days –2 and –1). Mesna was used in patients treated with CEM.

Supportive care

All patients were housed in individual rooms with a high-efficiency particulate air-filtration system. Gut decontamination was accomplished with oral nonabsorbable antibiotics (vancomycin and polymyxin-B), oral trimethoprim-sulfamethoxazole, and a heated low-bacteria diet starting approximately on day –14. Reverse-isolation techniques with masks and gowns were used when neutrophil counts decreased to $<500/\mu l$. Broad-spectrum antibiotics were used for the first febrile episode, and all patients received amphotericin B syrup. All blood products were irradiated with 25 Gy before infusion and were infused with filters to capture lymphocytes. Immunoglobulin was given i.v. to all patients at a dose of 500 mg/kg per week from day –7 to day +30. Purified peripheral progenitor cells were rapidly thawed at 37 °C at 24 h after the completion of pretransplant conditioning and were given i.v., after which all patients received 5 μg/kg G-CSF given i.v. from day 1 until neutrophil recovery (defined as a WBC of $>10{,}000/\mu l$).

Response criteria

Malignant lymphomas

Patients surviving for >1 month posttransplantation were evaluated for response by computed tomography scanning, gallium scintigram, and, if necessary, bone marrow aspiration. These tests were done pretransplantation, every 3 months for the 1st year, and every 4 months thereafter or until disease progression or relapse. A CR was defined as the disappearance of all clinical evidence of disease and the normalization of all laboratory values and radiography results that had been abnormal before treatment for ≥4 weeks. A PR was defined as a reduction of ≥50% in the sum of the products of the cross-sectional diameters of all known lesions that lasted for ≥4 weeks. No response (NR) was defined as any response less extensive than a PR. Disease progression (PD) was defined as the occurrence of new lesions or as an increase of ≥25% in the sum of the products of the cross-sectional diameters of all known lesions.

Acute leukemias

A CR was defined as the disappearance of leukemic cells from bone marrow and peripheral blood and the normalization for ≥4 weeks of all laboratory values and radiography results that had been abnormal before treatment. A PR was defined as a reduction in leukemia cells to <5% in bone marrow and peripheral blood that lasted for ≥4 weeks. Failure was defined as any response less significant than a PR.

Toxicity assessment

Toxicity during and after infusion was assessed and graded according to the Japan Clinical Oncology Group toxicity criteria [34].

Flow-cytometric analysis and cell sorting

Density-separated cells were stained using fluorescein isothiocyanate (FITC)-conjugated anti-CD45 antibody (Fujisawa Pharmaceuticals Co. Ltd., Osaka, Japan) and phycoerythrin (PE)-conjugated anti-CD34 antibody HPCA-2 (Becton-Dickinson Immunocytometry Systems, San Jose, Calif., USA). Samples were run on a FACScan flow cytometer (Becton-Dickinson), and 10,000 events were acquired; data were analyzed using Lysis 11 software (Becton-Dickinson). CD34$^+$ cells were defined using histogram analysis and analysis, gates were set using the CD34 bright cluster obtained after immunoadsorption. To detect B-cell lymphoma cells the Simultest (Becton-Dickinson), which contains a combination of two monoclonal antibodies, was used. Anti-kappa is conjugated to FITC, whereas anti-lambda is linked to PE. These conjugates bind to the kappa light chains and lambda light chains, respectively.

Results

Patients' characteristics, response, and survival

Table 1 shows the type, stage, and status of disease determined in the eight patients. At the time of PBSCT, two individuals (patients 1 and 4) were in PR, and a CR was achieved after transplantation in both patients. The other six

Table 1 Patients' characteristics (*M3v* M3 variant)

Patient	Diagnosis	Status	Age (years)/ gender	Time between diagnosis and entry (months)
1	Follicular mixed	PR2	29/M	77
2	T-LBL	CR1	41/M	3
3	NK	CR2	46/M	21
4	MCL	PR1	49/F	3
5	AML (M3v)	CR2	39/M	31
6	Follicular small cleaved	CR2	41/M	41
7	T-LBL	CR1	26/M	2
8	ATLL	CR1	47/M	6

patients were in first or second CR at the time of PBSCT. The median duration of survival from the time of transplantation was 9 months (range 2–25 months). Patients 1 and 3 relapsed at 5 and 9 months after transplantation, respectively. All eight patients were alive at the time of the study.

Peripheral blood processing and cell-purification results

Results of cell harvesting and purification are listed in Table 2. The number of total peripheral-blood nucleated cells harvested ranged from 3.3×10^9 to 1.56×10^{10} cells/apheresis (median 9.7×10^9 cells/apheresis) and from 1.0×10^{10} to 3.06×10^{10} cells/patient (median 2.02×10^{10} cells/patient). After CD34$^+$ cell selection using microspheres the number of purified CD34$^+$ cells ranged from 4.3×10^6 to 1.88×10^8 cells/apheresis (median 3.8×10^7 cells/apheresis) and from 5.7×10^7 to 3.4×10^8 cells/patient (median 9.37×10^7 cells/patient).

Positive selection after the second course of apheresis in patient 3 was not performed, but apheresis for unprocessed back-up peripheral-blood graft preparation was done. The first and second courses of apheresis attempted in patient 8 failed, probably due to a nonspecific reaction between anti-CD34 antibody and platelets and/or monocytes (data not shown). The 21 purified CD34$^+$ cell preparations obtained were of median purity 92% (range 63.6–99.1%). The median percentage of recovery of CD34$^+$ cells from the starting peripheral blood nucleated cells (CD34$^+$ cell recovery, yield) was 46% (range 11–100%; values calculated to be $>100\%$ were defined as 100%). The CD34$^+$ peripheral-blood grafts transplanted into these eight patients contained a median of 1.75×10^6 CD34$^+$ cells/kg body weight (range 0.825×10^6 to 5.17×10^6 cells/kg).

Toxicity

All eight patients transplanted with CD34$^+$ cell grafts tolerated infusion of CD34$^+$ cells without suffering bradycardia, hypotension, hypertension, or signs of anaphylaxis. Although patients 2 and 8 had grade 3 and 2 hemorrhagic cystitis attributed to adenovirus infection as of day 47 and day 97 after transplant, respectively, they recovered fully.

Table 2 CD34$^+$ cell selection

Patient	Course	Number of cells harvested ($\times10^9$)	Number of cells selected ($\times10^7$)	Purity (%)	Yield (%)	Number of cells infused ($\times10^6$/kg)
1	1	11.4	2.8	79.5	112	0.82
	2	8.9	2.0	78.2	111	
	3	10.2	2.9	63.6	46	
2	1	8.5	8.2	91.6	124	5.17
	2	9.3	18.8	97.4	17	
3	1	12.0	3.7	92.9	31	0.95
	3	10.0	2.5	82.0	42	
4	1	3.3	0.43	92.0	11	1.85
	2	6.6	9.1	96.0	48	
5	1	15.3	4.4	92.2	44	1.25
	2	11.3	2.6	95.5	87	
6	1	15.0	17.2	99.1	57	5.00
	2	15.6	17.0	98.8	49	
7	1	6.1	5.0	93.7	67	1.73
	2	12.3	6.5	87.4	45	
8[a]	3	3.6	1.6	84.8	24	1.76
	4	6.8	3.9	91.6	25	
	5	6.9	3.7	91.2	33	
Median	2	9.7	3.8	92	46	1.75

[a] Presence of CD34$^+$ selection inhibitor

All patients experienced mucositis and received i.v. alimentation routinely and, if pain was severe, i.v. opiate analgesia. All patients had profound myelosuppression, and associated fevers were treated empirically with i.v. antibiotics. However, severe infection, defined as infections of severity exceeding grade 3, was not observed, and no patient had a blood culture that was positive for bacteria or fungi.

Two patients (2 and 4) had grade 3 hypoxemia with interstitial pneumonia (IP). The IP occurring as of day 39 after PBSCT in patient 2 was probably attributable to TBI; patient 4 had idiopathic IP (no evidence of cytomegalovirus) beginning at 4 months after PBSCT. Both patients recovered fully after administration of 50 mg/kg PSL. Patient 4 had grade 3 pericarditis, probably attributable to TBI, beginning at 8 months after PBSCT and recovered fully on PSL treatment.

In summary, most patients experienced only transient toxicities previously observed with the preparative chemoradiotherapy regimens used. There was no episode of venoocclusive disease of the liver, and no patient developed grade 4 or fatal toxicities in the immediate peritransplant period.

Hematopoietic engraftment

All transplanted patients were engrafted (Table 3). The median time until posttransplantation recovery of the WBC to $\geq1000/\mu l$ was 11 days (range 8–12 days). The platelet count recovered to $\geq50{,}000/\mu l$ by a median of 19 days [range 13 days to not yet reached (patient 5 with

Table 3 Hematopoietic recovery after CD34+ PBSCT[a] (*NR* Not reached)

Patient	Recovery of:			
	Number of CD34+ cells infused ($\times 10^6$/kg)	WBC $>1000/\mu l$ (day)	Reticulocyte count $>10\%$ (day)	Platelet count $>50{,}000/\mu l$ (day)
1	0.82	12	14	29
2	5.17	8	18	16
3	0.95	12	17	21
4	1.85	11	13	13
5	1.25	11	16	NR
6	5.00	9	15	15
7	1.73	11	15	41
8	1.76	11	18	18
Median	1.75	11	15	19

[a] All patients received G-CSF after PBSCT

APL)]. The median time until posttransplant recovery of the reticulocyte count to ≥10‰ was 15 days (range 13–18 days). As can be seen from Table 3, the most rapid engraftment was seen in the 2 patients (2 and 6) in whom the number of CD34+ cells infused was $\geq 5\times 10^6$ cells/kg. However, delayed recovery of the platelet count after transplantion did not correlate with the number of nucleated cells infused.

Discussion

This paper reports the results of a phase I/II trial designed to test the feasibility of using CD34+ PBSCs selected by the immunomagnetic bead method for PBSCT after the administration of myeloablative high-dose chemoradiotherapy regimens for consolidation in patients with high-risk or chemosensitive relapsed NHL or APL.

The superiority of high-dose chemotherapy with ABMT over salvage chemotherapy has clearly been established in patients with chemosensitive relapsed NHL [25]. Although the role of myeloablative therapy as first-line therapy for first-CR or first-PR patients with diseases of poor prognosis, such as high-risk aggressive or indolent NHL, LBL, MCL, and ATLL, has not been established, some trials have been reported [13, 26]. Three patients had highly aggressive NHL (two TLBL and one ATLL), and all were in first CR. One other patient in first CR with stage IV MCL was included due to the poor prognosis of the disease, as were two patients with chemosensitive relapsed follicular NHL and one patient with chemosensitive relapsed APL.

Transplantation of concentrated CD34+ stem cells, rather than infusion of autologous bone marrow cells or leukapheresis products, has the advantage of reducing the volume, which facilitates storage and decreases the amount of DMSO and cell-lysis products. This decreased volume has been shown to reduce cardiovascular side effects (specifically effects on the maximal heart rate and blood pressure) that are observed with standard bone-marrow cell infusions [29]. More importantly, the risk of infusing tumor cells while performing an autograft has led to the development of multiple techniques aimed at the removal or destruction of occult tumor cells that potentially contaminate the graft. The level of toxicity of these methods (referred to as negative selection) toward the normal stem cell compartment required to ensure engraftment is variable but potentially high, particularly when CPA derivatives (e.g., mafosfamide) are used.

Positive selection of CD34+ stem cells reduces the risk of damaging stem cells and, if needed, would facilitate further manipulation of the graft for purposes such as gene transfer. With the notable exception of acute leukemias, most hematological tumor cells do not express CD34 antigen on their surfaces [19]. However, one APL patient was included in this study because his leukemia cells were demonstrated to possess no CD34 antigen. Low-grade follicular lymphoma and MCL are particular challenges because they generally present as disseminated diseases with high rates of bone marrow and blood infiltration, which are easily detectable using molecular biology techniques, and both diseases have been reported to be incurable with conservative chemotherapy [15, 28]. Furthermore, although controversial, a recent analysis of outcome has shown lower relapse rates posttransplantion in patients with follicular lymphomas and the traceable bcl-2 rearrangement after effective purging of the bone marrow using a cocktail of mAbs [15].

Several techniques have been developed to concentrate CD34+ stem cells for autografting. All rely first on the recognition of these cells through their immunophenotype, including CD34+ alone [17, 21] or, for example, CD34+ Thy1+ [2] or CD34+/CD38– [33]. Subsequently, cells are selected using panning [21], avidin columns with a biotinylated anti-CD34 antibody system [14], highly efficient cell sorting [2], or the Isolex device with immunomagnetic beads used in the current study [8].

Considerable in vitro data have been reported on the CD34+ and the CD34– subpopulations obtained using these different approaches. These results indicate that the CD34+ fraction is heterogeneous and contains both mature committed progenitors (granulocyte-macrophage colony-forming units and erythrocyte blast-forming units), ensuring early recovery from aplasia, and immature progenitors, ensuring long-lasting hematopoietic reconstitution. Successful engraftment has been obtained in several animal models, including monkeys, using the CD34+ fraction [3, 4] but not the CD34– fraction. In humans, consistent engraftment in a large series of patients has been reported using the biotin-avidin immunoadsorption technique and the immunomagnetic bead method [8]. The simplicity of these techniques makes them easy to use in a hematology laboratory.

In a comparative study of engraftment kinetics in 44 breast cancer patients who received ablative chemotherapy followed by infusion of CD34+ cells concentrated from bone marrow with or without infusion of growth factors, Shpall et al. [30] have reported median times to neutrophil recovery to 0.5×10^9 cells/l of 23 days with bone marrow alone, 16 days when granulocyte-macrophage colony-stimulating factor is added, and 10 days when G-CSF is added

posttransplantion. Civin et al. [8] have reported a median time to neutrophil recovery to 1,000/μl of 32 days after transplantation of $CD34^+$ cells purified using the immunomagnetic bead technique without hematopoietic growth factors in patients with advanced solid tumors [8].

Our study provides further evidence that $CD34^+$ cell concentrates are capable of reconstituting hematopoiesis in patients receiving myeloablative chemoradiotherapy. The study also provides data on several practical aspects regarding the preparation of the concentrates. The purity and recovery of $CD34^+$ cells varied from patient to patient, similar to other results obtained using the immunomagnetic bead method [8] and other techniques [19]. These results are probably related to our observation that the purity of the selected fraction was related to the duration of prior chemotherapy. The 3 patients in whom the number of $CD34^+$ cells concentrated and infused was $<1.5\times10^6$/kg had a long history of combination chemotherapy. Most rapid engraftment (particularly in terms of platelet count) was seen in the 2 patients (2 and 6) in whom the number of $CD34^+$ cells infused was $\geq5\times10^6$/kg, although delayed recovery of the platelet count after transplantation was not demonstrated to correlate with the number of infused nucleated cells. The only patient whose platelet count had not recovered by 6 months after transplantation received 1.25×10^6 $CD34^+$ cells/kg. This dose exceeded the minimal threshold defined in our protocol (0.5×10^6 $CD34^+$ cells/kg); moreover, in the patient who received the lowest dose of $CD34^+$ cells (0.82×10^6 $CD34^+$ cells/kg) the platelet count recovered at day 29. These results suggested the possibility of a significant delay in the recovery of platelet counts in patients who had previously received an excessive number of courses of combination chemotherapy, particularly for acute leukemia, even if the dose of CD34 cells infused was 2 times the threshold dose.

Although polymerase chain reaction-mediated RNase protection assays [20, 35] at the 10^{-4} level demonstrated tumor contamination in 2 (patients 1 and 4) of 5 purified $CD34^+$ cell samples analyzed, a reduction in the number of contaminating lymphoma cells from the autograft of at least 1,000 to 10,000 orders of magnitude was achieved using $CD34^+$ selection by the immunomagnetic bead method (data not shown).

This phase I/II trial of high-dose chemoradiotherapy with PBSCT of $CD34^+$ cells selected using the immunomagnetic bead method shows that this is an active and safe therapy for refractory hematological malignancies with bone marrow or peripheral blood involvement. However, it is too early for any conclusion to be drawn regarding the long-term survival benefit.

References

1. Armitage JO (1989) Bone marrow transplantation in the treatment of patients with lymphoma. Blood 7:1749
2. Baum CM, Weissman IL, Tsukamoto AS, Buckle AM, Peault B (1992) Isolation of a candidate human hematopoietic stem cell population. Proc Natl Acad Sci USA 89:2804
3. Berenson RJ, Bensinger WI, Kalamasz T, Schuening D, Deeg HJ, Graham T, Storb R (1987) Engraftment of dogs with la-positive marrow cells isolated by avidin-biotin immunoadsorption. Blood 69:1363
4. Berenson RJ, Andrews RG, Bensinger WI, Kalamasz D, Knitter G, Buckner CD, Bernstein ID (1988) Antigen CD34+ marrow cells engraft lethally irradiated baboons. J Clin Invest 81:951
5. Berenson RJ, Bensinger WI, Hill RS, Andrews G, Garcia-Lopez J, Kalamasz DF, Still BJ, Spieer G, Buckner CD, Bernstein ID, Thomas ED (1991) Engraftment after infusion of CD34+ marrow cells in patients with breast cancer or neuroblastoma. Blood 77:1717
6. Berenson RJ, Andrews RG, Bensinger WI, Kalamasz DF, Hill RS, Still BJ, Weiden P, Shulman HM, Wamke R, Buckner CD, Bernstein ID (1994) Selection of CD34+ marrow cells for autologous marrow transplantation. Hematol Oncol Ann 2:78
7. Cabanillas F, Jagannath S, Philip T (1989) Management of recurrent or refractory disease. In: Magrath I (ed) Advances in lymphoma. National Cancer Institute, Washington, D.C., p 359
8. Civin CI, Trischmann T, Kadan NS, Davis J, Noga S, Cohen K, Duffy B, Groenewegen I, Wiley J, Law P, Hardwick A, Oldham F, Gee A (1996) Highly purified CD34-positive cells reconstitute hematopoiesis. J Clin Oncol 14:2224
9. Colombat P, Gorin NC, Lemonnier MP, Binet C, Laporte JP, Douay L, Desbois I, Lopez M, Lamagnere JP, Najman A (1990) The role of autologous bone marrow transplantation in 46 adult patients with non-Hodgkin's lymphomas. J Clin Oncol 8:630
10. Fisher RI, Dahlberg S, Nathwani BN, Banks PM, Miller TP, Grogan TM (1995) A clinical analysis of two indolent lymphoma entities: mantle-cell lymphoma and marginal zone lymphoma (including the mucosa-associated lymphoid tissue and monocytoid B-cell subcategories): a Southwest Oncology Group study. Blood 85:1075
11. Fouillard L, Gorin NC, Laporte JP, Douay L, Lopez M, Isnard F, Jouet JP, Noel MP, Morel P, Fenaux P, Aoudjhane M, Stachowiak J, Devidas A, Bauters F, Najman A (1991) Feasibility of autologous bone marrow transplantation for early consolidation of follicular non-Hodgkin's lymphoma. Eur J Haematol 46:279
12. Freedman A, Rie J, Neuberg D, Anderson KC, Rabinowe SN, Mauch P, Takvorian T (1991) Autologous bone marrow transplantation in 69 patients with a history of low grade B cell non-Hodgkin's lymphoma. Blood 77:2524
13. Freedman AS, Gribben JG, Rabinowe S, Neuberg D, Mauch P, Spector N, Anderson K, Soiffer R, Pesek K, Robertson M, Whelan M, Ritz J, Nadler L (1993) Autologous bone marrow transplantation in advanced low grade B cell non-Hodgkin's lymphoma in first remission. Blood 82[Suppl 1]:332A (abstract)
14. Gorin NC, Lopez M, Laporte JP, Quittet P, Lesage S, Lemoine F, Berenson RJ, Isnard F, Grande M, Stachowiak J, Labopin M, Fouillard L, Morel P, Jouet JP, Walter MPN, Detourmignies L, Aoudjhane M, Bauters F, Najman A, Douay L (1995) Preparation and successful engraftment of purified CD34+ bone marrow progenitor cells in patients with non-Hodgkin's lymphoma. Blood 85:1647
15. Gribben JG, Freedman AS, Neuberg D, Denis SD, Roy DC, Blake KW, Woo SD, Grossbard ML, Rabinowe SN, Coral F, Freeman GJ, Ritz J, Nadler AM (1991) Immunologic purging of marrow assessed by PCR before autologous bone marrow transplantation for B cell lymphoma. N Engl J Med 325:1525
16. Gulati SC, Shank B, Black P, Yopp J, Kosiner B, Straus D, Filipa D, Kempin S, Castro-Malaspina H, Cunningham I (1988) Autologous bone marrow transplantation for patients with poor-prognosis lymphoma. J Clin Oncol 8:1303
17. Hardwick AR, Kulcinski D, Mansour V, Ishisawa L, Law P, Gee AP (1992) Design of large scale separation systems for positive and negative selection of cells using superparamagnetic microspheres. J Hematother 1:379
18. International Non-Hodgkin's Lymphoma Prognostic Factors Project (1993) A predictive model for aggressive non-Hodgkin's lymphoma. N Engl J Med 329:987
19. Krause DS, Fackler MJ, Civin CI, May WS (1996) CD34: structure, biology and clinical utility. Blood 87:1

20. Kurokawa T, Kinoshita T, Ito T, Saito H, Hotta T (1996) Detection of minimal residual disease in B cell lymphoma by a PCR-mediated RNase protection assay. Leukemia 10:1222
21. Lebkowski JS, Schain LK, Okrongly D, Levinisky R, Harvey MJ, Okarma T (1992) Rapid isolation of human CD34 hematopoietic stem cells. Purging of human tumor cells. Transplantation 53:1011
22. Non-Hodgkin's Lymphoma Pathologic Classification Project (1982) National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphoma. Summary and description of a Working Formulation for clinical usage. Cancer 49:2112
23. Ogura M, Kagami Y, Suzuki R, Koike K, Tsuboi K, Uehira K, Ariyoshi Y, Ogawa M (1996) Mobilizing effect of peripheral blood stem cells by biweekly CHOP therapy with G-CSF for aggressive non-Hodgkin's lymphoma patients. Ann Oncol 7:172 (abstract)
24. Philip T, Armitage JO, Spitzer G, Chauvin R, Jagannath S, Cahn JY, Colombat P, Goldstone AH, Gorin NC, Flesh M, Laporte JPh, Maraninchi D, Pico J, Bosly A, Anderson C, Schots R, Biron P, Cabanillas F, Dicke K (1987) High dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate grade or high grade non Hodgkin's lymphoma. N Engl J Med 316:1493
25. Philip T, Guglielmi C, Hagenbeek A, Somers R, Van Der Lelie H, Bron D, Sonneveld P, Gisselbrecht C, Cahn JY, Harousseau JL, Coiffier B, Biron P, Mandelli F, Chauvin F (1995) Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med 333:1540
26. Rohatiner AZS, Freedman AS, Nadler L, Lim J, Lister TA (1994) Myeloablative therapy with autologous bone marrow transplantation as consolidation therapy for follicular lymphoma. Ann Oncol 5 (Suppl):143
27. Schiller G, Vescio R, Freytes C, Spitzer G, Sahebi F, Lee M, Hua Wu C, Cao J, Lee JC, Hong CH, Lichtenstein A, Lill M, Hall J, Berenson R, Berenson J (1995) Transplantation of CD34+ peripheral blood progenitor cells after high dose chemotherapy for patients with advanced multiple myeloma. Blood 86:390
28. Shimoyama M et al. (1988) The Lymphoma Study Group (1981–1983). Major prognostic factors of adult patients with advanced T-cell lymphoma/leukemia. J Clin Oncol 6:1088
29. Shpall EJ, Ball ED, Champlin RE, Le Maistre CF, Holland HK, Saral R, Hoffman E, Berenson RJ (1993) A prospective randomized phase III study using the Ceprate SC stem cell concentrator to isolate CD34+ hematopoietic progenitors for autologous marrow transplantation after high dose chemotherapy. Blood 82[Suppl 1]:83a (abstract)
30. Shpall EJ, Jones RB, Franklin WA, Archer PG, Cunel T, Bitter M, Claman H, Beamman S, Stemmer S, Purdy M, Myers S, Hami L, Taffs S, Heimfeld S, Hallagan J, Berenson RJ (1994) Transplantation of enriched CD34+ autologous marrow into breast cancer patients following high-dose chemotherapy: influence of CD34+ peripheral blood progenitors and growth factors on engraftment. J Clin Oncol 12:28
31. Takvorian T, Canellos GP, Ritz J, Freeman AS, Anderson KC, Mauch P, Tarbell N, Coral F, Daley H, Yeap B, Schlossman SF, Nadler LM (1987) Prolonged disease free survival after autologous bone marrow transplantation in patients with non-Hodgkin's lymphoma with a poor prognosis. N Engl J Med 316:1499
32. Teodorovic I, Pittaluga S, Kluin-Nelemans JC, Meerwalut JH, Hagenbeek A, Glabbeke M van, Somers R, Bijnens L, Noordijk EM, De Wolf Peeters C for the European Organization for the Research and Treatment of Cancer Lymphoma Cooperative Group (1995) Efficacy of four different regimens in 64 mantle-cell lymphoma cases: clinicopathologic comparison with 498 other non-Hodgkin's lymphoma subtypes. J Clin Oncol 13:2819
33. Terstappen LWMM, Huang S, Safford M, Lansdorp PM, Loken MR (1991) Sequential generations of hematopoietic colonies derived from single nonlineage-committed CD34+/CD38- progenitor cells. Blood 77:1218
34. Tobinai K et al. (1993) Toxicity grading criteria of the Japan Clinical Oncology Group. Jpn J Clin Oncol 23:250
35. Veelken H, Tycko B, Sklar J (1991) Sensitive detection of clonal antigen receptor gene rearrangements for the diagnosis and monitoring of lymphoid neoplasms by a polymerase chain reaction-mediated ribonuclease protection assay. Blood 78:1318