厚生労働科学研究費補助金　再生医療等研究事業

H17－再生－一般－016

＜研究課題名＞

同種造血幹細胞移植治療の成績向上を目指した包括的臨床研究

平成 17 年度～平成 19 年度
総合研究報告書

主任研究者　　高上　洋一
（所属機関　国立がんセンター中央病院）

平成 20（2008）年 4 月

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『　同種造血幹細胞移植治療の成績向上を目指した包括的臨床研究　』

主任研究者：	国立がんセンター中央病院	高上 洋一	（平成17年度～19年度）
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分担研究者：	九州大学病院	長藤 宏司	（平成17年度）
分担研究者：	近畿大学医学部	金丸 昭久	（平成17年度）
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研究要旨

骨髄移植などの同種造血幹細胞移植は、患者の年齢が50歳を超える場合や移植前に既に臓器の働きが低下している場合には、通常の方法で移植を行うと早期死亡率が著しく高くなる。これを克服する方法として、ドナー由来のリンパ球を介する移植片対白血病(GVL)効果を利用したミニ移植がヒトゲノム・再生医療等研究事業(以下、本事業と略す)において開発されて急速に普及している。本研究では、ミニ移植の標準化と安全性の向上を目指した臨床試験、移植領域に必須の薬剤の適応拡大、ならびに患者診療に密接に関連した基礎研究を行った。同時に、移植治療や再生医療の科学的根拠を検証する基盤研究を飛躍的に進める有力なツールとなる緑色蛍光タンパク質遺伝子導入マウス移植系による投与細胞の生体内での細胞動態解析システムを新たに開発した。これらの成果を移植医療の均てん化に利するために、全国の施設からの研修者教育にも力を注いだ。

A. 研究目的

ミニ移植を含めた各種の造血幹細胞移植の標準化を目指した臨床試験、安全性向上のための合併症対策、ならびに移植医療という希少領域に必須の薬剤の適応拡大を図り、また付随した基礎的研究も推進する。

具体的には、海外などのエビデンスを収集、解析して必須薬剤の適応を取得すると同時に、治療の標準化と適正化を図るための臨床試験を遂行する。同種免疫反応には人種差があるため、欧米の試験成績をわが国の移植現場に直接当てはめることはできず、我が国固有のエビデンスを必要としている。本研究で行う臨床試験の最大の特徴は、質の高いエビデンスを得るために既に稼動している臨床研究母体に拠って厳正なデータ管理を実施することである。これにより、我が国の移植領域における臨床試験体制の基盤を確立すると同時に、治療技術の伝播、医療の均てん化にも貢献する。

合併症の中でも、特にGVHDとサイトメガロウィルス（CMV）などの感染症は多くの患者に発症し、高齢者においては特に重篤化し易いため、重点対策を講じて移植治療の安全性を確保することは極めて重要である。しかし、GVHDは治療益であるGVL効果も合わせ持つため、その完全な予防が必ずしも治療成績の向上にはつながらない。よって、患者特性に合わせたGVHD予防と治療法、あるいは安全なGVL効果の増強法を開発する必要がある。併せて、移植治療に科学的根拠を付与するための各種の基礎研究を遂行し、新たな解析システムを開発する。

B．研究方法

他に有効な治療法がない白血病、悪性リンパ腫や骨髄異形成症候群、あるいは難治性の固形腫瘍患者を対象として臨床試験と基礎研究を遂行する。骨髄非破壊的なミニ移植前処置として、プリン誘導体であるフルダラビン(30 mg/kg/day x 6日) あるいはクラドリビン(0.11 mg/kg/day x 6日)に加えてブスルファン(4 mg/kg x 4日)を、また臍帯血移植の場合にはメルファラン(70 mg/kg x 2日)と4 Gyの全身放射線照射を投与した後に血縁者の末梢血幹細胞、非血縁者の骨髄あるいは臍帯血を用いた同種造血幹細胞移植を行い、得られた臨床データと患者検体などを用いて以下の研究を行う。現在、本事業において進行中の以下の臨床試験を完結させる。

＜倫理面への配慮＞

本研究を実施するにあたっては、ヘルシンキ宣言、米国ベルモントレポート等の国際的倫理原則、あるいは我が国のヒトゲノム・遺伝子解析研究に関する倫理指針、遺伝子治療臨床研究に関する指針に従い以下を遵守する。対象患者については、いずれも患者本人に説明同意文書の内容を極力分かり易い言葉で説明し、説明同意文書2部を作製して本人に渡したうえで文書による同意を得る。この際に、患者の費用負担が増えることはないこと、この研究への参加は自由で、参加しなくても不利益は受けないこと、この研究へ参加した場合でも、いつでもやめられること、データの取り扱い上、患者氏名等直接個人が識別できる情報を用いず、かつデータベースのセキュリティを確保し、個人情報（プライバシー）保護を厳守することも説明する。

C．研究結果

本年度は、以下のような成果を挙げた。

1) 高齢者白血病・悪性リンパ腫に対するミニ移植臨床試験
本試験の中間解析結果に基づいて、現在、該当企業が適応拡大申請中。

2) HLA一座不適合血縁ドナーを用いたミニ移植
19名を登録して試験を終了し、論文を執筆中。2008年度の日本血液学会総会にも演題を提出予定。

3) GVHD予防のためのシクロスポリンとタクロリムスの比較試験 (RIST0301)
68名を登録して試験を終了し、論文を執筆予定。2008年全米血液学会に演題提出予定。日本造血細胞移植学会総会にも演題提出予定。

4) 非血縁者間骨髄を用いたミニ移植の開発研究 (IST0305)
27名を登録して試験を終了。
日経BP社がんナビ
(ttp://cancernavi.nikkeibp.co.jp/news/hla1.html)も掲載し、全米血液学会で誌上発表し、論文執筆中。日本臨床腫瘍学会総会ではポスターディスカッション演題に採択。

5) 臍帯血ミニ移植 (RICBT0501)
5名を登録試験して終了し、2008年度からは新規試験を開始予定。

6) 移植後インターフェロンα投与の再発予防効果 (IFN2005) 2名を登録した時点で試験を終了し、新規試験に移行。

7) 自殺遺伝子を導入したドナーリンパ球輸注療法の開発研究
国立がんセンター倫理審査委員会に研究審査を申請。

8) ウィルスや真菌感染症治療薬やサイトカインの適応拡大試験
移植領域における適応外使用医薬品の承認を促進する対策を施すために、日本造血細胞移植学会と共同作業中。

9) 栄養・代謝障害を予防、治療する臨床研究 (NST01) 試験進行中で12名を登録。

10) シクロスポリン血中濃度の適正化試験 (RIST0401) 試験終了し、論文化作業中。

その他、移植直後のCRP値がGVHD発症の簡便なマーカーとなる可能性、2) 移植患者の血糖値や3) 体重のコントロールが、移植後の予後に影響することを世界でも初めて報告し、治療成績の向上の手かがりとする前向き試験を計画した。基礎研究としては、ミニ移植後の免疫機能を解析する過程で、GFP（緑色蛍光タンパク質）遺伝子導入マウス移植系による投与細胞の生体内での細胞動態解析システムを開発した。その結果、投与後早期より、リンパ節およびパイエル板等に集積することが単一細胞レベルで確認できるなど、今後、移植治療や再生医療の科学的根拠を検証する基盤研究が飛躍的に進むことが期待できる。

D. 考察

同種免疫反応には人種差があるため、欧米の試験成績をわが国での移植現場に直接当てはめることはできない。本研究においては、ミニ移植を含めた各種の造血幹細胞移植の標準化のための厳正な臨床試験と安全性向上のための合併症対策の検討を、既に稼動している臨床研究母体に拠って推進し、我が国固有のエビデンスを蓄積することを目指した。同時に、科学的妥当性を備えた対策を打ち出して治療の安全性を高めるために基礎研究を遂行した。移植医療の均てん化も目指して、全国の施設からの研修者を受け入れた。各種の臨床研究を推進しているが、特に高齢患者を対象とした白血病ミニ移植開発試験に関しては、本班研究成果を基にミニ移植に必須の薬剤が適応拡大承認を得る見通しとなった。学会と協働して稀少的な医療領域に有用な薬剤を臨床導入する手法を示した点は画期的と考える。同時に、治療を受ける患者検体を用いる基礎研究を並行して行うことで、治療学的妥当性を与え、新規治療開発にも貢献すると考える。

E. 結論

造血幹細胞移植治療の標準化、新規治療の開発並びに安全性向上を目指した臨床試験と基礎研究を推進して一定の成果を挙げることができた。得られた成果をもとに、有用な薬剤を臨床導入する道筋をつけて国民医療の向上に寄与した。また、全国の施設からの研修者教育も行うことで、移植医療の均てん化にも寄与した。同時に、治療の科学的根拠を検証するための画期的ツールを開発した。

F. 健康危機情報

該当事項なし

G. 知的財産権の出願・登録状況

該当事項なし

H. 研究発表

- 海外学会発表＝４２件
- 著書論文による発表＝４５件
- 上記のうち、主なものを下記に記載する。

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InterScience
Research article

Prospective phase II trial to evaluate the complications and kinetics of chimerism induction following allogeneic hematopoietic stem cell transplantation with fludarabine and busulfan

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This prospective trial assessed the safety and efficacy of allogeneic hematopoietic stem cell transplantation from a HLA-matched donor with a reduced-intensity regimen (RIST) consisting of iv fludarabine 30 mg/m^2 for 6 days and oral busulfan 4 mg/kg/day for 2 days in patients older than 50 years with hematological malignancies. Cyclosporine alone or cyclosporine with short-term methotrexate was randomized for graft-versus-host disease prophylaxis. After 30 patients had been enrolled, an interim analysis was performed, and this report focuses on a precise evaluation of the toxicity profile and chimerism kinetics. Sustained engraftment in all patients, no severe regimen-related toxicity (RRT) within 20 days, and no transplant-related mortality through Day 100 were observed. T-cell (CD3+) full-donor (over 90%) chimerism was observed in 22 of the 30 patients, while the remaining eight had mixed-donor chimerism over 77% on Day 90. Thereafter, five subsequently converted to full-donor chimerism without donor lymphocyte infusion by day 120 (n = 4) or Day 180 (n = 1). Two showed persistent mixed chimerism without relapse through Day 180. Grade III–IV acute graft-versus-host disease and extensive chronic graft-versus-host disease occurred in 10% and 73%, respectively. With a median follow-up of 1.5 years, overall survival and disease-free survival at 1 year was 83% and 62%, respectively. Seven patients hematologically relapsed overall, and five of them had myelodysplastic syndrome with poor prognostic factors. In older patients, RIST with fludarabine and busulfan was associated with acceptable toxicities and a satisfactory antileukemia effect, regardless of the early chimerism status. Am. J. Hematol. 00:000–000, 2007. © 2007 Wiley-Liss, Inc.

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative treatment of choice for hematological malignancies. However, many centers limit HSCT to younger patients because of the threat of a higher risk of treatment-related toxicities including graft-versus-host disease (GvHD), nonrelapse mortality, and lower disease-free survival (DFS) in the older population, although the median age of onset of chronic myeloid leukemia (CML) is in the sixth decade of life, and the peak incidence of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) is in the seventh decade. To overcome this obstacle, allogeneic HSCT with a reduced-intensity (RIST) or nonmyeloablative conditioning regimen has recently been explored for patients who are ineligible to receive conventional myeloablative HSCT (CIST) due to age limits or comorbidities. Many studies suggested that RIST is a reasonable option for older patients or patients with comobidities with acceptable treatment-related complications or morbidity, while preserving adequate antitumor effects [1–12]. However, these studies mostly pursued different variables including disease types, stages [1,4–6, 8,12], donor type [1,2,5,10], graft source [1,2], conditioning regimens [4,5,7,9], and/or GvHD prophylaxis [1,4,9]. This

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TABLE I. Patient and Donor Characteristics

UPN	Patient age/sex	Donor age/sex	Stage and diagnosis	IPSS/cytogenetic risk in MDS patients	CD34+ cells (10^6)	Blood type patient/donor
1	60/M	46/F	MDS (RA)	Intermediate-2/Poor	3.92	A/A
2	61/M	54/F	MDS (RAEB)	Intermediate-2/Poor	5.84	O/O
3	67/M	60/F	AML (M4) in 2CR		2.74	B/O
4	60/M	55/F	AML (M2) in 2CR		4.58	B/B
5	63/M	60/M	CML in 2CP		12.59	O/O
6	54/M	59/F	MDS (RAEB)	High/Intermediate	5.4	O/A
7	52/M	55/F	AML (M2) in 1CR		6.77	A/A
8	61/M	54/M	AML (M1) in 1CR		3.29	B/A
9	58/F	64/F	CML in 1CP		2.9	A/AB
10	64/F	59/F	ALL (L2) in 1CR		5.54	A/A
11	55/M	44/M	AML (M1) in 1CR		3.13	A/A
12	55/F	51/F	CML in 1CP		4.94	A/O
13	52/F	42/M	AML (M4) in 1CR		3.59	A/A
14	59/M	64/M	MDS (RAEB)	Intermediate-2/intermediate	3.58	A/AB
15	59/M	56/M	MDS (RA)	Intermediate-1/Good	3.58	AB/A
16	53/F	55/F	MDS (RA)	Intermediate-2/Poor	2.2	O/O
17	55/F	68/M	AML (M3) in 2CR		2.63	A/A
18	54/M	50/M	MDS (RA)	Intermediate-1/Poor	3.74	O/B
19	51/M	44/F	AML (M1) in 1CR		4.86	AB/A
20	64/F	66/M	CML in 2CP		3.59	O/A
21	68/F	64/M	MDS (RAEB)	Intermediate-1/Good	3.56	B/B
22	53/M	44/M	MDS (RAEB)	High/Intermediate	7.2	B/B
23	60/F	53/M	AML (M2) in 1CR		2.83	A/B
24	59/M	62/M	AML (M4) in 2CR		5.47	A/O
25	51/F	47/F	MDS (RAEB)	Intermediate-2/Poor	5.93	A/A
26	59/M	62/F	MDS (RA)	Intermediate-2/Poor	4.02	B/O
27	59/M	48/M	AML (M2) in 2CR		4.94	B/A
28	56/M	62/F	MDS (RAEB-t)	High/Good	4.38	AB/A
29	53/F	62/F	AML (M2) in 1CR		3.06	O/O
30	54/F	63/M	AML (M2) in 1CR		6.47	A/O

M, male; F, female; MDS, myelodysplastic syndrome; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; RA, refractory anemia; RAEB, refractory anemia with excess blasts; RAEB-t, refractory anemia with excess blasts in transformation; CR, complete remission; CP, chronic phase. All donors were HLA-matched siblings.

makes overall interpretation of studies difficult. Additionally, there has been no study to prospectively assess whether RIST consisting of 180 mg fludarabine plus 8 mg/kg busulfan without antithymocyte globulin actually produces less significant organ toxicities and treatment-related toxicities in an older patient population. Information regarding the impact of the speed and degree of lineage-specific donor chimerism on clinical outcomes after RIST in older patients has been limited [3,8,13–17]. Moreover, even studies evaluated with more homogeneous patient population, type of GvHD prophylaxis and/or tempo of withdrawal of immunosuppressive agents varied depending on transplant centers and a feasible prophylaxis regimen for acute GvHD has not been well evaluated in RIST, which is considered to require a sophisticated balance between GvHD and a graft-versus-leukemia (GvL) effect.

To address these points, we conducted a prospective randomized clinical trial to evaluate the safety and efficacy of RIST with fludarabine and oral busulfan in patients aged over 50 years and with appropriate GvHD prophylaxis. In this report, the results of an interim analysis, including clinical outcomes, complications, and chimerism kinetics, were compared with those previously published in the literature.

Patients and Methods

Patient eligibility and accrual

Eligible patients ranged in age from 50 to 69 years (median 58.5, range 51–68 years) and had a hematological malignancy, including AML or acute lymphoblastic leukemia (ALL) in 1st or 2nd complete remission (CR), CML in 1st or 2nd chronic phase (CP), and MDS. They were required to have an HLA-identical related donor. The study protocol was reviewed and approved by the institutional review boards of the participating transplantation centers (Appendix). Eligible patients and their donors gave written informed consent before enrollment. The enrollment criteria included a performance status (PS) of the Eastern Cooperative Oncology Group (ECOG) of less than two, a serum creatinine concentration of less than 2.0 mg/dl, a cardiac ejection fraction of more than 50%, arterial oxygen saturation without supplemental oxygen of more than 93%, liver function tests less than fourfold the upper limit of normal, total bilirubin less than 2.0 mg/dl, no active infection, and no previous allergy for drugs used for conditioning or GvHD prophylaxis. Donors were required to have a normal physical examination, and normal values in the serum chemistry and blood counts, and negative results of serologic testing for human immunodeficiency virus and hepatitis B. The patient and donor characteristics are shown in Table I. Those with AML/ALL in 1st CR, CML in 1st CP, or MDS in refractory anemia were defined as low risk, and the others were defined as high risk. All 12 patients with MDS except one (UPN 22) were transfusion dependent, and all those were grouped according to the International Prognostic Scoring System (IPSS) into intermediate or high risk at the time of transplantation: Intermediate-1, $n = 3$; intermediate-2, $n = 6$; high risk, $n = 3$. By IPSS criteria, 3 patients had good-risk, 3 had intermediate-risk, and 6 had poor-risk cytogenetics.

Donor selection and blood stem cell harvest

Related donors were selected based on compatibility of HLA-A, B and DRB1 by intermediate- or high-resolution DNA typing. After G-CSF treatment, apheresis procedures were performed daily until at least 2.0 $\times$ 10^6 CD34+ cells per kilogram of the recipient's body weight, up to three times, and all of the collected cells were cryopreserved until stem cell infusion.

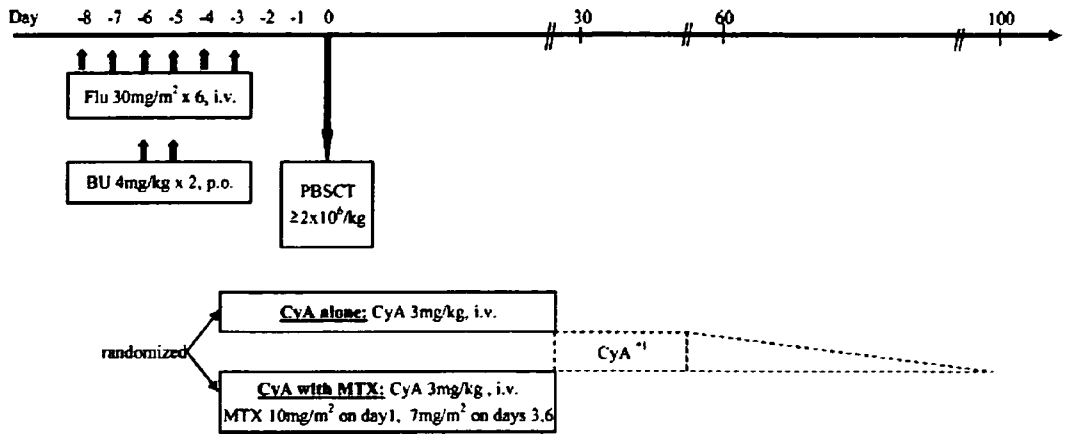

Figure 1. Treatment schedule. CyA; cyclosporine, MTX; methotrexate. *1: When acute GvHD was not observed, CyA was tapered by 10% a week starting at Day 28, and was eliminated by Day 100. When mixed chimerism was seen without active acute GvHD over Day 60, CyA was tapered and discontinued within 2 weeks. Patients who did not convert to complete chimerism after CyA withdrawal received donor lymphocyte infusion.

Treatment schedule

The treatment schedule is shown in Fig. 1. The conditioning regimen consisted of fludarabine (30 mg/m^2/day) infused over 30 min once a day on Days 8, 7, 6, 5, 4, and 3, and oral busulfan (4 mg/kg/day) on Days 6 and 5. To prevent seizures, the patients received oral valproate sodium, at a dose of 600 mg divided into 3 doses 2 days before busulfan administration, and this was continued until 24 hr after the last dose of busulfan.

Patients were randomized to receive either cyclosporine (CyA) alone or CyA plus short-term methotrexate (MTX) for GvHD prophylaxis. Randomization was performed by stratifying according to disease (AML, ALL, CML or MDS), transplant center, age (less than 60 years or more than or equal to 60 years), and sex (male or female). All patients received 3 mg/kg/day CyA by continuous iv infusion daily from Day 1 to maintain a therapeutic trough level of 250–400 ng/ml, and thereafter orally in an attempt to maintain a therapeutic trough level of 150–250 ng/ml. The patients who were assigned to CyA plus short-term MTX received a dose of 10 mg/m^2 iv MTX on Day +1, and 7 mg/m^2 on Days +3 and +6 after stem cell infusion. CyA was tapered starting at Day 28 in the absence of acute GvHD and was discontinued by Day 100 after transplantation. When a patient did not achieve complete donor chimerism by Day 60, CyA was tapered rapidly and discontinued within 2 weeks if clinically feasible, since anti-leukemic effect was presumed to occur after development of complete donor chimerism [14]. Cases of Grade II–IV acute GvHD were treated with 2 mg/kg/day of methylprednisolone in addition to CyA.

Supportive care

The following infection prophylaxis was recommended: prophylactic antibiotics (fluoroquinolones) were given during cytopenia, fluconazole (200 mg/day) was given at the start of conditioning and continued until the discontinuation of immunosuppressant, and oral acyclovir (1,000 mg/day) or iv acyclovir (750 mg/day) was given for prophylaxis of herpes simplex virus (HSV) and varicella zoster virus (VZV) from Day −7 to Day 35. Prophylaxis against *Pneumocystis carinii* was consisted of trimethoprim-sulfamethoxazole after neutrophil engraftment ($\geq 0.5 \times 10^9$ L^{-1}) and was continued until the discontinuation of immunosuppressant. During the first 100 days after transplantation, cytomegalovirus antigenemia assay with HRP-C7 or C10/C11 monoclonal antibody was performed weekly after neutrophil engraftment until Day 100 after transplantation. Pre-emptive therapy with ganciclovir was recommended upon the detection of positive antigenemia and was continued until it became negative. Patients were treated with G-CSF from Day +6 to neutrophil engraftment.

Chimerism analysis

Hematopoietic chimerism was evaluated with regard to peripheral T cell (CD3+) fraction by an analysis of DNA microsatellite polymorphisms by polymerase chain reaction (PCR) with D18S51, D20S471, and D22S684 fluorescence-labeled primers, which identified differences between patient and donor (on the basis of polymorphisms found in pretransplant patient/donor samples) using an BECKMAN COULTER CEQ8000 GENETIC ANALYSIS SYSTEM. T cell (CD3+) chimerism studies post HSCT were performed on Days 30, 60, 90, 120, and thereafter every other month through 1 year.

Assessment of response

Day 0 was defined as the day of stem cell infusion day. The day of neutrophil engraftment was defined as the first of two consecutive days on which the patient's absolute neutrophil count was above 0.5×10^9 L^{-1}. The day of platelet engraftment was defined as the first of seven consecutive days on which the platelet count was above 20×10^9 L^{-1} without platelet transfusion.

Regimen-related toxicity (RRT) was graded using the Seattle criteria [18] on the day before the initiation of conditioning regimens and at least 3 days a week until Day 20 after transplantation. All other observed adverse events were graded according to the National Cancer Institute Common Toxicity Criteria version 2.0 (NCI-CTC ver. 2.0) until Day 100 after transplantation. Infectious diseases were diagnosed based on any positive blood culture or histologic evidence of tissue invasion.

To evaluate the general condition of patients associated with the toxicity profile, PS, and dietary oral intake were also reported at least three times a week during the initial hospitalization and once a week afterwards up to Day 100 post-transplant.

The diagnosis and grading of acute and chronic GvHD was made based on the date of onset (within or beyond 100 days) and clinical findings in conjunction with biopsy of the skin and digestive tract using the published criteria [19,20]. Patients who survived 100 days or longer were evaluable for the assessment of chronic GvHD.

Pharmacokinetic studies of fludarabine phosphate and busulfan

Blood sampling for pharmacokinetic studies was done on Day −5 to investigate the effect of concomitant busulfan administration on the pharmacokinetics of 2-fluoro-ara A (2F-ara-A), which is the major metabolite of fludarabine phosphate. Blood samples for determining the 2F-ara-A plasma level were collected at 0, 0.5, 1, 2, 5, and 23.5 hr after the 4th infusion of fludarabine. We also obtained blood samples for determining the busulfan plasma level at 0, 0.5, 1, 1.5, 2, 3, and 6 hr after the sixth administration of busulfan (1 mg/kg/dose for 8 times). Blood samples were taken in tubes containing heparin and erythro-9-(2-hydroxy-3-nonyl)adenine. Plasma was obtained by centrifugation, and then transported to the laboratory and were stored at −20°C until analysis. Plasma levels of 2F-ara-A and busulfan were determined using high-performance liquid chromatography with fluorescence and UV detection, respectively. The accuracy and precision of the assays for 2F-ara-A and busulfan were confirmed by measuring QC samples of both before this study. The maximum concentration of drug in plasma after drug administration (C_{max}, C_{peak}) and the time to reach

the maximum concentration following drug administration (T_{max}) were observed. The area under the plasma concentration-versus-time curve (AUC) for 2F-ara-A or busulfan was calculated by dividing the administered dose by the final plasma clearance estimate, whereas the plasma clearance was determined by modeling all plasma concentration versus time data. Terminal half-lives ($T_{1/2}$) were calculated from the primary parameters.

Statistical analysis

The primary endpoint of this study was to determine the percentage of patients who were alive at 100 days after transplantation with complete donor chimerism (over 90%) achieved by Day 90. Secondary endpoints included the time to engraftment of neutrophils and platelets, the incidence and severity of RRT, the incidence and severity of acute and chronic GvHD, the anti-leukemia effect, DFS, and overall survival (OS). A descriptive statistical analysis was performed to assess patient baseline characteristics and disease. Time to engraftment, complete chimerism, acute or chronic GvHD, OS, and DFS were calculated using the Kaplan–Meier method. OS was defined as the time between stem cell infusion to death from any cause. DFS was defined as the time between stem cell infusion to relapse and death from any cause, whichever occurred first. After 30 patients had been enrolled in the study, a data and safety monitoring committee undertook an interim analysis. This analysis, completed in October 2004, included data for the primary endpoint, i.e. survival at Day 100 and chimerism status at Day 90, and data on acute and chronic GvHD, survival, chimerism status, and anti-tumor effect through Day 180. Neither of the predefined criteria for stopping the study was met; however, a review of available safety data including incidence and severity of RRT and Day 100 mortality indicated that this conditioning regimen was adequately safe for older patients. According to the recommendation of the committee, we decided to continue the study and published an interim report when 30 patients were enrolled and evaluated without comparing the two different GvHD prophylaxis procedures. This report includes data on these 30 patients with all available follow-up data through December 2005, and does not include the results of a comparison of the two different GvHD prophylaxis procedures.

Results

Engraftment and chimerism analysis

The results are summarized in Table II. One and four patients were not evaluated for neutrophil and platelet engraftment, respectively, because they did not show a nadir. The remaining patients achieved sustained engraftment and none experienced graft failure. The median number of days to achieve a neutrophil count $\geq 0.5 \times 10^9\ L^{-1}$ was 13 (range, 10–25 days), and this was 18 (range, 11–24 days) for a platelet count $\geq 20 \times 10^9\ L^{-1}$ without transfusion. Full-donor (over 90%) T-cell (CD3+) chimerism was observed in 2 and 9 of the 30 patients on Day 30 and Day 60, respectively (median [range], Day 30:71 [40 to ≥90] %, day 60:81 [41 to ≥90] %). Twenty-two patients achieved full-donor chimerism, while the remaining eight patients had mixed chimerism ranging from 78% to 88% on Day 90. Among those with mixed chimerism on Day 90, five subsequently converted to full-donor chimerism without early CyA withdrawal because of the severe acute GvHD (n = 2: UPN 1 and 15) and/or donor lymphocyte infusion (DLI) by day 120 (n = 4) or day 180 (n = 1). One achieved full-donor chimerism on Day 120 after DLI since the patient did not respond to the discontinuation of immunosuppressive drugs, and two had persistent mixed chimerism without relapse through 180 days after transplantation (71% and 75% donor-type chimerism on Day 180). The diagnoses of two patients with persistent mixed chimerism through Day 180 were CML and MDS, and they had not received proceeding cytotoxic chemotherapy; the patient with CML (UPN 12) received immunomodulators, imatinib mesylate and hydroxyurea, and the patient with MDS (UPN 21) received low-dose cytarabine and aclarubicin in combination with granulocyte colony stimulating factor before RIST.

Regimen-related toxicities, complications, and general condition

The frequencies of Grade I–IV organ toxicities within 20 days after transplantation are listed in Table III. Although non-fatal toxicities including Grade I/II were seen in all 30 patients, all of the observed episodes were reversible and in no case required suspension of fludarabine. Stomatitis was the most frequently observed organ toxicity (57%, 17/30), with 47% of them (8/17) had Grade II events. None of the patients experienced veno-occlusive disease of the liver (VOD). Twenty patients had at least one episode of infectious complications within the first 100 days, with a total of 44 documented episodes (median, 2; range, 1–7 episodes) within the first 100 days after transplantation. These included proven bacterial infection (1 episode), suspected bacterial infection (1), suspected fungal infection (2), cytomegalovirus antigenemia (6), HSV infection (1), suspected viral infection (1), and uncertain causes (33). All infectious complications were recovered with or without appropriate antibiotic therapy.

The median PS for the first 28 days was 0 (range, 0–3). The worst PS of 2 (n = 5) or 3 (n = 2) within the first 28 days was experienced temporarily due to infection (n = 2), Grade III GvHD (n = 1), and nausea/vomiting (n = 4). Those (n = 6) observed from Day 29 to Day 100 were all caused by Grade II or III acute GvHD. A one-thirds reduction in dietary oral intake was temporarily seen in 20 and 11 patients within the first 28 days and from 29 days to 100 days post HSCT, respectively, which resulted from nausea/vomiting (n = 18) and treatment-related mucositis (n = 2) within Day 28, and Grade II–III acute GvHD (n = 9), prolonged infection with Grade II acute GvHD (n = 1) and gastroesophageal reflux disease (n = 1) between Day 29 and Day 100.

GvHD

Grade I–IV acute GvHD at 100 days was documented, respectively, in 5 (17%), 15 (50%), 3 (10%), and 0 (0%) patients. The median time to the occurrence of Grade II–IV acute GvHD was 74 days (range, 18–100 days). All 30 patients survived beyond Day 100 and were evaluated for chronic GvHD. Twenty-six of the 30 patients (87%) developed chronic GvHD (limited type in four cases and extensive type in 22 cases) with the onset at a median of 123 days after transplantation (range, 116–217 days).

Disease response, survival, and cause of death

No patient died within the first 100 days, and the median follow-up period was 555 days (149–1114 days) after transplantation. Twenty-nine of the 30 patients achieved CR within 100 days after transplantation, but two of them with MDS, who had poor-risk cytogenetics and were classified into intermediate-2, subsequently relapsed on Day 141 (UPN 26) and Day 156 (UPN 25). One was treated with DLI (UPN 25) and showed a temporary response, but died because of the disease progression on Day 401. The other patient (UPN 26) did not respond to DLI and died of progressive disease on Day 412. One patient (UPN 22) with MDS with high risk IPSS achieved full-donor chimerism on Day 90, but could not achieve CR on Day 98 and died with progressive disease on Day 306. This patient showed full-donor chimerism through Day 180. Five other patients died between 100 days and 1 year after transplantation (149, 151, 169, 187, and 354 days). In six patients who died within the first year, two patients were over 60 years and four patients were classified into high risk disease group. Causes of death included progressive disease of MDS with poor IPSS in 1, GvHD and/or its complications in 4, and recurrence of interstitial pneumonia in 1. In four patients, who died of GvHD and/or its complications, all had experienced

TABLE II. Summary of Clinical Outcomes

UPN	Chimerism analysis			Post transplant DLI (reason)	GvHD		Infection until day 100 (etiological agent)	Relapse	Outcome (Cause of death)	Follow up
	Day 90(%)	Day 120(%)	Day 180(%)		Acute	Chronic				
1	88.40	≥90	≥90		Gr II (S, G)	Extensive	–	–	Alive	1,114
2	85	≥90	≥90	Yes (d662, relapse)	Gr II (S)	Extensive	Yes (unknown)	Yes (d402)	Dead (recurrent disease and its complication)	652
3	≥90	≥90	≥90		–	Extensive	Yes (S. maltophilia, unknown)	–	Alive	735
4	≥90	≥90	D		Gr II (S)	–	–	–	Dead (IP)	169
5	≥90	≥90	≥90		–	Extensive	–	–	Alive	731
6	≥90	≥90	≥90		Gr II (L)	Extensive	–	–	Alive	716
7	≥90	≥90	≥90		Gr II (S, G)	Extensive	Yes (CMV antigenemia)	–	Dead (GvHD)	354
8	≥90	≥90	≥90		Gr III (S, G, L)	Extensive	Yes (bacteremia susp., fungal susp., CMV antigenemia, unknown)	–	Alive	431
9	≥90	≥90	≥90		–	Extensive	Yes (unknown)	–	Alive	592
10	≥90	≥90	≥90		Gr II (S, G)	Extensive	Yes (unknown)	–	Dead (GvHD)	757
11	≥90	≥90	≥90		Gr II (S)	Extensive	–	–	Alive	360
12	88	79	71		Gr III (S, L)	Extensive	Yes (unknown)	–	Alive	720
13	≥90	≥90	≥90		Gr I (S)	Extensive	Yes (HSV, unknown)	–	Dead (GvHD)	517
14	≥90	≥90	≥90		Gr II (S, G, L)	Limited	Yes (fungal susp., unknown)	–	Dead (GvHD and its complication)	187
15	85	88	≥90		Gr III (S, G)	–	–	–	Alive	702
16	84	88	≥90		–	Limited [a]	Yes (CMV antigenemia)	–	Alive	642
17	80	≥90	D	Yes (d98, mixed chimerism)	Gr II (S, G)[b]	–	–	–	Dead (GvHD and its complication)	149
18	≥90	88	≥90		Gr II (S)	Extensive	Yes (CMV antigenemia)	–	Alive	729
19	≥90	≥90	≥90		Gr I (S)	Limited	–	–	Alive	737
20	≥90	≥90	≥90		Gr II (G)	–	Yes (CMV antigenemia)	Yes (d147)[c]	Alive	688
21	78	77	75		Gr II (S, L)	Extensive	–	Yes (d364)	Dead (BOOP)	593
22	≥90	≥90	≥90		Gr II (S, G)	Extensive	Yes (unknown)	Yes (d98)	Dead (progressive disease)	306
23	≥90	≥90	D		–	Extensive	Yes (unknown)	–	Dead (GvHD and its complication)	151
24	≥90	≥90	≥90		Gr II (G)	Extensive	–	Yes (>d365)[d]	Dead (recurrent disease)	825
25	≥90	87	≥90	Yes (d186, d238, relapse)	Gr II (S)	Extensive	Yes (CMV antigenemia)	Yes (d156)	Dead (recurrent disease)	401
26	≥90	≥90	≥90	Yes (d204, relapse)	Gr I (S)	Extensive	Yes (unknown)	Yes (d141)	Dead (recurrent disease and its complication)	412
27	84	≥90	≥90		–	Extensive	Yes (unknown)		Alive	371
28	≥90	≥90	≥90		Gr II (S)	Extensive	Yes (viral susp., unknown)	–	Alive	365
29	≥90	≥90	≥90		Gr I (S)	Extensive	Yes (unknown)	–	Alive	366
30	≥90	≥90	≥90		Gr I (S)	Limited	Yes (unknown)	Yes (d370)	Alive	370

ND, not done; D, dead; DLI, donor lymphocyte infusion; Gr, grade; GvHD, graft-versus-host disease; GvHD site codes, S-skin, G-gut, L-liver; CMV, cytomegalovirus; susp., suspected; unknown, no microbiological evidence despite symptoms; IP, interstitial pneumonia.

[a]This patient developed a GvHD starting on day 112 after receiving DLI for mixed chimerism.

[b]This patient developed gut GvHD starting on day 92.

[c]CNS relapse without hematological relapse.

[d]This patient relapsed after day 365, but the exact date of relapse is unknown.

TABLE III. Regimen-Related Toxicities Within 20 Days After HSCT According to the Seattle Criteria in 30 Patients

	Grade			
Toxicity	1	2	3	4
Heart	1	0	0	0
Bladder	0	1	0	0
Kidney	5	1	0	0
Lung	2	0	0	0
Liver	8	0	0	0
CNS	1	0	0	0
Stomatitis	9	8	0	0
GI toxicity	4	1	0	0

HSCT, hematopoietic stem cell transplantation; CNS, central nervous system; GI, gastro-intestinal.

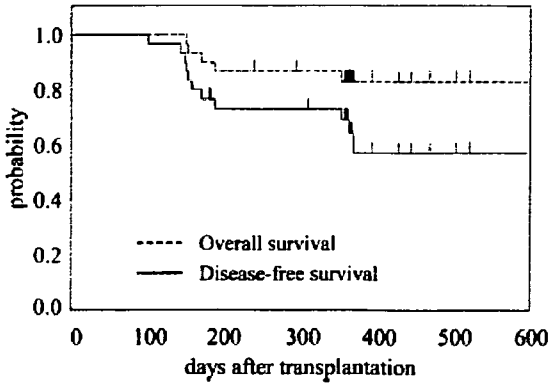

Figure 2. Kaplan–Meier product estimates of overall survival and disease-free survival.

gut GvHD, three of those developed extensive chronic GvHD and all were treated with corticosteroid.

The Kaplan-Meier estimated probability of OS and DFS at 1 year was, respectively, 83% and 62% (Fig. 2). Both patients age (≤55 years versus >55 years) and CD34+ cell dose (>5.0 $\times 10^6$ kg^{-1} versus ≤5.0 $\times 10^6$ kg^{-1}) were not associated with better outcomes by a stratified analysis (data not shown).

Pharmacokinetic results for fludarabine and busulfan

2F-ara-A and busulfan PK parameters were calculated from data obtained from blood samples from six consenting patients (UPN 1, 3–7). After the start of the 4th infusion of fludarabine phosphate (30 mg/m^2/dose), the maximum plasma level of 2F-ara-A was 3.12 ± 1.08 nmol/ml, with a subsequent decline to $T_{1/2}$ of 8.59 ± 1.57 h. The AUC (0–24 hr) and CL were 17.7 ± 2.82 nmol hr/ml and 78.9 ± 13.1 ml/min/m^2, respectively. After the 6th administration of busulfan (1 mg/kg/dose for eight times), the maximum plasma level of busulfan was 1.37 ± 0.34 nmol/ml, with a subsequent decline to a $T_{1/2}$ of 2.88 ± 0.65 hr. The AUC (0–6 hr) and CL were 4.85 ± 1.07 nmol hr/ml and 3.60 ± 0.88 ml/min/m^2, respectively. Since these parameters are similar to those in a previous study with the repeated administration of fludarabine phosphate alone at 15, 20, and 25 mg/m^2/dose (data not shown), combination with busulfan seemed to have no effect on the pharmacokinetics of 2F-ara-A. The steady-state plasma level of busulfan (808 ± 178 ng/ml) was observed to remain within a therapeutic level (600–900 ng/ml) in adults [21].

Discussion

In this prospective study, we showed that a combination of fludarabine (180 mg/m^2) and oral busulfan (8 mg/kg), despite the omission of antithymocyte globulin from the original regimen by Slavin et al. [6], can be successfully used to help prepare patients older than 50 years with hematological malignancies for HSCT from an HLA-matched related donor: All patients achieved sustained engraftment without graft failure, only an insignificant occurrence of RRT and treatment-related complications were seen, and PS and dietary intake were well maintained, which agrees with published observational studies on RIST with fludarabine and busulfan [16,22,23].

The rapid induction of complete donor-type chimerism was considered as an essential part of the RIST procedure. Although all of our patients rapidly developed conventional neutrophil and platelet engraftment, two of the 30 patients without preceding cytotoxic chemotherapy remained in mixed T-cell chimerism during the first 6 months after transplantation. A more rapid induction of T-cell chimerism has been observed in other studies of RIST in patients who had been previously treated with chemotherapy for diseases other than CML or MDS [24]. Although a close association between the occurrence of acute GvHD and the induction of higher levels of donor T-cell chimerism has been reported [14], in our experience over 50% of patients did not achieve complete chimerism at the onset of acute GvHD, demonstrating that mixed chimerism status did not provide absolute protection from GvHD, which is in agreement with data published by Baron et al. [15]. We speculate that differences in the conditioning regimen and GvHD prophylaxis may result in different observations.

While our less intensive regimen was associated with less toxicity, this strategy will only work if modifications to the conditioning regimen intensity that allow early clinical benefits do not also lead to reduced induction of GvL effect or other complications that increase relapse rate or result in worse survival in later time period [25]. A recent observational study from European Group of Blood and Marrow Transplantation Registry compared treatment-related mortality (TRM) and other outcomes between 315 RIST recipients and 407 CIST recipients, who were over 50 years and transplanted from a HLA matched sibling donor [26], and suggested that lower TRM but higher relapse rate were seen in RIST recipients. Given the fact that all three patients, who relapsed within 6 months after transplantation, were MDS with poor prognostic factors, the incidence of relapse in our study seems to be no higher than that in published data for CIST [27–30]. Taussig et al. evaluated the feasibility and safety of the fludarabine based RIST regimen in 16 patients with standard risk diseases [31]. In this study, TRM rate within 100 days was 0%, however, OS and DFS at 1 year read from Fig. 2 were 69% and 56%, respectively, where most of the patients included in this study had early stage diseases and over 30% of patients were aged less than 50 years. Despite the older patient population, our data showing no treatment-related mortality (TRM) within the first 100 days after transplantation and OS and DFS at 1 year of 83% and 62%, respectively, was encouraging.

In a previous report, we suggested that the development of GvHD is not essential for the control of low-risk myeloid malignancies, and that GvHD and infection, rather than relapse, are more important problems to be addressed in these patients [25]. Although our data showed favorable outcomes, six patients with four low risk disease and three patients aged less than 55 years died of GvHD or its complication within the first year should be interpreted with care. The incidence of Grade II–IV acute GvHD in this

study was somewhat higher than that in published literature and our own observational data with elder patients and high risk diseases [25]. However, Grade III–IV acute GvHD was infrequent and none died from acute GvHD. The incidence of chronic GvHD was higher than that in our previous experience (56%) [32] or in other reports [31,33] even after considering inevitable differences in the ethnicity, GvHD prophylaxis and matching practice of HLA, or disease risk. G-CSF mobilized peripheral blood stem cells may have been associated with an increased incidence of GvHD, particularly in its chronic form [34,35]. Conditioning regimen excluded antithymocyte globulin was also a possible explanation of this finding [23]. Most importantly, patients undergoing RIST are usually older than those undergoing CIST, which leads to a higher risk for GvHD [36,37]. Early CyA withdrawal regulation to get speedy achievement of complete donor chimerism after RIST in our protocol might have influenced the increased incidence of Grade II–IV acute GvHD, which might have affected the rate of chronic GvHD [33,35,38,39]. Although severe GvHD will be unavoidable for some patients including MDS with poor prognostic factors [40,41], the balance between GvHD and GvL is a significant concern in RIST and we should seriously evaluate the type and tapering speed of immunosuppressive agents after RIST. Current findings suggested GvHD control might be improved simply by extending the duration of CyA administration. Additionally, we noticed that the clinical features of GvHD are different in RIST than in CIST, i.e. a syndrome compatible with acute GvHD occurs well after Day 100. Hence, the current grading system for GvHD, which was developed on the basis of experience in ablative settings, may not be an optimal tool for assessing GvHD after RIST. We observed a late onset of acute GvHD and an early onset of chronic GvHD, and therefore believe that a significant number of late-onset acute GvHD may have been judged as chronic GvHD in this study simply because the onset of GvHD was over 100 days after transplantation. Our results support the current proposition by Mielcarek and Storb concerning the abandonment of the traditional Day 100 cutoff for separating acute from chronic GvHD [35].

In this prospective study, we confirmed the short-term safety and efficacy of our RIST procedure for hematological malignancies in the elderly. Long-term follow-up of patients to evaluate disease control and the consequence of therapy is mandatory, and the development of optimal GvHD prophylaxis, with the use of novel assessment criteria, will be of primary importance for the wider application of the RIST procedure. RIST may also be beneficial in young patients, since organ damage, including infertility, might be milder and less frequent in RIST than in CIST, which should be confirmed by further prospective clinical trials. Although the number of patients studied was limited, the analysis of fludarabine pharmacokinetics has for the first time provided reliable information on the interaction of key drugs, and we found no evidence to suggest that synergic or specific toxicities were associated with increased exposure to the concomitant use of busulfan, or vice versa. This information should be useful in future studies in which different drugs are combined with fludarabine.

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Appendix

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Comparable Antileukemia/Lymphoma Effects in Nonremission Patients Undergoing Allogeneic Hematopoietic Cell Transplantation with a Conventional Cytoreductive or Reduced-Intensity Regimen

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ABSTRACT

To evaluate the potential of allogeneic hematopoietic cell transplantation (HCT) with a reduced-intensity conditioning regimen (RIST) for the treatment of patients with hematologic malignancies not in remission, we retrospectively reviewed the medical records of 132 patients (89 leukemia or myelodysplastic syndrome, 40 malignant lymphoma, and 3 others) who received conventional myeloablative HCT (CST, n = 52) or RIST (n = 80). The median age of the RIST group was significantly higher than that of the CST group (53 years versus 40 years, $P < .01$). The RIST group also included a higher proportion of patients with an HCT-specific comorbidity index (HCT-CI) of 1 or more than the CST group (65% versus 37%, $P = .03$). The probabilities of achieving complete remission and the incidences of grades II-IV and III-IV acute graft-versus-host disease (aGVHD) in the CST and RIST groups were, respectively, 77% and 64%, 50% and 50%, and 23% and 28%, with no significant differences. Similarly, there was no difference in the 2-year probabilities of nonrelapse mortality (NRM, 36% and 38%), progressive disease or relapse (PD 51% and 49%), overall survival (OS, 31% and 38%), and progression-free survival (PFS, 28% and 29%). Multivariate analyses revealed that a higher HCT-CI score and transplant from donors other than HLA-matched relatives were associated with increased risks of NRM and poor OS, and patients who received chemotherapy within 2 months before HCT were associated with increased risks of PD, poor OS, and PFS after transplantation. After adjusting for these variables, the risks of NRM, PD, OS, and PFS in the RIST group were not significantly different from those in the CST group. In conclusion, these results suggest that the antileukemia/lymphoma effect associated with RIST is comparable to that associated with CST. RIST appears to be feasible for the treatment of hematologic malignancies not in remission.

KEY WORDS
Transplantation • Leukemia • Lymphoma

INTRODUCTION

Allogeneic hematopoietic cell transplantation (HCT) has the potential to achieve long-term cure of hematologic malignancies by pretransplant conditioning and a graft-versus-leukemia/lymphoma (GVL) effect. It has been well established that the disease status at the time of transplantation is the most important prognostic factor, and the rates of relapse and nonrelapse mortality (NRM) significantly increase in patients with hematologic malignancies who were not in remission. Therefore, conventional stem cell transplantation (CST) using a myeloablative conditioning

regimen has been universally used in the hope of maximally reducing the tumor burden before HCT in patients not in remission. However, CST may not be an option for many patients because of their older age or associated comorbidities. Alternatively, over the past few years, nonmyeloablative and reduced-intensity conditioning stem cell transplantation (RIST) have been offered to these patients undergoing HCT, on the assumption that RIST would be better tolerated [1-4].

There have been several reports that the outcome of older patients who underwent RIST while in remission was comparable to that of patients who received CST [5-9], which suggests that the GVL effect associated with RIST might be adequate for controlling chemosensitive or slowly progressing disease. On the other hand, it still remains controversial whether RIST is feasible for patients not in remission, although small pilot studies have shown that RIST was unsuccessful for advanced hematologic malignancies [3,10-14]. To address this issue, we retrospectively analyzed 132 patients who were not in remission at the time of CST or RIST.

PATIENTS AND METHODS

Study Patients

We retrospectively reviewed the medical records of 132 patients with various hematologic malignancies who underwent allogeneic HCT (CST, n = 52; RIST, n = 80) while not in remission at our institution from January 2000 to December 2004. Patients with chronic myelogenous leukemia (CML) in the chronic phase, myelodysplastic syndrome (MDS)-refractory anemia, and those with lymphoma in partial remission (PR) were not included because the response to treatment and the outcome of these patients is generally considered to be similar to those in patients who are in complete remission (CR). Bone marrow or granulocyte colony-stimulating factor (G-CSF)-mobilized peripheral blood stem cells (PBSC) were harvested from donors according to protocols approved by the guidelines of the Japan Marrow Donor Program, the Japanese Society for Hematopoietic Cell Transplantation, and the Japanese Society of Blood Transfusion. Informed consent was obtained according to the Declaration of Helsinki.

Transplantation Procedures

The conditioning regimens used in CST included the combination of cyclophosphamide (CY; 60 mg/kg i.v. daily for 2 days) and fractionated total body irradiation (TBI; 12 Gy in 6 fractions over 3 days) in 34 patients, CY and oral busulfan (BU; 16 mg/kg divided over 4 days) in 13 patients, and other combinations in 5 patients (Table 1). Targeted dose adjustment of BU was not performed. Patients who underwent RIST were older than 50 years of age or those who had comorbidities or prior transplantation. The conditioning regimens for RIST consisted of fludarabine (30 mg/m^2 i.v. daily for 6 days) or cladribine (0.11 mg/kg i.v. daily for 6 days) plus 8 mg/kg of oral BU [15] with (n = 27) or without (n = 53) 4 Gy TBI. In Japan, only bone marrow is permitted as a stem cell source in transplantation from an unrelated healthy volunteer donor. In the setting of nonmyeloablative stem cell transplantation from an unrelated donor, the sustained engraftment rate has been reported to be lower for recipients of bone marrow than for those given PBSC [13]. Therefore, low-dose TBI was also added to the conditioning regimen for RIST from an unrelated donor to facilitate engraftment.

Day 0 was defined as the day of stem cell infusion. G-CSF was administered after transplantation in all patients until neutrophil engraftment. Most patients who underwent CST were given cyclosporine (CSP) with methotrexate (MTX) [16], and all patients who underwent RIST were given CSP with or without MTX for graft-versus-host disease (GVHD) prophylaxis (Table 1). GVHD was treated with 1 to 2 mg/kg/day prednisolone equivalents, resumption of full-dose CSP administration if applicable, or both. Initial doses of corticosteroids and tapering schedules of immunosuppressive medications were modified at the discretion of the attending physicians according to the presence or absence of malignant cells and the severity of GVHD. Treatment for relapse after transplantation was left to the discretion of the attending physicians.

All patients received ciprofloxacin (200 mg orally 3 times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Patients who had positive serologic test results for herpes simplex virus or varicella zoster virus received prophylactic low-dose acyclovir until the cessation of immunosuppressive agents [17]. Prophylaxis against *Pneumocystis jiroveci* infection was provided with trimethoprim-sulfamethoxazole from the first day of conditioning to day −3 of transplantation, and from day 28 until day 180 or the cessation of immunosuppressive agents. Patients were monitored with weekly cytomegalovirus (CMV) pp65 antigenemia testing, and positive antigenemia was treated with ganciclovir as described previously [18,19].

Definitions

Chemotherapy within 2 months before HCT was defined as chemotherapy to control the disease except for rituximab alone for lymphoma and imatinib mesylate alone for CML. Pretransplantation comorbidities were determined by the HCT-specific comorbidity index (HCT-CI) [20] with a minor modification [21]. Neutrophil engraftment was defined as the first

Table 1. *Patient Characteristics*

	CST	RIST	P-Value
No. of patients	52	80	
Sex, male/female	25/27	50/30	
Median age, years (range)	40 (3-55)	53 (20-68)	<.01
Disease status at conditioning, N (%)			
AML	20 (38)	15 (19)	
Relapse I	12	6	
Relapse ≥2	5	5	
Primary refractory	3	4	
MDS (including overt AML)	15 (29)	24 (30)	
Relapse I	1	2	
Untreated	6	11	
Primary refractory	8	11	
ALL	5 (10)	2 (3)	
Relapse I	4	1	
Relapse 2	1	1	
CML	5 (10)	3 (4)	
Accelerated phase	3	0	
Blastic crisis	2	3	
NHL	7 (13)	33 (40)	
Relapse I	2	6	
Relapse ≥2	2	16	
Primary refractory	3	11	
Others*	0	3 (4)	
Chemotherapy within 2 months before HCT, N (%)	33 (63)	52 (65)	
Leukemia/MDS	30	23	
Lymphoma	3	26	
Others*	0	3	
HCT-CI score, N (%)			.03
0	33 (63)	28 (35)	
1-2	11 (21)	31 (39)	
≥3	8 (16)	21 (26)	
Conditioning regimen, N (%)			
TBI/CY	34 (65)	0	
BU/CY	13 (25)	0	
Fludarabine-based (± TBI)†	0	68 (85)	
Cladribine-based (±TBI)‡	0	12 (15)	
Others	5 (10)	0	
Donor type, N (%)			.1
HLA-matched related donor	17 (33)	41 (51)	
HLA-mismatched related donor	5 (9)	7 (9)	
Unrelated donor	30 (58)	32 (40)	
Stem cell source, N (%)			<.01
G-CSF mobilized PBSC	21 (40)	49 (61)	
BM	27 (52)	20 (25)	
CB	4 (8)	11 (14)	
GVHD prophylaxis, N (%)			<.01
Cyclosporine§	1 (2)	52 (65)	
Cyclosporine/MTX¶	49 (94)	28 (35)	
Tacrolimus	1 (2)	0	
Tacrolimus/MTX	1 (2)	0	
Prior HCT, N (%)	4 (8)	8 (10)	.65

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; AML, acute myelogenous leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; CML, chronic myelogenous leukemia; NHL, non-Hodgkin's lymphoma; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; TBI, total-body irradiation; CY, cyclophosphamide; BU, busulfan; HLA, human leukocyte antigen; G-CSF, granulocyte colony-stimulating factor; PBSC, peripheral blood stem cell; BM, bone marrow; CB, cord blood; GVHD, graft-versus-host disease; MTX, methoterexate.

*Others included 1 chronic lymphocytic leukemia and 2 multiple myeloma patients.

†Twenty-three patients received 4 Gy TBI.

‡Four patients received 4 Gy TBI.

§Including 7 with antithymocyte globulin.

¶Including 10 with antithymocyte globulin.

of 3 consecutive days after transplantation that the absolute neutrophil count exceeded 0.5 × 10^9/L of peripheral blood. The diagnosis and clinical grading of acute and chronic GVHD (aGVHD, cGVHD) were performed according to established criteria [22-24]. CR was defined as lower than 5% blasts in the bone marrow, with a neutrophil count >1.5 × 10^9/L and a platelet count >100 × 10^9/L in leukemia/MDS patients, and according to the International Workshop Criteria [25] in lymphoma patients.

Statistical Analysis

The endpoints of the study were progressive disease/relapse (PD), NRM, overall survival (OS), and progression-free survival (PFS). OS, NRM, and PD were defined as the time between stem cell infusion to the event. PFS was defined as the time between stem cell infusion to PD or death from any cause, whichever occurred earlier. OS and PFS were estimated by the Kaplan-Meier method [26]. NRM and PD were estimated by the cumulative incidence. The chi-square test or Fisher's exact test was used to evaluate the differences in the clinical characteristics of the CST and RIST groups. The log-rank test and the generalized Wilcoxon test were used to compare the probabilities of survival, NRM, and PD after HCT over time across patient subgroups.

Multiple Cox regression models were used for multivariate risk factor analysis for PD, NRM, OS, and PFS after HCT. Clinical factors evaluated in the PD, NRM, OS, and PFS analyses were patient age at the time of HCT (continuous), HCT-CI (0, 1-2, 3 or more), conditioning (CST, RIST), donor (HLA-matched related, HLA-mismatched related or unrelated), disease type (leukemia/MDS, lymphoma), and chemotherapy within 2 months before HCT (yes, no). Logistic regression analysis was performed to identify prognostic factors that were associated with the achievement of CR. In addition to the variables examined in the Cox analysis, blast percentage (≥20%, <20%) in the bone marrow or peripheral blood and the serum lactate dehydrogenase (LDH) level (normal, elevation) before HCT were included for the analysis of CR in patients with leukemia/MDS and those with lymphoma, respectively. We considered 2-sided *P*-values of <.05 to be statistically significant. Statistical analyses were performed with the SAS version 8.2 (SAS Inc, Cary, NC).

RESULTS

Patient Characteristics

The characteristics of all patients who underwent CST (n = 52) or RIST (n = 80) are summarized in Table 1. The median age of the RIST group was significantly higher than that of the CST group (53 years versus 40 years, $P < .01$). A large number of patients in both groups had acute myeloid leukemia (AML) or MDS (CST 67%, RIST 49%), and the RIST group included a higher population of patients with malignant lymphoma (CST 13%, RIST 40%). All malignant lymphomas (n = 40) were non-Hodgkin's lymphoma, including aggressive (n = 16), highly aggressive (n = 15), and indolent (n = 9) lymphomas. The distribution of lymphoma subtypes was similar between the 2 groups. Disease status at transplantation included primary refractory (n = 42), refractory relapse (n = 65), blastic crisis, or accelerated phase of CML (n = 8) and untreated disease (n = 17). The distribution of disease status and the proportion of patients who received chemotherapy within 2 months before HCT were similar between the 2 groups. The RIST group contained higher proportions of patients with an HCT-CI score of 1 or more (CST 37%, RIST 65%) and those who received G-CSF-mobilized PBSC (CST 40%, RIST 61%) than the CST group.

In the leukemia/MDS patients (n = 89), the median percentage of blasts (82 patients in bone marrow and 7 patients in peripheral blood) in both groups were similar (CST 29%, RIST 30%). In patients with malignant lymphoma, serum LDH was elevated above the upper normal limit in 3 of 7 (43%) in the CST group compared to 23 of 33 (70%) in the RIST group.

Engraftment and GVHD

The clinical course and response are detailed in Table 2. The median duration of follow-up in surviving patients is 1123 days (range: 367-2044 days) in the CST group and 899 days (range: 334-1961 days) in the RIST group. Neutrophil engraftment was observed in 48 patients (92%) and 75 patients (94%), at a median of 17 days and 12 days, respectively. Engraftment was not confirmed in the remaining 9 patients because of death or PD within 28 days after HCT. The incidences of grade II-IV and grade III-IV aGVHD were similar in the CST and RIST groups (50% versus 50% and 23% versus 28%, respectively). The incidences of cGVHD and chronic extensive GVHD were also similar (46% versus 49% and 34% versus 38%, respectively).

Disease Response

The probabilities of achieving CR as the best response were similar after CST and RIST (77% and 64%, respectively) (Table 2). To examine the possible risk factors for achieving CR, we separately analyzed patients with leukemia/MDS and those with lymphoma using a logistic regression analysis (Table 3). Conditioning regimen (RIST) did not influence the CR rate in patients with leukemia/MDS (odds ratio [OR] 1.11, 95% confidence interval [CI] 0.40-3.07,

Table 2. *Clinical Course and Response*

	CST (N = 52)	RIST (N = 80)
Median follow-up of surviving patients, days	1123 (367–2044)	899 (334–1961)
Engraftment of neutrophils, N (%)	48 (92)	75 (94)
Median day (range)	17 (10–35)	12 (5–43)
Acute GVHD, N (%)		
Grade II–IV	26 (50)	40 (50)
Grade III–IV	12 (23)	22 (28)
CR*, N (%)	40 (77)	51 (64)
Leukemia/MDS (n = 89), CR/total	35/45	35/44
Lymphoma (n = 40), CR/total	5/7	14/33
Causes of NRM, N (%)	15 (29)	26 (33)
GVHD	6	11
Infection		
fungus	0	4
CMV	0	1
bacterial	4	7
Interstitial pneumonitis	2	1
Others†	3	2

CST indicates conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; GVHD, graft-versus-host disease; MDS, myelodysplastic syndrome; CR, complete remission; NRM, nonrelapse mortality; CMV, cytomegalovirus.

*CR as the best response after transplantation.

†Others included acute myocardial infarction, subarachnoid hemorrhage, and pulmonary alveolar haemorrhage in the CST group, and cerebral hemorrhage and unknown in the RIST group.

P = .84) or in those with lymphoma (OR 0.29, 95% CI 0.05-1.75, P = .18). In the leukemia/MDS patients, those who received chemotherapy within 2 months before HCT (OR 0.32, 95% CI 0.09-1.05, P = .06) and transplant from donors other than an HLA-matched relative (OR 0.28, 95% CI 0.08-1.06, P = .06) tended to have a lower CR rate, whereas the blast percentage (≥20%) of bone marrow or peripheral blood was not associated with the CR rate. In lymphoma patients, chemotherapy within 2 months before HCT was the only factor that was significantly associated with a low CR rate (OR 0.04, 95% CI 0.005-0.40, P < .01), whereas serum LDH elevation did not influence the CR rate.

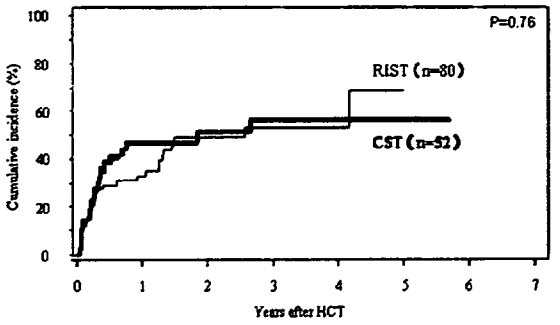

Figure 1. Cumulative incidence of PD. The 2-year probabilities of PD in the CST (51%) and RIST (49%) groups were not significantly different (P = .76).

As shown in Figure 1, the cumulative incidence of PD was not significantly different between the CST and RIST groups. The 2-year probabilities of PD were 51% in the CST group and 49% in the RIST group, which were not significantly different (P = .76). Cox regression analysis was performed to identify factors that were associated with PD. Multivariate analyses in all patients showed that those who received chemotherapy within 2 months before HCT were associated with an increased risk of PD (hazard ratio [HR] 3.93, 95% CI 1.97-7.83, P < .01) (Table 4). After adjusting for these variables, the intensity of conditioning (CST or RIST) did not influence the rate of PD in any of the patients. To further evaluate the association between risk factors and outcome, we performed a subset analysis in patients who underwent CST or RIST. As a result, chemotherapy within 2

Table 3. *Logistic Analysis of CR Rate in Leukemia/MDS and Lymphoma Patients*

		Leukemia/MDS (N = 89)		Lymphoma (N = 40)	
		Odds Ratio (95% CI)	P	Odds Ratio (95% CI)	P
HCT-CI	0	1.00		1.00	
	1–2	1.44 (0.43–4.87)	.56	3.33 (0.66–16.7)	.14
	3 or more	0.96 (0.28–3.35)	.95	2.22 (0.40–12.3)	.36
Age		1.00 (0.97–1.04)	.70	1.02 (0.97–1.07)	.49
Conditioning	RIST	1.11 (0.40–3.07)	.84	0.29 (0.05–1.75)	.18
Donor	Alternative*	0.28 (0.08–1.06)	.06	0.95 (0.26–3.42)	.93
Chemotherapy within 2 months before HCT	Yes	0.32 (0.09–1.05)	.06	0.04 (0.005–0.40)	<.01
Blasts†	≥20%	0.62 (0.21–1.80)	.38		
Serum LDH level	Elevation			0.35 (0.09–1.34)	.12

MDS indicates myelodysplastic syndrome; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; RIST, reduced-intensity stem cell transplantation; LDH, lactate dehydrogenase; CI, confidence interval.

*Non-HLA-matched related donor.

†Blast counts in bone marrow (N = 82) or peripheral blood (N = 7).

Table 4. *Multivariate Analysis of PD, NRM, OS, and PFS in All Patients*

Covariates*	N	PD HR (95% CI)	PD P	NRM HR (95% CI)	NRM P	OS HR (95% CI)	OS P	PFS HR (95% CI)	PFS P
Conditioning									
CST	52	1.00		1.00		1.00		1.00	
RIST	80	0.91 (0.53-1.55)	.72	0.99 (0.51-1.96)	.99	0.95 (0.60-1.51)	.83	0.95 (0.63-1.43)	.79
HCT-CI score									
0	65			1.00		1.00		1.00	
1-2	38			3.25 (1.43-7.40)	<.01	1.76 (1.08-2.89)	.02		
3 or more	29			6.61 (2.88-15.2)	<.01	2.62 (1.51-4.56)	<.01	1.63 (1.02-2.62)	.04
Donor									
MRD	58			1.00		1.00			
Alternative†	74			2.77 (1.39-5.54)	<.01	1.80 (1.15-2.82)	.01		
Chemotherapy within 2 months before HCT									
No	47	1.00				1.00		1.00	
Yes	85	3.93 (1.97-7.83)	<.01			1.73 (1.10-2.72)	.02	2.23 (1.44-3.45)	<.01

PD indicates progressive disease or relapse; NRM, nonrelapse mortality; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; MRD, HLA-matched related donor; HCT, hematopoietic cell transplantation.

*Factors analyzed included age at the time of HCT (continuous), HCT-CI (0, 1–2, 3, or more), conditioning (CST, RIST), donor (MRD, Alternative), disease type (leukemia/MDS, lymphoma) and chemotherapy within 2 months before HCT (yes, no).

†Non-HLA-matched related donor.

months before HCT was associated with an increased risk of PD only in the RIST group, and not in the CST group (Table 5).

NRM

Major causes of NRM for patients in both groups were GVHD and infection (Table 2). More patients died of fungal infection in the RIST group compared to the CST group, but the 2-year probabilities of NRM were not significantly different (36% and 38%, $P = .50$, Figure 2). A Cox regression analysis was performed to identify factors associated with NRM. Multivariate analyses in all patients showed that a higher HCT-CI score (1 or more) and transplant from an HLA-mismatched related or unrelated donor (alternative donor) were associated with an increased risk of NRM (Table 4). After adjusting for these variables, the intensity of conditioning (CST or RIST) did not influence the rate of NRM in any of the patients. A subset analysis revealed that a higher HCT-CI score (1 or more) was associated with increased NRM in the CST group, but not in the RIST group (Table 5). In contrast, transplant from an alternative donor was associated with increased NRM in the RIST group, but not in the CST group.

Survival

The 2-year probabilities of OS and PFS were not significantly different between the CST and RIST groups (31% and 38%, $P = .98$, for OS; 28% and

Table 5. *Multivariate Analysis of Outcomes after HCT in the CST and RIST Groups*

	Covariates	CST (N = 52) HR (95% CI)	CST (N = 52) P	RIST (N = 80) HR (95% CI)	RIST (N = 80) P
PD	Chemotherapy within 2 months before HCT		NS	6.16 (2.15–17.7)	<.01
NRM	HCT-CI (1–2)	4.48 (1.26–16.0)	.02		NS
	HCT-CI (3 or more)	10.2 (2.91–35.7)	<.01	2.41 (1.14–5.10)	.02
	Alternative donor*		NS	4.63 (1.96–10.9)	<.01
OS	HCT-CI (1–2)	2.69 (1.23–5.90)	.01		NS
	HCT-CI (3 or more)	4.84 (1.97–11.9)	<.01		NS
	Alternative donor*		NS	3.04 (1.73–5.35)	<.01
PFS	HCT-CI (3 or more)	2.26 (1.01–5.04)	.04		NS
	Chemotherapy within 2 months before HCT	2.10 (1.05–4.19)	.03	2.10 (1.19–3.70)	.01
	Alternative donor*		NS	1.79 (1.06–3.00)	.03

PD, indicates progressive disease or relapse; NRM, nonrelapse mortality; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CST, conventional stem cell transplantation; RIST, reduced-intensity stem cell transplantation; HCT, hematopoietic cell transplantation; HCT-CI, hematopoietic cell transplantation-specific comorbidity index; NS; not significant.

*Non-HLA-matched related donor.