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NOD mouse is that this strain has a polymorphic SIRPA capable of binding to human CD47.[26] Blocking the CD47-SIRPA interaction by antihuman CD47 Fc inhibited the engraftment of human HSCs in the NOD-SCID xenograft model. In fact, HSCs have contact with macrophages in the vascular or reticuloendothelial niche,[43,44] suggesting that self-recognition by macrophages could operate actively at the HSC niche. The impairment of antiphagocytic signals that involves HSCs specifically may be one of the primary mechanisms for the severe hypocellularity and pancytopenia seen in the BM of HLH patients.

A schematic of the proposed pathogenesis of HLH based on the results of the present study is provided in Figure 7. In HLH, elevated inflammatory cytokines activate macrophages. Mature blood cells with high levels of CRT expression might be readily ingested by macrophages on activation compared with immature cells. The prophagocytic CRT is not expressed in HSCs; however, HSCs cannot compensate for the loss of mature cells in HLH by enhancement of hematopoiesis, because HSCs are also targeted by BM macrophages through inhibition of surface CD47 expression by inflammatory cytokines. The down-regulation of CD47 in HSCs abrogates the antiphagocytic SIRPA signal, resulting in active engulfment of HSCs by macrophages, presumably at the HSC niche. Therefore, hemophagocytosis in HLH occurs by at least 2 independent pathways mediated by CRT or CD47.

We have reported previously that in HLH patients, activated macrophages and monocytes produce a high level of cytokines such as IL-6 and TNF-α, whereas activated T cells produce IFN-γ and M-CSF.[3] These cytokines may cause the down-regulation of CD47 in HSCs that further activate macrophages. Therefore, to trigger HLH development, activation of both acquired and innate immune systems might be required. Nonetheless, our present data highlight the importance of CD47-SIRPA axis in the development of HLH, by which the HSC, the source of all blood cells, becomes the target for engulfment. Accordingly, our results suggest that the CD47-Fc protein that can bind to SIRPA on activated macrophages may be able to suppress the deregulated engulfment of HSCs in HLH. Understanding the mechanism of HSC-specific down-regulation of CD47 will be critical to the development of future therapeutic approaches for HLH.

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Authorship

Contribution: T.K. and K.T. coordinated the project, designed and performed the experiments, analyzed the data, and wrote the manuscript; K.K., T.Y., S.D., G.Y., and Y.K. performed the experiments; J.K. analyzed the data; Y.A. and N.H. provided technical advice; and T.M., H.I., T.T., and K.A. designed the experiments, reviewed the data, and edited the manuscript.

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Correspondence: Katsuto Takenaka, MD, PhD, Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Sciences, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan; e-mail: takenaka@intmed1.med.kyushu-u.ac.jp.

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Letter to the Editor

Expansion of NK cells from cord blood with antileukemic activity using GMP-compliant substances without feeder cells

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Neonatal cord blood (CB) cells have been demonstrated to contain a high percentage of natural killer (NK) cells, but the NK cells are immature, with a low level of cytolytic activity. However, expression levels of perforin and granzyme have been reported to be high in CB NK cells, and it has been suggested that CB NK cells are phenotypically and functionally mature. It has been suggested that CB is a source of stem cells that is as safe and effective as bone marrow or mobilized peripheral blood.[1,2] Also, there are a number of progenitor cell populations in CB that can be differentiated to NK cells. Therefore, CB is a useful source to expand NK cells for adoptive immunotherapy, particularly against malignant cells that express a low level of human leukocyte antigen class I molecules. Resting CB NK cells rapidly respond to cytokine stimulation by increasing cytolytic activity. Adoptive transfer of allogeneic NK cells is a potential immunotherapy to induce a graft-versus-leukemia (GVL) effect, without causing a graft-versus-host disease (GVHD).[3] In this study, we tried to expand NK cells from CB with antileukemic activity using good manufacturing practice (GMP)-compliant substances without feeder cells. We used tacrolimus (FK506) and low molecular weight heparin (dalteparin sodium) for expansion of NK cells. Kim *et al.*[4] showed impaired interleukin (IL)-2 signaling and a reduction in activating receptors in NK cells by tacrolimus. However, we have reported that tacrolimus enhances the cytolytic activity of inhibitory NK cell receptor (CD94/NKG2A)-expressing CD8T cells.[5] Also, Wang *et al.*[6] showed that a related compound (cyclosporine A) has essentially no effect on cytolytic activity of NK cells. There is conflicting data on the effect of calcineurin inhibitors on NK cell. Anyways, tacrolimus can inhibit T-cell proliferation. Heparin was reported to bind to several types of cytokines and to activate them, and also to have an important role in the expansion of hematopoietic progenitor cells.[7] Also, heparin sulfate and its related compounds were recognized by natural cytotoxicity receptors such as NKp30, NKp44 and NKp46, and soluble heparin enhanced the secretion of interferon-γ by NK cells.[8] Spanholtz *et al.*[9] reported efficient expansion of NK cells from CB CD34$^+$ cells, using low molecular weight heparin-based media containing various cytokines. Therefore, we tried to use tacrolimus to inhibit T-cell proliferation and dalteparin to support NK-cell proliferation during NK-cell expansion using IL-2, IL-15 and OKT3 *in vitro*.

Umbilical CB cells (Hokkaido Cord Blood Bank, Sapporo, Japan; 1×10^6 per ml) were cultured with IL-15 (10 ng/ml; PeproTech Inc., Rocky Hill, NJ, USA), IL-2 (5 ng/ml; R&D Systems, Minneapolis, MN, USA) and anti-CD3 monoclonal antibody (mAb) (OKT3, 10-1000 ng/ml, Janssen Pharmaceutical Company, Tokyo, Japan), with or without tacrolimus (0.02-0.1 ng/ml, Fujisawa, Osaka, Japan) and dalteparin sodium (Fragmin, 5-10 U/ml, Pfizer Japan, Tokyo, Japan) in culture medium stem cell growth medium (SCGM) (CeeGenix, Freiburg, Germany), which was produced under GMP, with 5% human AB serum in 24-well plates or T25 flasks without feeder cells. Cell cultures were split approximately one-second to one-fourth after 3-4 days of culture, and fresh medium, cytokines and reagents were added. After 3 weeks culture of umbilical CB cells (1×10^6 per ml) with IL-15, IL-2 and anti-CD3 mAb without feeder cells, CD56$^+$CD3$^-$ NK cells had increased by more than 1000-fold with about 50% purity. Furthermore, addition of dalteparin sodium and tacrolimus efficiently augmented NK cell expansion (1700-fold expansion with 72.8% purity). Also, NK cell proportion was the highest (72.8%) after expansion with both dalteparin sodium and tacrolimus compared with expansion with cytokines only, dalteparin sodium only and tacrolimus only (Table 1, means ± s.d.s, $n = 5$). The proportion of CD56$^+$CD3$^-$ NK cells increased after more than 7 days of culture, and the proportion of CD56-expressing cells increased up to 90% after 3 weeks of culture (Figure 1a, bars indicate means ± s.d.s, $n = 5$). Finally, we could obtain about 40×10^6 NK cells from 1×10^6 unmanipulated CB cells under GMP-conditioned medium with 5% human AB serum without feeder cells. Furthermore, this method has also enabled to expand NK cells from adult peripheral blood mononuclear cells (PBMCs; preliminary data not shown).

These expanded NK cells expressed stimulatory NK cell receptor NKG2D and intracellular cytotoxic molecule granzyme (Figure 1b, bars indicate means ± s.d.s, $n = 5$). Also, the expanded CD16$^+$CD56$^+$ NK cells expressed high levels of inhibitory NK receptors, but significantly higher levels of stimulatory NK cell receptors including NKG2C, NKG2D, NKp30, NKp46 and especially, NKp44, than the levels of these receptors on CD16$^+$CD56$^+$ NK cells in resting CB before culture were noted (Figure 1c, $P < 0.01$, bars indicate means ± s.d.s, $n = 10$).

The cytolytic activities of expanded NK cells were tested against ^{51}Cr-labeled K562 human leukemic cell lines, patients' leukemic cells and allogeneic phytohemagglutin (PHA) blasts (5×10^3), using standard 4-h ^{51}Cr release assays. The expanded NK cells had

Table 1. Expansion of NK cells from CB samples

	Pre	*IL2+15*	*+Tacrolimus*	*+Fragmin*	*+T+F*
%	2.8 ± 0.8	48.3 ± 6.5	50.4 ± 3.7	65.0 ± 10.8^b	72.8 ± 9.6^{a,c,d}
Absolute number	0.028 ± 0.008	36.1 ± 10.4	34.7 ± 16.2	49.9 ± 17.7^e	43.5 ± 14.3
Fold expansion	1	1422 ± 316	1360 ± 581	1989 ± 678^e	1706 ± 389

Abbreviations: CB, cord blood; IL, interleukin; NK, natural killer. Values in the upper column indicate the percentage of CD56$^+$CD3$^-$ cells after 3-weeks expansion of CB cells with indicated factors (%). Significant differences were found in the values compared with the values for culture with only IL-2 and IL-15. Significant differences were also found in the values compared with the values for culture with 0.02 ng/ml of tacrolimus and 5 IU/ml of fragmin. Values in the middle and lower columns indicate the calculated absolute CD56$^+$CD3$^-$ cell number and fold expansion of NK cells after 3-weeks expansion of 1×10^6 CB cells. Significant difference was found only in the value for culture with IL-2, IL-15 and 0.02 ng/ml of tacrolimus, compared with the values for culture with IL-2, IL-15 and 5 IU/ml of fragmin (means ± s.d.s, $n = 5$). $^aP < 0.01$, $^bP < 0.05$, $^cP < 0.01$, $^dP < 0.05$ and $^eP < 0.05$.

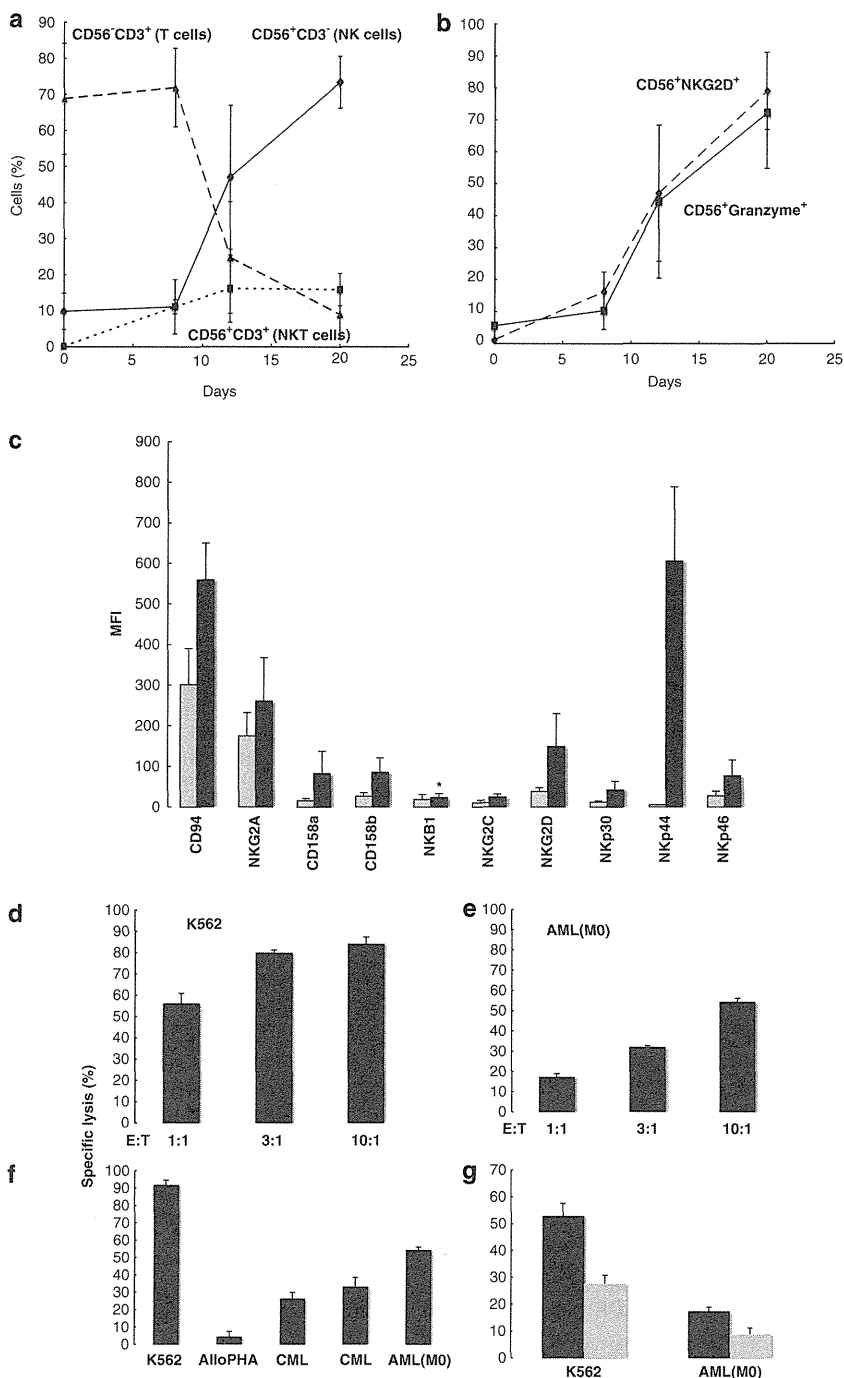

Figure 1. Time course profile of expanded cells during culture without feeder cells. $CD56^+CD3^-$ (NK cells), $CD56^-CD3^+$ (T cells) and $CD56^+CD3^+$ cells (NKT cells; **a**) and $CD56^+NKG2D^+$ cells and $CD56^+granzyme^+$ cells (**b**); bars indicate means ± s.d.s, $n = 5$. Mean fluorescence intensity (MFI) of NK receptors on $CD16^+CD56^+$ NK cells in CB before expansion (gray bars) and after expansion (black bars). There were significant differences between before and after expansion except for NKB1 *($P < 0.01$, $n = 10$; **c**). Cytolytic activities of expanded NK cells against K562 cells (**d**) and AML (M0) patient's leukemic cells (**e**). Data presented are means ± s.d.s (effector/target ratios of 1:1, 3:1 and 10:1). Cytolytic activities against K562 cells, allogeneic third-party PHA blasts and patients's leukemic cells from chronic myeloid leukemia and AML (M0; E:T ratio of 10:1; **f**). Inhibitory effect of anti-NKG2D monoclonal antibody (20 μg/ml, gray bars) against cytolytic activities of expanded NK cells (E:T ratio is 1:1; **g**).

a very high level of cytolytic activity against the K562 leukemic cell line, with specific lysis of more than 50% under the condition of an effector:target ratio (E:T ratio) of 1:1 and more than 80% under the condition of an E:T ratio of 3:1 (Figure 1d). Also, the expanded NK cells could attack patients' primary acute myeloid leukemia (AML; M0) and chronic myeloid leukemia (CP) leukemic cells, but could not attack allogeneic third-party PHA blasts (Figures 1e and f). Anti-NKG2D mAb (1D11, 20 μg/ml, Serotec, Oxford, UK)

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suppressed the cytolytic activity against K562 cells and also patients' primary leukemic cells (Figure 1g). Therefore, the cytolytic activity of these expanded NK cells depended at least partially on NKG2D-activating receptor.

Allogeneic NK cells have been reported to have a strong GVL effect after haploidentical hematopoietic stem cell transplantation (HSCT) in patients with advanced AML, without causing GVHD.[3] Adoptive transfer of allogeneic NK cells may be a promising immunotherapy. However, expansion of NK cells seems to be difficult compared with expansion of T cells. About 20-fold expansion of NK cells was achieved by culture of PBMCs with cytokines for a short time and co-culture with K562 cells that had been transfected with, and expressed membrane-bound IL-15 and 4-1BBL. These expanded NK cells using artificial feeder cells had cytolytic activity against human AML cells and also pediatric solid tumors such as Ewing sarcoma and rhabdomyosarcoma. On the other hand, there have been several reports of NK cell expansion from PBMCs without using feeder cells. Alici *et al.*[10] reported the possibility of expanding NK cells without feeder cells from PBMCs of multiple myeloma patients with significant cytolytic activity against primary autologous multiple myeloma cells. It is more beneficial for clinical use if it is not necessary to use feeder cells for efficient expansion of NK cells *in vitro*. Also, Ayello *et al.*[11] reported a 20-fold expansion of NK cells from CB cells with depletion of adherent monocytes by *ex vivo* culture with IL-2, IL-7 and IL-12 for 7 days. Therefore, NK cells can be expanded from not only PBMCs, but also CB.

Clinical-scale NK cell purification has so far been performed by donor leukapheresis followed by CD3 depletion with or without CD56 enrichment. There have been several clinical reports on adoptive transfer of allogeneic NK cells for hematological malignancies. Passweg *et al.*[12] first reported the feasibility of allogeneic NK cell purification and infusion in five myeloid malignant patients after haploidentical HSCT. Miller *et al.*[13] reported that haploidentical NK cell infusions after cyclophosphamide and fludarabine treatment resulted in expansion of donor NK cells and induction of complete hematological remission in 5 of 19 AML patients with poor prognosis. A recent pilot study showed good results in pediatric patients who received haploidentical NK cells to consolidate chemotherapy for AML patients. Also, Nguyen *et al.*[14] reported a persistent and massive expansion of infused alloreactive NK cells in an AML patient who had relapsed after haploidentical HSCT. Yoon *et al.*[15] reported patients who underwent human leukocyte antigen-mismatched HSCT and subsequently received donor NK cells that were generated from CD34+ cells from donor leukapheresis products by *ex vivo* culture for more than 6 weeks (9.3×10^6 per kg, $CD122/CD56^+$ 64%, $CD3^+$ 1%). There were no signs of acute toxicity in 14 adult patients infused with these cells 6-7 weeks after transplantation, with one patient developing acute GVHD and five patients developing chronic GVHD. Therefore, clinical-grade allogeneic NK cell infusion is safe and feasible. However, many issues remain to be resolved, including selection of donor, NK cell selection and expansion procedure, type of conditioning regimen, survival and expansion of NK cells in the recipient after infusion, their localization and finally, the clinical effect of NK cell infusion.

In this study, we could obtain about 40×10^6 NK cells from 1×10^6 unmanipulated CB cells using tacrolimus and dalteparin sodium without feeder cells (more than 1000-fold expansion with more than 70% purity). Thus, alloreactive Killer cell immunoglobulin-like receptor (KIR)-mismatched expanded NK cells from CB can be used for adoptive NK cell immunotherapy to induce a strong GVL/tumor effect without severe GVHD for patients who do not have KIR-mismatched related donors.

CONFLICT OF INTEREST

A patent application for the composition for expanding NK cells and the use of them has been filed with Junji Tanaka as a sole inventor.

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J Tanaka, J Sugita, S Shiratori, A Shigematu, S Asanuma, K Fujimoto, M Nishio, T Kondo and M Imamura
Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
E-mail: jutanaka@med.hokudai.ac.jp

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ORIGINAL ARTICLE

A comparison of oral mucositis in allogeneic hematopoietic stem cell transplantation between conventional and reduced-intensity regimens

Haruhiko Kashiwazaki · Takae Matsushita · Junichi Sugita · Akio Shigematsu · Kumiko Kasashi · Yutaka Yamazaki · Takashi Kanehira · Takeshi Kondo · Tomoyuki Endo · Junji Tanaka · Satoshi Hashino · Mitsufumi Nishio · Masahiro Imamura · Yoshimasa Kitagawa · Nobuo Inoue

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Abstract Severe oral mucositis developed in allogeneic hematopoietic stem cell transplantation (HSCT) accompanies intolerable pain and risk for systemic bacteremia infection. Conventional stem cell transplantation (CST) and reduced-intensity regimens for allogeneic HSCT (RIST) may differently affect the occurrence and severity of oral mucositis. Here, we comparatively examined oral mucositis in patients undergoing CST and that in RIST patients to search for measures to alleviate oral mucositis. We retrospectively analyzed the data of 130 consecutive patients undergoing HSCT (conventional, 60; RIST, 70). Oral mucositis was evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. We also investigated the risk factors for severe oral mucositis in each regimen. The incidence of oral mucositis was not significantly different between RIST and CST patients. The use of opioid analgesics to control pain due to oral mucositis was significantly less in patients undergoing RIST compared with those receiving CST. The risk factors for severe oral mucositis, determined by univariate and multivariate analyses, were "younger age (<40)" in CST and "longer duration of neutropenia (≥14 days)" in RIST. Although the incidences of oral mucositis were almost the same, the need for opioid analgesics and the risk factors for severe oral mucositis differed between CST and RIST patients.

Keywords Hematopoietic stem cell transplantation · Oral mucositis · Reduced-intensity regimens

H. Kashiwazaki (✉) · T. Matsushita · N. Inoue
Gerodontology, Division of Oral Health Science,
Hokkaido University Graduate School of Dental Medicine,
Kita-13 Nishi-7, Kita-ku,
Sapporo, Hokkaido 060–8586, Japan
e-mail: kashi@den.hokudai.ac.jp

J. Sugita · A. Shigematsu · T. Kondo · T. Endo · J. Tanaka ·
S. Hashino · M. Nishio · M. Imamura
Stem Cell Transplantation Center, Hokkaido University Hospital,
Kita-14 Nishi-5, Kita-ku,
Sapporo 060–8648, Japan

K. Kasashi
Division of Pharmacy, Hokkaido University Hospital,
Kita-14 Nishi-5, Kita-ku,
Sapporo 060–8648, Japan

Y. Yamazaki · Y. Kitagawa
Oral Diagnosis and Oral Medicine,
Division of Oral Pathobiological Science,
Hokkaido University Graduate School of Dental Medicine,
Kita-13 Nishi-7, Kita-ku,
Sapporo 060–8586, Japan

T. Kanehira
Preventive Dentistry, Division of Oral Health Science,
Hokkaido University Graduate School of Dental Medicine,
Kita-13 Nishi-7, Kita-ku,
Sapporo 060–8586, Japan

Introduction

Oral mucositis is one of the most common complications associated with allogeneic hematopoietic stem cell transplantation (HSCT). It was seen in 60–90% of patients who had received stem cell transplantation [1–3]. The oral mucositis in HSCT accompanies so severe pain that it can lead to anorexia and dehydration, and a large population of patients with severe oral mucositis requires total parenteral nutrition and opioid analgesics [4]. Severe oral mucositis is

also associated with worse clinical and economic outcomes, especially systemic bacteremia infection [5].

Recently, reduced-intensity conditioning regimens for allogeneic HSCT (RIST) have been developed for patients who are considered unsuitable for conventional stem cell transplantation (CST) because of advanced age or medical contraindications [6, 7]. The conditioning regimens typically include a purine analog, such as fludarabine (FLU), an alkylating agent, or low-dose total body irradiation (TBI). We need to consider the differences between CST and RIST protocols in the effects on oral mucositis because such a variety of RIST protocols have been developed and their toxicity profiles can make differences in the degree of immunosuppression or myeloablation [2, 3, 8–10].

The present study was a retrospective analysis to compare oral mucositis in 70 consecutive patients who had received RIST, which mainly consisted of FLU, busulfan (BU), and TBI, with that in 60 patients who had received CST during the same period. We also investigated risk factors for severe oral mucositis in each regimen.

Materials and methods

Patients

We retrospectively analyzed the data of 130 consecutive patients undergoing HSCT between March 2006 and December 2009 at Stem Cell Transplantation Center of Hokkaido University Hospital (M, 67; F, 63; 47.6± 15.2 years). CST and RIST were administered to 60 (M, 28; F, 32) and 70 (M, 39; F, 31) patients, respectively. Characteristics of the patients and transplantation are shown in Table 1. The ethical committee of Hokkaido University Hospital approved this study. An informed consent was obtained from each patient.

Table 1 Patients and transplantation characteristics

		CST (*n*=60)	%	RIST (*n*=70)	%	*P* value
Age, median (range)		36 (17–54)		55 (17–68)		<0.01
Patient, sex	Male	28	46.70	39	55.70	0.3
Underlying disease,	ALL	23	38.30	3	4.30	<0.01
	AML	28	46.70	22	31.40	
	MDS	3	5.00	7	10.00	
	CML	4	6.60	2	2.90	
	ML	1	1.70	24	34.30	
	ATLL	1	1.70	1	1.40	
	MM	0	0.00	5	7.10	
	Others	0	0.00	6	8.60	
Disease status at transplantation	CR	41	68.30	27	38.60	<0.01
	Non CR	15	25.00	31	44.30	
	Chronic phase/stable disease	4	6.70	12	17.10	
Conditioning regimen	Fludarabine/busulfan	0	0.00	62	88.60	-
	Fludarabine/melphalan	0	0.00	5	7.10	
	CY/VP16/TBI	27	45.00	0	0.00	
	CY/TBI	23	38.30	0	0.00	
	Others	10	16.70	3	4.30	
Total body irradiation		57	95	64	91.40	0.6
GVHD prophylaxis	Cyclosporine A + methotrexate	23	38.30	27	38.60	0.9
	Tacrolimus + methotrexate	37	61.60	43	61.40	
Stem cell source	Related BM	6	10.00	8	11.00	0.5
	Related PBSC	8	13.30	5	7.10	
	Unrelated BM	37	61.70	47	67.20	
	Unrelated CB	9	15.00	10	14.30	

CST conventional stem cell transplantation, *RIST* reduced-intensity stem cell transplantation, *ALL* acute lymphoblastic leukemia, *AML* acute myelogenous leukemia, *MDS* myelodysplastic syndrome, *CML* chronic myelogenous leukemia, *ML* malignant lymphoma, *ATLL* adult T cell leukemia/lymphoma, *MM* multiple myeloma, *CR* complete remission, *CY* cyclophosphamide, *VP16* etoposide, *TBI* total body irradiation, *GVHD* graft-versus-host disease, *BM* bone marrow, *PBSC* peripheral blood stem cell, *CB* cord blood

Conditioning regimens

Most of the conventional conditioning regimens consisted of TBI (12 Gy in six fractions) plus cyclophosphamide (60 mg/kg once daily i.v. for 2 days, total dose of 120 mg/kg) ±VP-16 (15 mg/kg once daily i.v. for 2 days, total dose of 30 mg/kg) [11, 12], and most of the reduced-intensity conditioning regimens consisted of FLU (30 mg/m^2 once daily i.v. for 6 days, total dose of 180 mg/m^2) plus oral BU (4 mg/kg p.o. in divided doses daily for 2 days, total dose of 8 mg/kg) or intravenous BU (3.2 mg/kg i.v. in divided doses daily for 2 days, total dose of 6.4 mg/kg) plus low-dose TBI (4 Gy in two fractions). Cyclosporine A (CsA, 3 mg/kg) or tacrolimus (FK, 0.03 mg/kg) and short-course methotrexate (MTX) were used for graft-versus-host disease (GVHD) prophylaxis. MTX was given at a dose of 15 mg/m^2 or 10 mg/m^2 on day 1, and 10 mg/m^2 or 7 mg/m^2 on day 3 and day 6.

Assessment of oral mucositis

Oral mucositis was graded as follows according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 3.0[13]:

Grade 1: Erythema of the mucosa
Grade 2: Patchy ulcerations or pseudomembranes
Grade 3: Confluent ulcerations or pseudomembranes, bleeding in response to minor trauma
Grade 4: Tissue necrosis, significant spontaneous bleeding, life-threatening consequences
Grade 5: Death

Grading was done daily by nurses under the instruction of dentists, and the consistency of assessments was double-checked by the dentists during their rounds at least once per week. Severe oral mucositis was defined as grades 3–4.

Assessment of use of opioid analgesics to control pain due to oral mucositis

The use of opioid analgesics to control pain due to oral mucositis was evaluated for all patients, and frequencies of its use were compared among HSCT types.

Oral management

All subjects were referred to dentists, and necessary dental treatment was completed before HSCT. Namely, at least two dentists examined the patients' oral health, including oral hygiene and potential causes of infections in the oral region by radiographic survey and by clinical examination of the hard and soft tissues and dental problems that might cause infection, such as periapical and marginal periodontitis, dental caries, and semi-impacted or impacted teeth, were treated by surgical procedures as much as possible until HSCT. All subjects received instruction regarding self-management of oral hygiene: tooth brushing after every meal and before going to bed, and oral rinsing with normal saline solution every 3 h during the day. The dentists and hygienists weekly performed an oral examination on the patients and monitored their compliance in a clean room.

Table 2 Incidence of oral mucositis

	Grades of oral mucositis				
	0	1	2	3	4
Total (*n*=130)	27	30	30	42	1
%	20.80	23.10	23.10	32.30	0.70
CST (*n*=60)	10	15	15	19	1
%	16.70	25.00	25.00	31.70	1.70
RIST (*n*=70)	17	15	15	23	0
%	24.30	21.40	21.40	32.90	0

CST conventional stem cell transplantation, *RIST* reduced-intensity stem cell transplantation

Statistical analysis

Univariate analyses were performed using the chi-square test and Fisher's exact test, as appropriate. The factors with a *P* value of 0.05 or less in the univariate analyses were included in the multivariate analysis. Multivariate logistic regression models were used to analyze the influence of

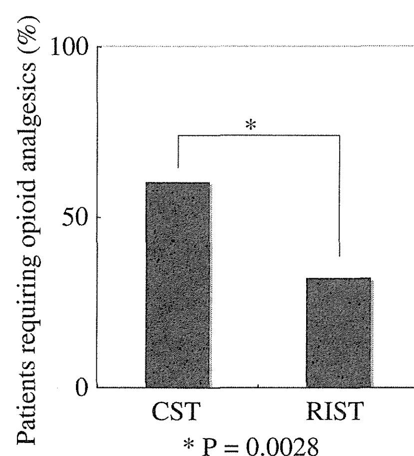

Fig. 1 Use of opioid analgesics to control pain due to oral mucositis in CST and RIST. Difference in frequencies of patients requiring opioid analgesics between CST and RIST were analyzed by the chi-square test

Table 3 Univariate and multivariate analysis for severe mucositis in CST (n=60)

Variables		Severe mucositis				Univariate	Multivariate	
		Yes	%	No.	%	*P* value	Odds ratio (95%CI)	*P* value
Age								
	<40	16	44.40	20	55.60	<0.05	5.6 (1.9–16.5)	<0.05
	≥40	4	16.70	20	83.30			
	<50	19	33.30	38	66.70	0.53		
	≥50	1	33.30	2	66.70			
Sex								
	Male	8	28.60	20	71.40	0.65		
	Female	12	37.50	20	62.50			
Disease status at transplantation								
	CR	14	34.10	27	65.90	0.93		
	Non CR	6	40.00	9	60.00			
Conditioning regimen								
	VP/CY/TBI	13	48.10	14	51.60	<0.05		
	non VP/CY/TBI	7	31.20	26	78.80			
GVHD prophylaxis								
	CsA + MTX	8	34.80	15	65.20	0.93		
	FK + MTX	12	32.40	25	67.60			
Dose of MTX								
	15 10 10	12	32.40	25	67.60	0.81		
	10 10 10	5	31.20	11	68.80			
	10 7 7	1	33.30	2	66.70			
Stem cell source								
	Related BM	3	50.00	3	50.00	0.93		
	Related PBSC	2	25.00	6	75.00			
	Unrelated BM	12	32.40	25	67.60			
	Unrelated CB	3	33.30	6	66.70			
Duration of neutropenia (<500/ml)								
	≥21 days	9	45.00	11	55.00	0.29		
	<21 days	11	27.50	29	72.50			
	≥14 days	20	39.20	31	60.80	<0.05		
	<14 days	0	0	9	100			

CI confidence interval, *CR* complete remission, *TBI* total body irradiation, *CsA* cyclosporine A, *MTX* methotrexate, *FK* tacrolimus, *CY* cyclophosphamide, *BM* bone marrow, *PBSC* peripheral blood stem cell, *CB* cord blood, *VP16* etoposide

selected variables on the risk for severe oral mucositis. For most of the statistical analysis, SPSS 14.0 for Windows (SPSS, Chicago, IL, USA) was used. The *P* value was set to <0.05 as significant.

Results

Patients and transplantation characteristics

Characteristics of the patients and transplantations are shown in Table 1. Median age, underlying disease, and disease status at transplantation were significantly different between CST and RIST patients. Other parameters such as sex, TBI, and GVHD prophylaxis were not different between CST patients and RIST patients.

Incidences and severity of oral mucositis in CST and RIST

As shown in Table 2, the incidences of oral mucositis (>grade 1) were not significantly different between CST and RIST patients according to the NCI-CTCAE; the frequencies were 83.3% (50/60) and 75.7% (53/70), respectively. Severe mucositis (grades 3 and 4) was

Table 4 Univariate and multivariate analysis for severe mucositis in RIST (n=70)

Variables		Severe mucositis				Univariate	Multivariate	
		Yes	%	No.	%	*P* value	Odds ratio (95%CI)	*P* value
Age								
	<40	2	28.60	5	71.40	0.78		
	≥40	21	33.30	42	66.70			
	<50	4	21.10	15	78.90	0.32		
	≥50	19	37.30	32	62.70			
	<60	17	30.90	38	69.10	0.72		
	≥60	6	40.00	9	60.00			
Sex								
	Male	10	25.60	29	74.40	0.15		
	Female	13	41.90	18	58.10			
Disease status at transplantation								
	CR	10	37.00	17	63.00	0.7		
	non CR	10	32.30	21	67.70			
Conditioning regimen								
	FLU/BU	21	33.90	41	66.10	0.88		
	FLU/LPAM	1	20.00	4	80.00			
Total Body Irradiation								
	Yes	21	32.80	43	67.20	0.67		
	No	2	33.30	4	66.70			
GVHD prophylaxis								
	CsA + MTX	7	25.90	20	74.10	0.47		
	FK + MTX	16	37.20	27	62.80			
Dose of MTX								
	15 10 10	6	17.10	29	82.90	0.06		
	10 10 10	13	52.00	12	48.00			
	10 7 7	0	0	2	100			
Stem cell source								
	Related BM	0	0	8	100	0.98		
	Related PBSC	2	40.00	3	60.00			
	Unrelated BM	16	34.00	31	66.00			
	Unrelated CB	5	50.00	5	50.00			
Duration of neutropenia (<500/ml)								
	≥21 days	11	57.90	8	42.10	0.015		
	<21 days	12	23.50	39	76.50			
	≥14 days	21	46.70	24	53.30	0.0024	12.4 (1.4-109)	0.024
	<14 days	2	8.00	23	92.00			

CR complete remission, *FLU* fludarabine, *BU* busulfan, *LPAM* L-phenylalanine mustard, *CsA* cyclosporine A, *MTX* methotrexate, *FK* tacrolimus, *BM* bone marrow, *PBSC* peripheral blood stem cell, *CB* cord blood

observed in 33.3% (20/60) of CST patients and 32.9% (23/70) of RIST patients, which showed no significant difference. However, a significantly lower percentage of patients undergoing RIST (32.2%) required opioid analgesics to control pain due to oral mucositis compared with those undergoing CST (60.4%) as shown in Fig. 1 (P=0.0028).

Univariate and multivariate analyses for severe oral mucositis in CST and RIST

To identify the risk factors for severe mucositis in CST and RIST, a univariate and multivariate analyses were performed in each regimen. The results in CST are summarized in Table 3. The univariate analysis showed that "younger

age (<40)", "VP-16 regimen", and "longer duration of neutropenia (≥14 days)" were significantly associated with a high incidence of severe oral mucositis in CST. Of those, only "younger age (<40)" remained significant in multivariate analysis (odds ratio, 5.6; 95%CI, 1.9–16.5; $P<0.05$). With regards to RIST, the results are summarized in Table 4. Only "longer duration of neutropenia (≥14 days)" was significantly associated with sever oral mucositis in RIST in both univariate and multivariate analyses (odds ratio, 12.4; 95% CI, 1.4–109; $P=0.02$).

Discussion

The results of this study are summarized as follows: (1) The incidence of oral mucositis was almost the same between CST and RIST patients; (2) The use of opioid analgesics to control pain due to oral mucositis was significantly less in patients undergoing RIST compared with those receiving CST; (3) Univariate and multivariate analyses revealed that the risk factors for severe oral mucositis were "younger age (<40)" in CST and "longer duration of neutropenia (≥14 days)" in RIST.

While Takahashi et al. reported that the severity of oral mucositis was reduced in RIST patients compared with CST patients [1], no significant difference was observed in the incidence of severe oral mucositis between patients who received CST and those who received RIST in our study. Several studies reported that severe oral mucositis was correlated with TBI [14, 15]. One of the reasons for this "no significant difference" in our study might be associated with the use of TBI in most RIST patients. The patients who received our RIST regimen including TBI tended to have a longer neutropenic period and more mucosal injury than those in patients who received other RIST regimens [16, 17]. Furthermore, both CST and RIST regimens in the present cases used the same doses of MTX on days 1, 3, and 6 as GVHD prophylaxis.

Severe oral mucositis causes intolerable pain, which is often controlled by the administration of opioid analgesics. As recent trends in cancer pain control recommend the appropriate use of narcotics to minimize pain, the use of opioid analgesics in RIST patients was significantly less compared with that in CST patients. As RIST tends to dispense with narcotics, their major side effects such as ileus could be also avoided.

In multivariate analysis, "younger age (<40)" was significantly associated with severe oral mucositis in CIST patients (odds ratio, 5.6; 95%CI, 1.9–16.5; $P<0.05$). This confirms the report of Vagliano where severe oral mucositis was observed more in adult patients than in the elderly patients [18]. Sonis reported that young patients, who typically have a higher proliferating fraction of basal cells, are three times more likely to develop mucositis than elderly adults in whom the basal cell proliferation is slow [19]. In RIST patients, the "duration of neutropenia (more than 14 days)" was significantly associated with severe oral mucositis in multivariate analysis (OR=12.4, 95%CI 1.4–109, $P=0.024$). Once patients developed oral mucositis, it continued to worsen during neutropenia. In those patients, it is important to prevent the development of oral mucositis.

Although our analysis has limitations due to its retrospective nature and the small sample size, our results showed that the need for opioid analgesics and the risk factors for severe oral mucositis differed between CST and RIST patients. Further prospective controlled studies are needed to assess the differences between CST and RIST for better management of oral mucositis in HSCT patients.

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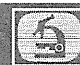

Retrospective analysis of an efficient peripheral blood stem cell collection and the relation between infused cell dose and clinical outcome in patients with malignant lymphoma and multiple myeloma

Y. TSUTSUMI*, R. OGASAWARA*, S. ITO*, J. SASAKI†, A. MORITA†, N. SENOO†, N. MURATA†, J. TANAKA‡, M. ASAKA§, M. IMAMURA‡

*Department of Hematology, Hakodate Municipal Hospital, Hakodate, Japan
†Department of Transfusion, Hakodate Municipal Hospital, Hakodate, Japan
‡Department of Hematology and Oncology, Hokkaido University Graduate School of Medicine, Sapporo, Japan
§Department of Gastroenterology, Hokkaido University Graduate School of Medicine, Sapporo, Japan

Correspondence:
Yutaka Tsutsumi, Department of Hematology, Hakodate Municipal Hospital, 1-10-1, Minato-cho, Hakodate 041-8680, Japan.
Tel.: +81 138 43 2000;
Fax: +81 138 43 4426l;
E-mail: yutsutsu@shore.ocn.ne.jp

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Keywords
Peripheral blood stem cell collection, non-Hodgkin's lymphoma, multiple myeloma, CD34

SUMMARY

Introduction: Etoposide (VP16) is a drug used not only for the treatment of lymphoma but also for the collection of peripheral blood stem cells (PBSCs). We analysed the efficacy and adverse effects of collecting PBSCs and the relation between the infused cell dose and the clinical outcome in lymphoid malignancies.
Method: Investigating 30 patients with non-Hodgkin's lymphoma, one patient with Hodgkin's lymphoma, and five patients with multiple myeloma, we compared the effects of several doses of etoposide with those of CHOP or CHOP-like treatments or salvage treatments. We also analysed the relation between the amount of $CD34^+$ cells collected (above or below 5.0×10^6/kg/day) and prognosis of these patients.
Results: We found the collected cell count to be highest in patients treated with 500 mg/m^2 of VP16 and lowest in those not treated with VP16 ($P = 0.0073$). A $CD34^+$ cell count above 100/μL on the collection day indicates that the target amount of $CD34^+$ cells (4.0×10^6/kg) can be readily obtained and was reached most rapidly by the patients who had received 500 mg/m^2 of VP16 ($P = 0.01$). The longer duration of neutropenia in those patients ($P = 0.000006$) resulted in longer antibiotic treatment ($P = 0.0052$). Both progression-free survival (PFS) and overall survival (OS) were better for the patients who yielded more than 5.0×10^6 $CD34^+$ cells/kg/day ($P = 0.087$ for PFS and $P < 0.033$ for OS).
Conclusion: We show here that 3 days of VP16 at 500 mg/m^2 was useful for the collection of PBSCs and that patients who yielded more than 5.0×10^6 $CD34^+$ cells/kg/day survived longer than those who yielded less.

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INTRODUCTION

Peripheral blood stem cells (PBSCs) are now one of the grafts of choice for autologous stem cell transplantation (ASCT) in patients with malignant lymphoma and multiple myeloma. At least 2×10^6 $CD34^+$ cells/kg need to be used to prevent failure or delayed engraftment (Haas *et al.*, 1994; Laport *et al.*, 1996; Schwella *et al.*, 1996; Kiss *et al.*, 1997; Watts *et al.*, 1998), and the use of more than 5×10^6/kg has been suggested for successful engraftment in nearly all patients (Bensinger *et al.*, 1995; Weaver *et al.*, 1995; Sutherland *et al.*, 1999). Efficient PBSC collection without serious adverse effects is thus important when patients require ASCT.

While several collection regimens have been reported, either a single-agent treatment or a combination treatment with cyclophosphamide followed by granulocyte colony-stimulating factor (G-CSF) is commonly used (Goldschmidt *et al.*, 1996, 1997; Malone *et al.*, 2003). Etoposide (VP16) is a drug used not only for the treatment of lymphoma but also for the collection of PBSCs (Confer *et al.*, 1998; Raiser *et al.*, 1999; Shied *et al.*, 2000; Jungians *et al.*, 2001), and in the present study we retrospectively analysed the collection efficacy of low doses of VP16 and evaluated their adverse effects. We also compared the clinical outcome between patients who yielded more than 5×10^6 $CD34^+$ cells/kg and patients who yielded $<5 \times 10^6$ $CD34^+$ cells/kg. We found that a collection regimen using 3 days of 500 mg/m^2 of VP16 was effective for collecting PBSCs without producing any serious adverse effects.

PATIENTS AND METHODS

Patients

From February 2005 to March 2010, we performed PBSC collections in 36 patients with non-Hodgkin's lymphoma, Hodgkin's lymphoma, or multiple myeloma. The patient characteristics are listed in Table 1. Before the PBSC collection, 30 of the patients received CHOP (cyclophosphamide,750 mg/m^2; vincristine, 1.4 mg/m^2; doxorubicine, 50 mg/m^2 on day 1; prednisolone 100 mg/body on day 1–5) or CHOP-like treatments and five received radiation therapy. Almost all the patients with lymphoma (29 of 31) or multiple myeloma (4 of 5) were at the most advanced stage (stage IV lymphoma or stage III multiple myeloma). The Committee for Ethics of Medical Experiments on Human Subjects of Hakodate Municipal Hospital approved this study and all the patients in this study were enroled after obtaining their informed consent.

Table 1. Characteristics enrolled patients

Median age (range)	57 (37–67)
Sex (M/F)	25/11
Disease	
DLB	20
MCL	3
IVL	2
MALT	1
FL	1
PL	1
AILT	2
HD	1
MM	5
Stage	
Lymphoma (I/II/III/IV)	0/1/1/29
Myeloma (I/II/III/)	1/0/4
Previous treatment	
R-CHQP or CHOP like regimen	30
R-ESHAP	1
CASER	1
R-EPOCH	1
Vel + DEX	4
FND	1
BEACOPP	1
Cyclo-VAMP	2
VAD	2
MP or MCNU-VMP	2
Median previous treatment count (range)	3 (2–14)
Radiation	5
PESC count ($CD34^+$ cells/day)	4.73×10^6/μL/kg (0.215–25.4)

Collection treatment and PBSC collection

Before PBSCs were collected, 26 patients were given 500 mg/m^2, 350 mg/m^2, or 250 mg/m^2 of VP16 for 6 h, with or without rituximab, on three consecutive days (Table 2) and the other 10 patients were given CHOP or CHOP-like treatments or salvage treatments. All patients were hospitalized during VP16 treatments. PBSC collection treatments were started 3–4 weeks after the last chemotherapy. Subcutaneous injection of 200 μg/m^2 G-CSF (Filgrastim; Kyowa Hakko Kirin

Table 2. Efficacy of $CD34^+$ cells collection

Factor	N	Average ($\times 10^6/\mu L/kg$) (range: $\times 10^6/\mu L/kg$)	P
500 mg/m^2 VP16	17	10.5 (1.44–25.36)	0.0073
350 mg/m^2 VP16	4	8.10 (1.38–13.98)	
250 mg/m^2 VP16	5	4.42 (0.465–6.84)*	
The others	10	2.53 (0.215–7.76)**	
Age			
≥56	22	6.97 (0.215–17.94)	0.07176
≤55	14	6.75 (0.85–25.4)	
B-cell lymphoma	28	8.25 (0.215–25.36)	0.2895
T-cell lymphoma	2	2.32 (1.44–3.20)	
Multiple myeloma	5	4.42 (0.465–6.84)	
Previous treatment			
≥4	16	5.06	0.0763
≤3	20	17.9	
CD34 cell court			
>100/μL	14	13.0	0.00001
30–100/μL	7	6.39**	
<30/μL	15	2.17**	

ANOVA with Tukey's/Scheffe's test: *$P<0.05$; **$P<0.01$.

Co, Ltd, Kyowa, Tokyo, Japan) was given twice a day until the day before the last leukapheresis. The $CD34^+$ cell count in a morning peripheral blood sample was monitored daily by flow cytometry, and leukapheresis was performed with a continuous-flow blood cell separator (AS TEC 204; Fresenius, Bad Homburg, Germany). The blood volume processed, at 50–60 mL/min, in each leukapheresis was 150–200 mL/kg. Venous blood was obtained through a double-lumen catheter in the femoral vein (Arrow International Inc, Reading, PA, USA). The PBSC count determined by leukapheresis of $CD34^+$ cells was 4.73×10^6/kg/day (range, $0.215–25.4 \times 10^6$/kg/day).

Statistical analysis

Statistical tests were performed with StatMate IV (ATMS Co, Ltd, Tokyo, Japan) software, and data are expressed as mean ± SD or median (range). Differences were evaluated by ANOVA, and P values <0.05 were considered statistically significant.

Survival

Survival and progression-free survival (PFS) probabilities were estimated by the Kaplan–Meier methods. Overall survival (OS) was calculated from the date of diagnosis to the date of the patient's last follow-up or death for any reason. PFS and relapse were determined with cumulative incidence estimates, treating relapse and death as competing risk events. All time-to-event end points were censored at the time of last contact.

RESULTS

PBSC collection efficacy

The relation between several factors and the efficacy of $CD34^+$ cell collection is shown by the values listed in Table 2. The 500-mg/m^2 dose of VP16 was the one most effective for the collection of $CD34^+$ cells, and the 350 and 250-mg/m^2 doses were also more effective than treatments other than VP16 ($P = 0.0073$ in the top row, 0.01 in the second row, and 0.05 in the third). Age and disease type did not affect the efficacy of $CD34^+$ cell collection, but the collection efficacy tended to be better in the group that had received previous treatments fewer than four times than in the group that had received them four or more times ($P = 0.0763$). A $CD34^+$ cell count above 100/μL on the collection day was a good marker for being able to collect a sufficient amount of $CD34^+$ cells ($P = 0.00001$), and a $CD34^+$ cell count of at least 30/μL appeared to be needed for collecting the target amount ($P < 0.01$).

PBSC collection efficacy and patient characteristics in the treatment groups

Clinical stage, international prognosis index, and previous treatment frequency did not differ significantly between the groups. Although the rates of complete remission (CR) were higher for the groups treated with VP16, none of the CR rates differed significantly between groups. Obtaining a sufficient amount of $CD34^+$ cells, however, requires more collecting days in the groups receiving treatments other than VP16 than it did in the groups treated with VP16 ($P = 0.018$).

Adverse effects

The duration of neutropenia (neutrophil count below 500/μL) and the lowest neutrophil count correlated

with the VP16 dose, and the neutropenia duration and lowest neutrophil count for the group treated with other regimens were between the corresponding values for the groups treated with 250 and 350 mg/m^2 of VP16. The duration of the below-500/μL neutrophil count was shortest in the group treated with 250 mg/m^2 of VP16 ($P = 0.000006$) and the neutrophil count was highest in the group treated with 250 mg/m^2 of VP16 ($P = 0.0016$). The numbers of febrile days and C-reactive-protein-positive days did not differ significantly between any of the treatment groups. Although the number of days in which antibiotic treatment was required was smallest for the group that did not receive VP16 ($P = 0.0052$), uncontrollable infection was not observed in any of the VP16-treated groups. Antibiotic treatments were started at the time of fever or the elevation of C-reactive protein. This is the reason for the differences in the duration of the administration of antibiotics.

Survival differences between treatment groups

Survival analysis was performed for patients receiving front-line peripheral blood stem cell transplantation (PBSCT), patients receiving salvage PBSCT, and patients receiving only chemotherapy (Table 3). Front-line PBSCT was given to patients who in their first CR were at high or high-intermediate risk according the international prognostic index (IPI). The chemotherapy group contained patients who did not hope for a transplant because their progress was good as well as patients who died before receiving a transplant. The salvage PBSCT group contained the three recurrence patients and three chemotherapy-refractory patients. Age and stage were almost the same in these three groups, but the numbers of patients scoring a high or high-intermediate risk in the IPI were much larger in the patients receiving only chemotherapy. A low-risk-score patient with testis lymphoma received front-line PBSCT. The chemotherapy regimen consisted of rituximab plus an MCEC regimen (ranimustine, 200 mg/m^2 for 2 days; carboplatin, 300 mg/m^2 for 4 days; etoposide, 500 mg/m^2 for 3 days; and cyclophosphamide, 50 mg/kg for 2 days) (Numata *et al.*, 2010). Both the PFS and OS were best in the group of patients receiving front-line PBSCT and worst in the patients receiving salvage PBSCT (Figure 1a, $P < 0.00001$; and Figure 1b, $P = 0.0016$). Although the PFS in the group treated with front-line PBSCT differed significantly from that in the group treated with chemotherapy alone (Figure 1a, $P < 0.026$), the OS did not (Figure 1b, $P = 0.089$).

Analysing the relation between the amount of CD34$^+$ cells collected (above or below 5.0×10^6/kg/day) and the characteristics of 15 patients with non-Hodgkin's lymphoma, we found that the amount of cells collected was lower in men than in women but there were no other significant differences between the two cell-count groups (Table 4).

We then analysed the relation between the amount of CD34$^+$ cells collected and the PFS and OS. There were no significant differences in age, sex, stage, or IPI between the group yielding more than 5.0×10^6 CD34$^+$ cells/kg/day and the group yielding $<5.0 \times 10^6$ CD34$^+$ cells/kg/day, but the PFS and OS were better for the patients who yielded more than 5.0×10^6 CD34$^+$ cells/kg/day (Figure 1c, $P = 0.087$; and Figure 1d, $P < 0.033$).

Table 3. Characteristics of patients in survival-analysis groups

	Front-line PBSCT	Salvage PBSCT	Chemotherapy only
Age	57 (37–67)	54 (43–65)	59 (42–66)
Sex	5/4	5/1	9/5
Disease			9
DLB		3	1
IVL	7		1
FL	1		2
MCL		1	
MALT		1	
PL		1	1
AITL	1		
Stage (I/II/III/IV)	0/0/0/9	0/0/0/6	0/1/0/13
IPI (L/LI/IH/H)	1/1/4/3	1/2/1/2	2/4/5/3

IPI, international prognostic index; PBSC, peripheral blood stem cell.

DISCUSSION

High-dose chemotherapy with autologous hematopoietic stem cell rescue is one of the standard treatment options for several hematopoietic malignancies, and PBSCs are autologous hematopoietic stem cells useful for transplantation. Among the advantages of using PBSCs are faster engraftment, a shorter hospital stay,

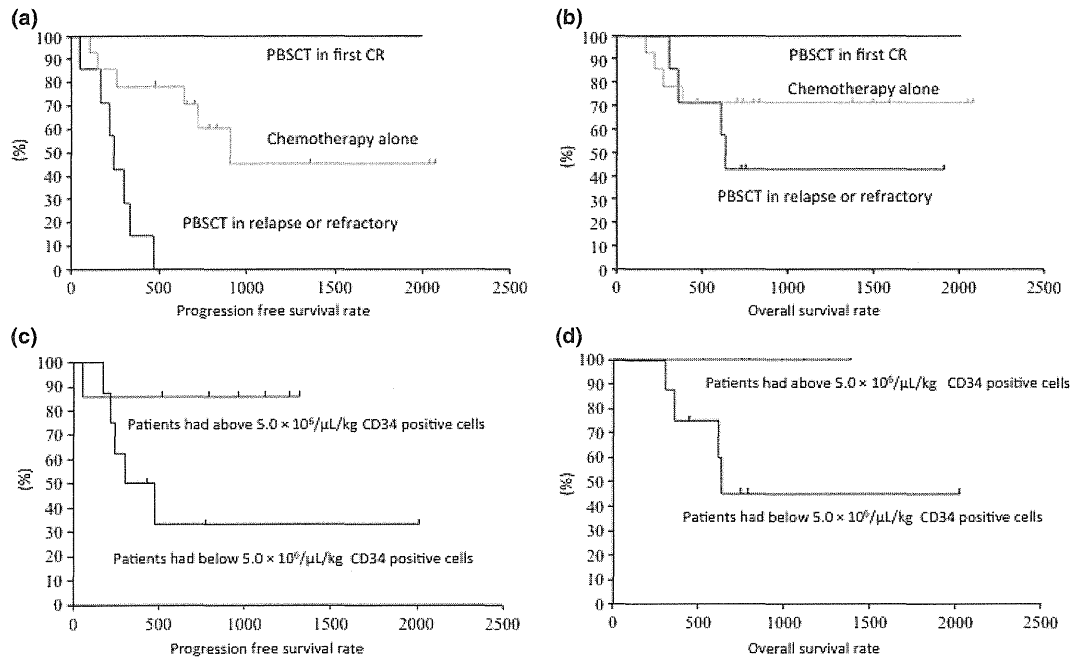

Figure 1. (a, b) Progression-free and overall survival (OS) of patients receiving front-line PBSCT (PBSCT at first complete remission), PBSCT as salvage therapy (PBSCT at relapsed or refractory disease), or chemotherapy alone. (c, d) Progression-free and OS of patients yielding $CD34^+$ cell counts above and below 5.0×10^6/μL/kg/day.

Table 4. Characteristics of non-Hodgkin's lymphoma patients with large and small amounts of $CD34^+$ cells collected in a day

Factor	Daily collection $CD34^+$ cell dose: Above 5.0×10^6 μL/kg/day	Below 5.0×10^6 μL/kg/day
Age	55 (37–67)	56 (52–65)
Sex (male/female)	4/3	6/2
Disease		
DLB	6	4
IVL		1
FL		
MCL		1
MALT	1	
PL		1
AITL		1
Duration of engraftment (average: days)	10.4	9.4
Stage (I/II/III/IV)	0/0/0/7	0/0/0/8
IPI (L/LI/IH/H)	0/2/3/2	2/1/2/3

IPI, international prognostic index.

convenient stem cell collection, and a lower chance of tumour contamination (Gordan *et al.*, 2003). Chemotherapy combined with G-CSF is one of the useful methods of collecting PBSCs, and several combinations of chemotherapy and G-CSF have been studied (Bensinger, Dipersio & McCarty, 2009). A preparative regimen comprising cyclophosphamide and/or VP16 plus G-CSF is commonly used before PBSCs are collected, but several authors have reported that the standard collection treatment with cyclophosphamide failed in 10–30% of heavily pre-treated patients.

Several studies have investigated the effectiveness and toxicity of VP16 used alone and combined with other agents for stem cell collection (Confer *et al.*, 1998; Raiser *et al.*, 1999; Shied *et al.*, 2000; Jungians *et al.*, 2001; Mollee *et al.*, 2002; Li *et al.*, 2009), and most have recommended VP16 doses ranging from 1.0 to 2.0 g/m^2. Infections sometimes occur during the neutropenia associated with these doses, and although reducing the dosage of VP16 decreases the chances of infection and adverse effects, it also reduces the mobilized $CD34^+$ cell count. In the present study, the 500 and 350 mg/m^2 doses given for 3 days caused longer

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lasting neutropenia and lower neutrophil counts but uncontrollable infection was not observed. These results showed that a certain amount of VP16 was necessary in collection treatments. This analysis also confirmed that CHOP or CHOP-like therapy may not be recommended as a mobilizing regimen and that PBSC collection is easy when the VP16 dose is low.

High-dosage chemotherapy combined with autologous PBSCT is also considered better than chemotherapy alone in terms of long-term remission and better survival (Philip *et al.*, 1995; Villanueva & Vose, 2006; Rohatiner *et al.*, 2007; Bensinger, Dipersio & McCarty, 2009). In this retrospective analysis, front-line PBSCT turned out to be the best option for patients with lymphoma and chemotherapy alone turned out to be their second-best option. For the refractory or relapsed patients, salvage PBSCT was the worst option. These results showed that front-line PBSCT is one of the superior treatment options for patients with lymphoma at high or intermediately high risk. Although the PFS and OS of patients treated with salvage PBSCT were inferior to those of patients treated with front-line PBSCT or chemotherapy alone, 40% of relapsed or refractory patients treated with salvage PBSCT were still alive at the end of this study. Overall survival of patients treated with salvage PBSCT was not inferior to that in a previous report (Bensinger, Dipersio & McCarty, 2009). Some relapsed and refractory patients might be rescued by high-dosage chemotherapy plus autologous PBSCT.

The correlation between the collected $CD34^+$ cell count and the prognosis is unclear. Some investigators have reported that higher stem cell doses are associated with better survival (Pavone *et al.*, 2006; Bensinger, Dipersio & McCarty, 2009), but others have reported that there is no significant correlation between prognosis and high-dosage therapy with autologous stem cell rescue (Blystad *et al.*, 2004; Wang *et al.*, 2007). Patients who yielded more than $5.0 \times 10^6/\mu L/kg$ $CD34^+$ cells showed longer PFS and OS. Some investigators have reported delayed engraftment because of the bone marrow stromal damage caused by high-dosage chemotherapy before stem cell transplantation (Galotto *et al.*, 1999; Awaya *et al.*, 2002), so stromal damage may be less in patients who yielded higher numbers of $CD34^+$ cells, resulting in a rapid hematopoietic recovery. But no significant difference in the length of time between PBSCT and engraftment was observed. In the present study, we usually infused about $4.0 \times 10^6/\mu L/kg$ $CD34^+$ cells, which appeared to be enough to accomplish a successful engraftment by day 10. It therefore seems that the speed of engraftment may be influenced more by the infused cell count than the marrow environment.

In conclusion, treatment with 500 mg/m^2 of VP16 for 3 days in this study was the best regimen for the collection of $CD34^+$ cells. Although the result of upfront PBSCT was satisfactory, the suitable regimen for PBSC collection and the suitable recipient for autologous PBSCT will need to be clarified in further studies.

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ORIGINAL ARTICLE: CLINICAL

Clinical evaluation of WT1 mRNA expression levels in peripheral blood and bone marrow in patients with myelodysplastic syndromes

Yasunori Ueda[1], Chisato Mizutani[1], Yasuhito Nannya[2], Mineo Kurokawa[2], Sumiko Kobayashi[3], Jin Takeuchi[3], Hideto Tamura[4], Kiyoyuki Ogata[4], Kazuo Dan[4], Hirohiko Shibayama[5], Yuzuru Kanakura[5], Keiko Niimi[6], Ko Sasaki[7], Masato Watanabe[8], Nobuhiko Emi[8], Masanao Teramura[9], Toshiko Motoji[9], Michiko Kida[10], Kensuke Usuki[10], Satoru Takada[11], Toru Sakura[11], Yoshikazu Ito[12], Kazuma Ohyashiki[12], Hiroyasu Ogawa[13], Takahiro Suzuki[14], Keiya Ozawa[14], Kiyotoshi Imai[15], Masaharu Kasai[15], Tomoko Hata[16], Yasushi Miyazaki[16], Yasuyoshi Morita[17], Akihisa Kanamaru[17], Akira Matsuda[18], Kaoru Tohyama[19], Daisuke Koga[20], Hiroya Tamaki[13], Kinuko Mitani[7], Tomoki Naoe[6], Haruo Sugiyama[21] & Fumimaro Takaku[22]

[1]*Department of Hematology/Oncology, Transfusion and Hemapheresis Center, Kurashiki Central Hospital, Okayama, Japan,* [2]*Department of Hematology and Oncology, University of Tokyo Graduate School of Medicine, Tokyo, Japan,* [3]*Department of Hematology and Rheumatology, Nihon University School of Medicine, Tokyo, Japan,* [4]*Division of Hematology, Department of Medicine, Nippon Medical School, Tokyo, Japan,* [5]*Department of Hematology and Oncology, Osaka University Graduate School of Medicine, Osaka, Japan,* [6]*Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan,* [7]*Department of Hematology and Oncology, Dokkyo Medical University Hospital, Tochigi, Japan,* [8]*Department of Hematology, Fujita Health University School of Medicine, Aichi, Japan,* [9]*Department of Hematology, Tokyo Women's Medical University, Tokyo, Japan,* [10]*Division of Hematology, NTT Kanto Medical Center, Tokyo, Japan,* [11]*Department of Hematology, Saiseikai Maebashi Hospital, Gunma, Japan,* [12]*Division of Hematology, Tokyo Medical University Hospital, Tokyo, Japan,* [13]*Division of Hematology, Department of Internal Medicine, Hyogo College of Medicine, Hyogo, Japan,* [14]*Division of Hematology, Department of Medicine, Jichi Medical University, Tochigi, Japan,* [15]*Department of Hematology, Sapporo Hokuyu Hospital, Sapporo, Japan,* [16]*Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan,* [17]*Division of Hematology, Kinki University School of Medicine, Osaka, Japan,* [18]*Department of Hemato-Oncology, Saitama International Medical Center, Saitama Medical University, Saitama, Japan,* [19]*Department of Laboratory Medicine, Kawasaki Medical School, Okayama, Japan,* [20]*Diagnostic Division, Otsuka Pharmaceutical Co., Ltd., Tokushima, Japan,* [21]*Department of Functional Diagnostic Science, Osaka University Graduate School of Medicine, Osaka, Japan, and* [22]*Jichi Medical University, Tochigi, Japan*

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Abstract

A study to evaluate WT1 mRNA expression levels in peripheral blood (PB) and bone marrow aspirate (BM) was conducted in 172 patients, including 115 with myelodysplastic syndromes (MDS), in Japan. The level of WT1 mRNA expression was evaluated according to the French–American–British (FAB) and World Health Organization (WHO) classifications (2001, 2008) and using the International Prognostic Scoring System and the WHO Prognostic Scoring System scales. WT1 mRNA expression levels in PB and BM were well correlated ($r = 0.85$), and they tended to increase with disease stage progression and in those at higher risk of leukemic transformation. WT1 mRNA expression can be a useful marker for the diagnosis and risk evaluation of MDS.

Keywords: Myelodysplastic syndromes, WT1 mRNA expression, classification system, peripheral blood, bone marrow

Introduction

Myelodysplastic syndrome (MDS), a clonal disorder of pluripotent hematopoietic stem cells, is a blood disease characterized by dysplasia and ineffective hemopoiesis. Approximately 20–30% of cases of MDS undergo transformation to acute myeloid leukemia (AML) [1].

The expression of Wilms' tumor gene (WT1) has been found to be a new prognostic factor and marker for the detection of minimal residual disease (MRD) in acute leukemia, including AML and acute lymphocytic leukemia (ALL) [2]. A recent study has revealed the clinical relevance of measuring WT1 mRNA for monitoring MRD in AML, primarily due to its high rate of expression (93.9%) in the peripheral blood (PB) of incipient untreated patients with AML, secondarily due to its ability to predict relapse after complete remission (CR), and finally because its levels after consolidation therapy

Correspondence: Yasunori Ueda, MD, PhD, Department of Hematology/Oncology, Transfusion and Hemapheresis Center, Kurashiki Central Hospital, 1-1-1 Miwa, Kurashiki, Okayama 710-8602, Japan. Tel: + 81-86-422-0210. Fax: + 81-86-425-9879. E-mail: ueda-y@kchnet.or.jp

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