厚生労働科学研究費補助金　がん臨床研究事業

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＜研究課題名＞

再発等の難治性造血器腫瘍に対する同種造血幹細胞移植を用いた効果的治療法確立に関する研究

平成 19 年度～平成 21 年度
総合研究報告書

研究代表者　　森　慎一郎

国立がんセンター中央病院

平成 22 年（2010 年）3 月

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Ⅰ．総合研究報告

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『 再発等の難治性造血器腫瘍に対する同種造血幹細胞移植を用いた効果的治療法確立に関する研究 』

研究代表者　森 慎一郎　国立がんセンター中央病院／臨床検査部細菌免疫検査室 医長

研究要旨

わが国における同種造血幹細胞移植の治療成績を飛躍的に向上させる事を目的として、移植前処置法、ならびに免疫抑制療法の個別化、最適化を図るための臨床研究を実施した。移植前処置には致死量をはるかに超える大量の抗がん剤がもちいられ、免疫抑制には薬物動態の個人差が極めて大きい各種免疫抑制剤が用いられる。これらの薬物は同種造血幹細胞移植の成功の鍵となる最も重要な要素であり、薬理動態試験などの臨床試験に基づいて最適化する事が可能である。しかし、わが国固有のエビデンスは驚くほど少なく、人種差が大きいために海外でのエビデンスを直接利用することには限界がある。そこで本研究班では、同種造血幹細胞移植に用いられる基本的薬剤についての多数の臨床薬理学的試験を実施し、以下の研究成果を得たので、これらの研究より得られた結論を列挙する。

1. 免疫抑制剤の使用方法の最適化に関する検討

●経口シクロスポリンを高齢者に投与する場合、5mg/kg/日が至適投与開始量であり、薬剤添付文書に記載されている投与量は明らかに過量である

●従来用いられている trough 濃度ではなく、シクロスポリン点滴開始後 3 時間後の血中濃度を HLA 一致血縁者間移植においては、800ng/mL 以上、非血縁者間移植では 1,000ng/mL 以上となるように調節する事により、重症急性 GvHD の発症を抑えられる。

●本邦の造血幹細胞移植例では、タクロリムスクリアランス (CLTac)は 0.044±0.037L/hr/kg と海外の報告よりも低値である。タクロリムスクリアランスは年齢による差を認めないが、男女差があり女性例ではクリアランスが早い。

●血清ビリルビン値が CLTac に与える影響は少なく、ビリルビンの上昇がみられただけでは、投与量の減量の必要性は低い。

●静注タクロリムスを経口薬に切り替える際には、平均値では静注量の 4 倍が適量となるが、個人差が大きく、4 倍投与が適量となるケースは 40%程度に過ぎない。静注投与時に CLTac を算出する事により、個別化したより適切な投与量設定が可能である。

●ボリコナゾール、イトラコナゾールをタクロリムスと併用する際には、一般的にタクロリムスの減量が必要であるが、個人差のため添付文書の記載の通りに機械的に減量する事は不適切であり、頻回の血中濃度測定による調節が必要である

2. 移植前処置薬としての静注 Busulfan の有効性と安全性に関する検討

●静注ブスルファンの小児への投与量設定については体重を用いた現行の添付文書の用法、用量に一定の妥当性がある。

●静注ブスルファンの小児例における薬物動態の個人差は大きく、血中濃度が極端に高値となり、重篤な有害事象が出現する例がみられる。ブスルファンの 1 投与分を事前に投与し（テストドーズ）、薬理動態を調べておく事により、この様な症例を事前に予測する事が可能である。しかし、テストドースの薬物動態は、その後の投与の薬物動態を必ずしも精確に反映しておらず、より厳密な個別化の方法論の確立が必要である。

3. 合併症治療薬の検討

●サイトメガロウイルス感染症に対する経口 Valganciclovir の有用であるが、有害事象の頻度等を静注ガンシクロビルと比較した場合、薬剤添付文書の用量は適切でない可能性がある。

●ウイルス性出血性膀胱炎の治療に、シドフォビルは極めて有用であるが、国内未承認のため、わが国での導入が必要である。

4. 臨床試験体制の基盤整備に関する研究

多数の臨床課題に対して、比較的少数例から必要なエビデンスを得るためには、臨床薬理学的エンドポイントを用いるここと や、ベイズ流の試験デザインを用いる事が有用である。

A. 研究目的

本研究班では、移植に用いられる幹細胞の種類（血縁者/非血縁者由来、骨髄、末梢血幹細胞、臍帯血など）によらず、同種造血幹細胞移植を実施する際に共通の基本的薬物療法である、前処置薬と免疫抑制剤の個別化、最適化をはかる事により、わが国における同種造血幹細胞移植の治療成績を飛躍的に向上させることを目的とした。移植前処置薬については、比較的少数例の国内治験データをもとに最近承認された静注用ブスルファン製剤の適正使用を目指し、特に適正な投与量が未確立の小児例に関して、前向きの薬物動態試験を行い、その適正使用を確立する事を目指した。また、高齢者や臓器機能低下患者等の特別な患者軍に対する適正使用方法についても、薬物動態試験を計画した。免疫抑制剤については、シクロスポリンについて、固形臓器移植分野で蓄積されたエビデンスをもとに、造血幹細胞移植患者に対して多施設共同前向き試験を実施した。また、タクロリムスについては、静注製剤から経口投与に切り替える際の投与量について確立したデータが存在せず、薬剤添付文書にも記載がない事から、これを明らかにする薬物動態試験を実施した。これらの臨床試験結果により薬剤の適正使用の方法論が確立した段階においては、各薬剤の添付文書にその成果を反映することを目的とする。これによって研究成果が広く速やかに臨床現場に普及し、治療技術の均霑化に大きく寄与するものと思われる。

B. 研究方法

1) 高齢者を対象とし、経口シクロスポリンの用量設定試験を実施した。
2) シクロスポリン投与3時間後の血中濃度に基づいて、投与量を調節する多施設共同研究を実施した。
3) タクロリムス血中濃度とGvHD合併頻度の関係、ならびに本邦の患者におけるタクロリムスクリアランスを測定する薬理学的試験を実施した。
4) 静注タクロリムスを経口に切り替える際の適切な投与量、ならびにクリアランスとの関係を後方視的に検討した
5) タクロリムス使用下にボリコナゾール、イトラコナゾールが使用された患者においてタクロリムス血中濃度の変化、ならびにクリアランスの変化を測定した
6) 静注ブスルファン血中濃度が、生着や有害事象に与える影響を小児患者において検討した。
7) あらかじめ静注ブスルファンを一回投与し、その際の血中濃度と、その後の投与時の血中濃度を比較し、テストドーズの有用性を検討する試験を実施した。
8) ウイルス性出血性膀胱炎に対するシドフォビルの有用性を検討する試験を実施した。
9) サイトメガロウイルス感染症に対してバルガンシクロビルが使用された症例を後方視的に検討し、効果と有害事象の頻度を静注ガンシクロビル使用例と比較する事により、添付文書の用量設定の妥当性を検討した。

＜倫理面への配慮＞

これらの基礎的あるいは臨床的研究の実施に当たっては、国の緒指針に基づいて被験者となる患者の人権に十分配慮するとともに、個人情報の厳格な管理を行った。

C．研究結果

●経口シクロスポリンを高齢者に投与する場合、5mg/kg/日が至適投与開始量であり、薬剤添付文書に記載されている投与量は明らかに過量である。

●従来用いられている trough 濃度ではなく、シクロスポリン点滴開始後3時間後の血中濃度をHLA一致血縁者間移植においては、800ng/mL以上、非血縁者間移植では 1,000ng/mL以上となるように調節する事により、重症急性 GvHD の発症を抑えられる。

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●多数の臨床課題に対して、比較的少数例から必要なエビデンスを得るためには、臨床薬理学的エンドポイントを用いることや、ベイズ流の試験デザインを用いる事が有用である。

D．考察

同種造血幹細胞は、生物製剤である造血幹細胞を除けば、純然たる薬物療法である。従って既に方法論が確立している薬物動態学・薬力学的な研究によって薬物治療の最適化をはかる事は重要であるとともに、確実に成果が得られる分野である。しかし、わが国におけるエビデンスは絶対的に不足しており、現場では個人の経験によるさじ加減や欧米のデータに基づいた治療レジメンがそのまま用いられているという現状がある。本研究班の研究成果はこういった状況を打開し、治療成績の向上と治療技術の均霑化に大きく寄与し得るものと期待される。

E．結論

本研究班の研究成果が最終的に薬剤添付文書に反映されれば、わが国の移植医療の質の向上に大きく貢献すると思われる。

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

Ⅱ．主な研究成果（論文発表）一覧

研究成果の刊行物（論文別刷）

＜ 主な研究成果（論文発表）一覧 ＞

著者名（研究者にｱﾝﾀﾞｰﾗｲﾝ）	論文タイトル	発表誌名	巻号	ページ	出版年
Onishi Y, **Mori S**, Kusumoto S, Sugimoto K, Akahane D, Morita-Hoshi Y, Kim SW, Fukuda T, Heike Y, **Tanosaki R**, Tobinai K, Takaue Y.	Unrelated-donor bone marrow transplantation with a conditioning regimen including fludarabine, busultan, and 4 Gy total body irradiation.	Int J Hematol	85	256-263	2007
Miyakoshi S, Kami M, Tanimoto T, Yamaguchi T, Narimatsu H, Kusumi E, Matsumura T, Takagi S, Kato D, Kishi Y, Murashige N, Yuji K, **Uchida N**, Masuoka K, Wake A, Taniguchi S.	Tacrolimus as prophylaxis for acute graft-versus-host disease in reduced intensity cord blood transplantation for adult patients with advanced hematologic diseases.	Transplantation	84	316-322	2007
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Nishikawa T, Okamoto Y, Tanabe T, Shinkoda Y, Kodama Y, Higashi M, Hirano H, Arita K, **Kawano Y.**	Unexpectedly high AUC levels in a child who received intravenous busulfan before stem cell transplantation.	Bone Marrow Transplantation	45	602-604	2010

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Unrelated-Donor Bone Marrow Transplantation with a Conditioning Regimen Including Fludarabine, Busulfan, and 4 Gy Total Body Irradiation

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Abstract

We investigated the feasibility of reduced-intensity conditioning with 4 Gy total body irradiation, fludarabine (30 mg/m^2 for 6 days), and busulfan (4 mg/kg for 2 days) for bone marrow transplantation from a serologically HLA-matched unrelated donor. Seventeen adult patients (median age, 55 years; range, 27-67 years) with various hematologic malignancies (6 in remission, 11 not in remission) were treated. Successful engraftment was achieved in all patients at a median of day 18 (range, day 14-35) after transplantation, although subsequent secondary graft failure was observed in 2 patients. The cumulative incidence of acute graft-versus-host disease (GVHD) of grades II to IV at day 100 was 48%. With a median follow-up of 286 days (range, 56-687 days), the rates of 1-year overall survival, 100-day nonrelapse mortality, and 1-year nonrelapse mortality were 41%, 14%, and 46%, respectively. Eleven patients died, and the causes of death were relapse (n = 4), pulmonary complications (n = 4), acute GVHD (n = 2), and sepsis (n = 1). The remaining 6 patients (at transplantation, 2 were in remission, and 4 were not in remission) are currently still in remission. These results suggest that this regimen reduces the risk of graft failure, but further studies are needed to ameliorate transplantation-related toxicities, primarily GVHD and/or pulmonary complications.

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Key words: Unrelated donor bone marrow transplantation; Fludarabine; Busulfan, TBI

1. Introduction

Although allogeneic hematopoietic stem cell transplantation (HSCT) is a possible curative approach for patients with various hematologic malignancies, only 30% to 40% of patients in Japan have an appropriate family donor available [1]. Hence, the application of unrelated-donor transplantation using bone marrow or cord blood cells has been expanding. Another area of current interest is the application of reduced-intensity conditioning regimens, mostly incorporating fludarabine as a primary agent, because conventional allogeneic HSCT using a conditioning regimen with high doses of systemic chemotherapy/radiation is associated with significant toxicities. In contrast, HSCT with a reduced-intensity conditioning regimen allows older patients and those who have contraindicating comorbidities to undergo HSCT [2-7].

Nevertheless, special consideration should be paid to developing reduced-intensity conditioning protocols for the unrelated-donor HSCT setting, because the incidences of both graft rejection and graft-versus-host disease (GVHD) are greater than in related-donor transplantation. In addition, the intensity of the reduced-intensity conditioning regimen influences transplantation-related toxicities and the relapse rate, and the stem cell source (ie, peripheral blood stem cells or bone marrow cells) influences engraftment [8]. Accordingly, several reduced-intensity conditioning protocols have been tested to address a variety of problems [8-17]. In this study, we investigated the feasibility of bone marrow transplantation (BMT) from a serologically HLA-matched unrelated donor with a regimen containing

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4 Gy of total body irradiation (TBI), fludarabine (Flu), and busulfan (BU).

2. Patients and Methods

2.1. Patients and Donors

The data for adult patients with hematologic malignancies who underwent unrelated-donor BMT through the Japan Marrow Donor Program between June 2002 and December 2003 at the National Cancer Center Hospital were analyzed retrospectively. This protocol was approved by the Ethics Committee, and written informed consent was obtained from each patient. The patients who were enrolled in this study were ineligible for conventional allogeneic HSCT because of age (older than 50 years) and/or concomitant diseases or preceding intensive therapies, such as autologous HSCT or multiple chemotherapies. Donor-recipient pairs were selected on the basis of serologic matching for HLA-A and HLA-B and molecular matching for HLA-DRB1. HLA allele typing was performed by intermediate-resolution polymerase chain reaction (PCR) analysis. The stem cell source, which was determined by the Japan Marrow Donor Program donor center, was bone marrow in all cases.

2.2. Treatment Plan and Evaluations

The conditioning regimen consisted of 30 mg/m^2 Flu intravenously daily for 6 days (day –8 to day –3), 4 mg/kg BU orally daily for 2 days (days –6 and –5, without BU dose adjustment), and 4 Gy TBI without lung shielding (day –9 or day –1, single dose or 2 divided doses). Non–T-cell–depleted bone marrow was infused on day 0. The time of neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $\geq 0.5 \times 10^9$/L, and the time of platelet engraftment was defined as the first of 7 consecutive days with a platelet count $\geq 20 \times 10^9$/L without transfusion support. Granulocyte colony-stimulating factor (G-CSF) was administered at 300 μg/m^2 from day 6 and continued until neutrophil engraftment. The degree of donor chimerism among peripheral blood mononucleated cells was evaluated by PCR analysis of short tandem repeat polymorphisms with fluorescently labeled primers. Secondary graft failure was defined as cytopenia with an absolute neutrophil count $< 0.1 \times 10^9$/L or decreasing chimerism not associated with relapsing disease in patients who had recovered in the early posttransplantation period.

GVHD prophylaxis consisted of cyclosporin A (CsA) from day –1 (daily administration of 3 mg/kg by continuous intravenous infusion or 6 mg/kg orally in 2 divided doses) and methotrexate (10 mg/m^2 intravenously on day 1 and 7 mg/m^2 on days 3, 6, and 11). The CsA dosage was adjusted according to the patient's renal function and to maintain therapeutic levels (250-350 ng/mL) with continuous infusion or trough levels (150-250 ng/mL) with oral administration. In patients without GVHD, CsA was tapered from day 100 over a 3- to 6-month period. Standard criteria were used to grade acute and chronic GVHD [18,19]. Chronic GVHD was evaluated in patients who survived at least 100 days and was classified as limited or extensive. Patients who developed acute GVHD ≥grade II were treated with methylprednisolone at 1 to 2 mg/kg per day.

2.3. Supportive Care

Antimicrobial prophylaxis consisted of ciprofloxacin, fluconazole, acyclovir, and trimethoprim/sulfamethoxazole according to our institutional protocol. All patients were nursed in a room equipped with high-efficiency air filtration of particulates. Monitoring for cytomegalovirus (CMV) antigenemia was performed once a week after neutrophil engraftment by means of the horseradish peroxidase–C7 method. Patients positive for CMV antigenemia were started preemptively on ganciclovir therapy.

2.4. Statistical Analysis

Overall survival was calculated from the time of transplantation until death from any cause. Progression-free survival was measured from transplantation until disease progression or death from any cause. Nonrelapse death was defined as death due to any cause other than relapse. Survival curves for overall survival and progression-free survival were estimated by the Kaplan-Meier method.

3. Results

3.1. Patients

The median age of the 17 patients was 55 years (range, 27-67 years; Table 1). The diagnoses were acute myeloid leukemia (AML) (n = 7), myelodysplastic syndrome (MDS) (n = 4), chronic myelogenous leukemia (n = 1), non-Hodgkin's lymphoma (n = 4), and multiple myeloma (n = 1). Six patients were in remission at transplantation, and the remaining 11 were not in remission. Three patients with MDS or AML following MDS underwent unrelated-donor BMT as a primary treatment. Seven donor-recipient pairs were fully matched for HLA-A, HLA-B, and HLA-DRB1 at the allele level, 4 donor-recipient pairs had an allele-level mismatch at the HLA-A locus, and 5 pairs had an allele-level mismatch at the HLA-DRB1 locus. One patient was mismatched with the donor at 3 HLA alleles.

3.2. Engraftment and Chimerism

The median number of infused nucleated cells was 2.7×10^8/kg (range, $0.65\text{-}5.5 \times 10^8$/kg). All patients achieved neutrophil recovery, but 5 patients did not become independent of platelet transfusion during their follow-up period (Table 2). The median times until neutrophil and platelet recoveries were 18 days (range, 14-35 days) and 26 days (range, 15-112 days), respectively (Figure 1). Late graft failure was observed in 2 patients, one of whom had secondary graft failure due to myelosuppression caused by ganciclovir treatment for CMV colitis. In this patient, donor chimerism was not assessed after day 30 when complete donor chimerism was confirmed. In the other case, donor chimerism decreased from 89% on day 30 to 33% on day 60, despite the tapering of CsA from day 30. Chimerism was

Table 1.
Patient and Disease Characteristics*

Patient No.	Age, y/Sex	Disease	Status	Time from Dx to HSCT, mo	HLA Allelic Mismatch	GVH Vector	HVG Vector	Contraindications to Conventional HSCT	Pretransplantation Comorbidities
1	55/F	AML	CR3	117				Age	No
2	52/F	AML	Primary Ref	13	DRB1	1	1	Age + comorbidity	Pneumonia
3	57/F	AML	Rel2	28				Age	Atrial fibrillation
4	55/M	MDS	Primary Ref	3				Age	Atrial fibrillation
5	57/M	MDS	CR1	8				Age	No
6	59/M	CML	CP2	8				Age	No
7	55/M	PTCL	PR	16	DRB1	1	1	Age	Gastric ulcer
8	58/M	AML	Untreated	10	DRB1	1	1	Age	Bronchial asthma, FEV_1 75%
9	59/M	AML	Untreated	33	DRB1	1	1	Age	Bilirubin 1.5 mg/dL
10	52/M	AML	CR1	11	A	1	1	Age	FEV_1 67%
11	57/M	MDS	CR1	13				Age	Prior gastric cancer
12	61/M	AML	CR2	58	A, both DRB1	3	3	Age	No
13	67/F	FL	Primary Ref	58	A	1	1	Age + comorbidity	Dyspnea requiring oxygen
14	27/M	DLBCL	Rel3	38	A	1	0	Prior autologous HSCT	No
15	48/F	MM	Primary Ref	80				Comorbidity	Ventricular septal defect
16	52/F	MDS	Untreated	130	A	1	1	Age	No
17	49/M	FL	Rel1	28	DRB1	1	1	Prior multiple chemotherapies	No

*Dx indicates diagnosis; HSCT, hematopoietic stem cell transplantation; GVH, graft-versus-host; HVG, host-versus-graft; AML, acute myeloid leukemia; CR3, third complete remission; Ref, refractory; Rel2, second relapse; MDS, myelodysplastic syndrome; CML, chronic myelogenous leukemia; CP2, second chronic phase; PTCL, peripheral T-cell lymphoma; PR, partial remission; FEV_1, forced expiratory volume in 1 second; FL, follicular lymphoma; DLBCL, diffuse large B-cell lymphoma; MM, multiple myeloma.

evaluated by analysis of short tandem repeats in 14 patients, and complete donor chimerism was confirmed in 12 of these patients. One patient who relapsed on day 32 had exhibited 54% donor chimerism on day 30. In the remaining 3 patients who relapsed after transplantation, complete donor chimerism had been achieved by day 30. In the patient who relapsed on day 78, donor chimerism decreased from 100% on day 30 to 64% on day 60. Mixed chimerism was not confirmed in the other 2 patients before disease progression or relapse. The patients without graft failure or relapse did not have mixed chimerism during their follow-up periods.

3.3. Regimen-Related Toxicities and Infections

Regimen-related toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 2.0, and maximum toxicities are shown in Table 3. Fifteen of the 17 patients had grade III oral/pharyngeal mucositis that required morphine as an analgesic. Reversible elevation (grades III-IV) in transaminase and bilirubin levels occurred in 35% and 12% of the cases, respectively. No veno-occlusive disease was observed. Four patients developed transient grade III hyponatremia within 28 days after transplantation. Four patients developed transient pulmonary infiltration or congestive heart failure due to hypercytokinemia at engraftment, and 2 of these patients developed grade II acute GVHD after engraftment. No histologic findings of acute GVHD were seen in the other 2 patients. One patient developed reversible paroxysmal supraventricular tachycardia. One patient developed bloody diarrhea and abdominal pain even after improvement of acute GVHD of the skin, and we diagnosed intestinal thrombotic microangiopathy from the results of a gut biopsy. This patient was successfully managed by diminishing immunosuppressive treatment. Four patients who had blood cultures positive for bacterial infection (*Pseudomonas aeruginosa*, *Acinetobacter lwoffii*, *Corynebacterium* sp, and *Staphylococcus* sp) within 28 days after transplantation were successfully treated with antibiotics. Invasive aspergillosis was encountered in 2 patients (1 proven and 1 possible case). In the proven case, the patient had bronchiolitis obliterans, which was the ultimate cause of death. Of the 17 patients, CMV antigenemia was detected in 12 patients, 2 of whom had CMV colitis.

3.4. Graft-versus-Host Disease

Acute GVHD of grades II to IV was diagnosed in 8 patients (48%; 95% confidence interval [CI], 36%-59%); the GVHD was grade II in 3 patients and grade IV in 5. The median time to the onset of acute GVHD was 32 days (range, 20-81 days) after transplantation (Figure 2A). Two of 4 patients who skipped methotrexate treatment on day 11 because of severe mucositis developed grade IV acute GVHD. Two of the 5 patients with grade IV acute GVHD subsequently died. One of these patients had acute GVHD after the withdrawal of CsA treatment at the time of leukemia relapse, and the other patient had received bone

Table 2.
Transplantation Outcomes*

Patient No.	Time to ANC >0.5 × 10^9/L, d	Time to Platelets >20 × 10^9/L, d	Acute GVHD						Chronic GVHD (Involved Organs)	Follow-up, d	Current Disease Status	Cause of Death
			Grade	Skin	Liver	Gut	mPSL, mg/kg	Response				
1	16	26	IV	3	4	4	2	PG	NE	121	Dead	Acute GVHD
2	35	30	0	0	0	0	—	—	NE	133	Dead	Relapse
3	17	—	0	0	0	0	—	—	NE	56	Dead	Relapse
4	14	15	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	439	Dead	BO
5	15	22	I	1	0	0	—	—	Ext (skin, mouth, liver)	286	Dead	IP
6	21	38	II	3	0	0	—	—	NE	260	Dead	Relapse
7	14	25	I	2	0	0	—	—	Ext (mouth, liver)	687+	CR, alive	
8	20	30	II	3	1	0	1	PR	Ext (skin)	667+	CR, alive	
9	22	—	II	3	0	0	—	—	Ext (skin, mouth, eyes)	336	Dead	Organizing pneumonia
10	18†	—	0	0	0	0	—	—	NE	94	Dead	Secondary graft failure
11	16	23	0	0	0	0	—	—	Ext (skin, mouth)	564+	CR, alive	
12	16†	—	IV	2	4	3	2	PG	NE	69	Dead	Acute GVHD
13	18	23	I	1	0	0	1	CR	Ext (mouth, eyes, liver)	525+	CR, alive	
14	18	—	IV	3	4	2	1	UE	NE	64	Dead	Relapse
15	14	16	0	0	0	0	—	—	Ext (mouth, eyes)	511+	CR, alive	
16	26	112	0	0	0	0	—	—	Lim (mouth)	463+	CR, alive	
17	22	38	IV	4	0	0	2	CR	Ext (skin, mouth, eyes, liver, lung)	276	Dead	BO + aspergillosis

*ANC indicates absolute neutrophil count; GVHD, graft-versus-host disease; mPSL, methylprednisolone; PG, progressive response; NE, not evaluable; CR, complete response; Ext, extensive disease; BO, bronchiolitis obliterans; IP, interstitial pneumonitis; PR, partial response; UE, unevaluated; Lim, limited disease.

†Secondary graft failure occurred after neutrophil recovery.

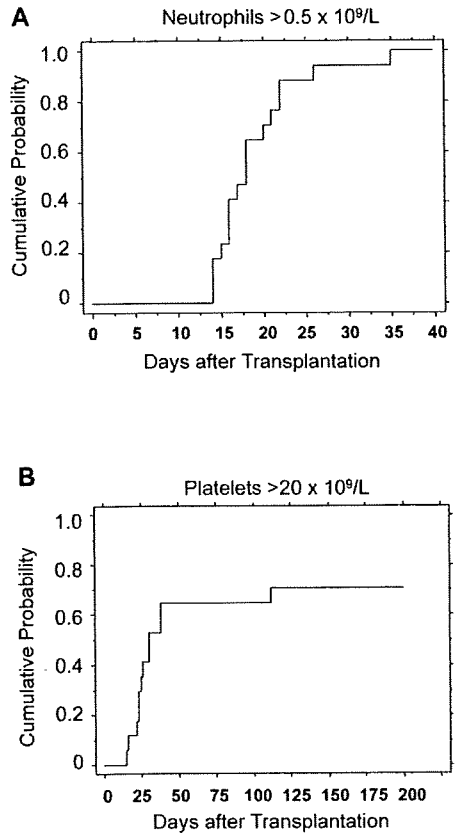

Figure 1. Engraftment after unrelated-donor bone marrow transplantation following reduced-intensity conditioning expressed as the cumulative probability of a neutrophil count >0.5 × 10^9/L (A) and a platelet count >20 × 10^9/L (B). All patients achieved neutrophil recovery, but 5 patients did not achieve platelet recovery. The median times until neutrophil and platelet recoveries were 18 days (range, 14-35 days) and 26 days (15-112 days), respectively. Late graft failure was observed in 2 patients.

marrow from a donor with allele-level mismatches at 3 HLA loci. Two patients with grade IV acute GVHD involving only the skin were successfully treated with methylprednisolone. Grade II acute GVHD involving only the skin was treated solely with CsA in 2 patients (Table 2). In 7 patients without relapse or secondary graft failure, CsA was tapered from a median of day 120 (range, day 96-169). Only 2 of the 7 patients were able to discontinue CsA (at days 203 and 288). Chronic GVHD was documented in all patients who survived beyond day 100 (1 with limited GVHD, 9 with extensive disease). There was no significant correlation between HLA disparity at the allele level and the incidence of GVHD, although it was difficult to analyze the data statistically because of the small number of patients in this study.

3.5. Survival and Causes of Death

The median follow-up period was 286 days (range, 56-687 days). Overall, 11 patients died, but 6 patients are currently in remission (2 in remission and 4 not in remission at the time of transplantation). The estimated 100-day and 1-year nonrelapse mortality rates were 14% (95% CI, 12%-17%) and 46% (95% CI, 33%-57%), respectively (Figure 2B). Estimated 1-year overall survival and progression-free survival rates were both 41% (95% CI, 32%-51%; Figure 3). There were 4 deaths due to recurrent or progressive disease at a median time of 55 days (range, 32-93 days). The causes of the 7 treatment-related deaths included acute GVHD (n = 2), secondary graft failure with sepsis (n = 1), interstitial pneumonitis (n = 1), organizing pneumonia (n = 1), bronchiolitis obliterans (n = 1), and bronchiolitis obliterans with invasive aspergillosis (n = 1).

4. Discussion

In our previous study in an unrelated-donor BMT setting, 5 patients underwent conditioning with a combination of Flu (30 mg/m^2 for 6 days) or cladribine (0.11 mg/kg for 6 days), BU (4 mg/kg for 2 days), and antithymocyte globulin (2.5 mg/kg for 4 days) without TBI, but secondary graft failure in 2 of these patients alerted us to a possible higher risk of graft rejection when we used bone marrow instead of peripheral blood cells as the stem cell source. In this study, we demonstrated that the addition of 4 Gy of TBI to the widely applied combination of Flu (30 mg/m^2 for 6 days) and BU (4 mg/kg for 2 days) reduces the risk of graft failure and enables the rapid achievement of full donor chimerism without donor lymphocyte infusion (DLI) and that the regimen-related toxicity was acceptable. Nevertheless, a relatively high incidence of nonrelapse mortality was observed. We lost 4 patients who developed extensive chronic GVHD and subsequent pulmonary complications in the later phase, more than 6 months after transplantation. Because many patients develop extensive GVHD, we assume that the pulmonary complications were primarily due to GVHD and not the consequence of our reduced-intensity stem cell transplantation (RIST) regimen incorporating 4 Gy of TBI. However, Deeg et al reported that more pulmonary compli-

Table 3.
Maximum Toxicities (N = 17)*

Grade	Cardiac, n	Mucositis, n	GI, n	Hepatic, n	CNS, n	Hyponatremia, n	Pulmonary, n	Renal, n
0	12	0	9	1	16	6	11	15
I	4	0	3	2	0	7	2	0
II	0	2	4	7	0	0	0	2
III	1	15	1	5	1	4	4	0
IV	0	0	0	2	0	0	0	0

*GI indicates gastrointestinal tract; CNS, central nervous system.

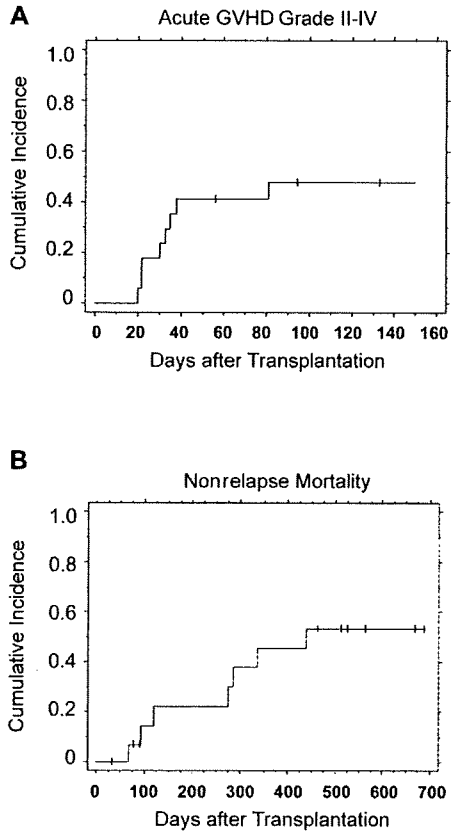

Figure 2. Cumulative incidence of acute GVHD (grades II-IV) (A) and nonrelapse mortality (B) after unrelated bone marrow transplantation following reduced-intensity conditioning. Acute GVHD (grades II-IV) was diagnosed in 8 patients (48%) (grade II in 3 patients and grade IV in 5) at a median of day 32 (range, day 20-81). The estimated 100-day and 1-year nonrelapse mortality rates were 14% and 46%, respectively.

cations developed in patients with aplastic anemia who received 4 to 6 Gy of TBI in combination with cyclophosphamide/antithymocyte globulin for unrelated-donor BMT than in patients who received 2 Gy TBI [20]. These investigators recommended that a 2-Gy TBI dose is sufficient to allow stable engraftment without increased toxicities, and this proposal should be evaluated in future studies. On the other hand, Maris et al described a nonmyeloablative conditioning regimen consisting of 2 Gy TBI and Flu (90 mg/m^2) for unrelated-donor HSCT [8]. In their study, the use of bone marrow rather than G-CSF–mobilized peripheral blood cells as the source of hematopoietic stem cells led to a lower engraftment rate (56% versus 85%), as well as lower rates of overall survival (33% versus 57%) and progression-free survival (17% versus 44%). Because bone marrow is currently the only stem cell source available from volunteer donors in Japan, we may need a more intensified regimen than the combination of 2 Gy TBI and 90 mg/m^2 Flu.

In this study, the rates of acute GVHD of grades II to IV and extensive chronic GVHD in patients who survived for more than 100 days were 48% and 90%, respectively. Grade IV acute GVHD was the primary cause of death in 2 patients. Moreover, the quality of life of patients who develop extensive chronic GVHD rapidly deteriorates, particularly in elderly patients. Although CsA was tapered from a median of day 120 in this series, it might be better to delay the start of CsA tapering in elderly patients, who are associated with higher GVHD rates. Studies have incorporated in vivo T-cell depletion through the addition of antithymocyte globulin or alemtuzumab in order to reduce the risk of GVHD [21-26]. In the study reported by Chakraverty et al, severe GVHD following RIST from an unrelated donor was decreased with in vivo use of alemtuzumab in the preparative regimen [23]. In their study, the rates of acute GVHD (grades II to IV) and chronic GVHD were 21% and 8%, respectively. The long half-life of alemtuzumab (15-21 days) may disturb the induction of full donor chimerism, however. If patients cannot achieve full donor chimerism, the usual option is DLI, which carries a risk of GVHD [26]. Moreover, lymphocytes for DLI are not always available for every patient, particularly in unrelated-donor transplantation settings. In this regard, we think that a regimen that routinely involves DLI after transplantation cannot be considered a universal strategy. In the present study, 2 patients who had

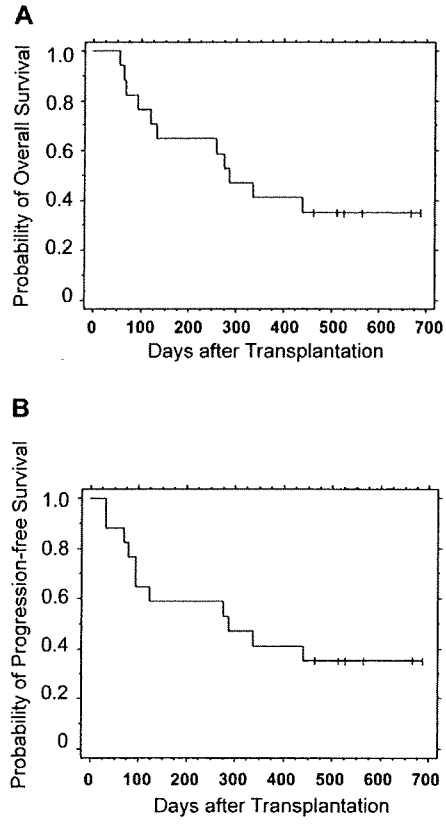

Figure 3. Kaplan-Meier actuarial probability of overall survival (OS) (A) and progression-free survival (PFS) (B) after unrelated-donor bone marrow transplantation following reduced-intensity conditioning. The median follow-up was 286 days (range, 56-687 days). The 1-year OS and PFS rates were both 41%. All 6 of the surviving patients (2 in remission and 4 not in remission at transplantation) remain in remission.

secondary graft failure did not receive DLI, because of grade IV acute GVHD in 1 patient and a reduced performance status in the other. Another approach to preventing severe GVHD is the use of novel immunosuppressive regimens. Several combinations of agents for GVHD prophylaxis, including CsA/mycophenolate mofetil [8,14,16] and tacrolimus/methotrexate [10,15,27], have been reported previously, and their value should be tested in prospective trials.

The induction of adequate antileukemic activity is another primary concern with a RIST procedure, particularly for patients with refractory diseases. de Lima et al reported a promising regimen that consisted of once-daily intravenous BU (130 mg/m^2 for 4 days) and Flu (40 mg/m^2 for 4 days) for patients with AML or MDS [27]. Replacement of oral BU with an intravenous preparation may result in an improved toxicity/survival profile. In our series, 4 patients achieved remission after RIST, although they were not in remission at the time of transplantation. Hence, it is likely that the antileukemic effect exerted by 4 Gy TBI in combination with Flu and BU is valuable even for the immediate control of leukemic blasts, although this possibility needs to be confirmed in further studies. The use of DLI has allowed the rescue of relapsed patients after allogeneic HSCT. In this study, however, we did not give DLI to 4 patients with progressive or relapsed diseases after transplantation because the relevance of the graft-versus-leukemia effect in rapidly proliferating diseases was not fully established and 2 of the patients had developed acute GVHD.

In conclusion, our regimen of 4 Gy TBI, Flu (180 mg/m^2), and BU (8 mg/kg) was effective in reducing the risk of graft failure following unrelated-donor transplantation. We confirmed, however, that a high incidence of nonrelapse mortality, primarily due to GVHD and/or pulmonary complications, still remains a major obstacle for the wider application of this procedure to elderly or medically infirm patients. Further studies to identify ways to ameliorate transplantation-related toxicities are urgently required.

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Tacrolimus as Prophylaxis for Acute Graft-Versus-Host Disease in Reduced Intensity Cord Blood Transplantation for Adult Patients With Advanced Hematologic Diseases

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Background. Myeloablative cord blood transplantation (CBT) for adult patients offers a 90% chance of engraftment with a 50% rate of transplant-related mortality, mostly attributable to infection. We have demonstrated the feasibility of reduced-intensity CBT (RI-CBT) for adult patients, in which cyclosporine was used for acute graft-versus-host disease (GVHD) prophylaxis. Transplantation-related mortality (TRM) was 27% within 100 days. Therefore our objective was to evaluate the feasibility of RI-CBT with tacrolimus as GVHD prophylaxis for adult patients with hematologic malignancies.

Methods. Thirty-four patients with a median age of 56.5 years (range; 22–68) with hematologic diseases underwent RI-CBT at Toranomon Hospital between November 2003 and September 2004. Preparative regimen comprised fludarabine 25 mg/m2 on days −7 to −3, melphalan 80 mg/m2 on day −2, and 4 Gy total body irradiation on day −1. GVHD prophylaxis was continuous intravenous infusion of tacrolimus 0.03 mg/kg, starting on day −1.

Results. Thirty-one patients achieved neutrophil engraftment at a median of day 20. Median infused total cell dose was 2.4×10E7/kg (range; 1.6–4.8). Thirty-two patients achieved complete donor chimerism at day 60. Grade II–IV acute GVHD occurred in 45% of patients, with a median onset of day 26. Primary disease recurred in five patients, and TRM within 100 days was 12%. Estimated 1-year overall survival was 70%.

Conclusion. This study demonstrated the possible improvement in transplant-related mortality by tacrolimus as GVHD prophylaxis in adult RI-CBT recipients.

Keywords: Tacrolimus, Acute graft-versus-host disease, Reduced intensity cord blood transplantation.

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Cord blood transplantation (CBT) represents an attractive alternative for patients with hematologic diseases who lack matched related or unrelated donors. The value of CBT using myeloablative preparative regimens has been confirmed for pediatric and adult patients (*1–5*). Myeloablative CBT for adult patients offers a 90% chance of engraftment with a 50% rate of transplant-related mortality, mostly attributable to infection (*1–4, 6*).

We and other groups have demonstrated the feasibility of reduced-intensity CBT (RI-CBT) for adult patients, in which cyclosporine was used for acute graft-versus-host disease (GVHD) prophylaxis (*7, 8*). Transplantation-related mortality (TRM) was 27% within 100 days (*7*). Posttransplant immune disorders including pre-engraftment immune reactions (PIR) and acute GVHD were problematic in RI-CBT for adult patients (*7, 9*). These reactions and/or additional immunosuppressive therapy might have increased the risk of infection and/or organ dysfunction, leading to a high TRM (*10, 11*).

We employed tacrolimus for acute GVHD prophylaxis in place of cyclosporine from November 2003 in RI-CBT. It might reduce the incidence and severity of PIR and acute GVHD. We will summarize the results of RI-CBT using tacrolimus to investigate its safety and efficacy for acute GVHD prophylaxis after RI-CBT.

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MATERIALS AND METHODS

Study Patients

The study population consisted of 34 adult patients with hematologic disorders, who underwent a protocol of RI-CBT with GVHD prophylaxis using tacrolimus alone at Toranomon Hospital between November 2003 and September 2004. All of the patients were incurable with conventional treatments and were considered inappropriate for conventional allogeneic stem-cell transplantation (allo-SCT) due to the lack of an human leukocyte antigen (HLA)-identical sibling or a suitable unrelated donor, age >50 years old, and/or organ dysfunction. Patients with no suitable HLA-matched related donor were eligible for this protocol if a matched unrelated bone marrow donor was unavailable as a first treatment option. If there was insufficient time for an unrelated bone marrow donor search due to rapidly progressive disease or if the preliminary search indicated a low possibility of obtaining a matched unrelated bone marrow donor, we attempted to locate cord blood grafts. Patients who received RI-CBT as second allo-SCT were excluded from this study. The clinical protocol was approved by the Institutional Review Board of Toranomon Hospital, and written informed consent was obtained from all patients in accordance with the Declaration of Helsinki.

HLA Typing and Donor Matching

HLA-A and HLA-B antigens were determined by serologic typing. HLA-DRB1 alleles were identified by high-resolution molecular typing using polymerase chain reaction method (PCR) with sequence-specific primers (SSP). All cord blood grafts were evaluated by HLA-A, HLA-B, and HLA-DRB1 typing and nucleated cell counts. Preferred cord blood units were those matched to 4 or 6 of 6 HLA loci and contained at least a cell count of 2×10E7 nucleated cells/kg of recipient body weight before freezing. All cord blood units came from cord blood bank in the Japan Cord Blood Bank Network (*12*).

Preparative Regimen and GVHD Prophylaxis

Preparative regimen comprised fludarabine 25 mg/m^2 on days −7 to −3, melphalan 80 mg/m^2 on day −2, and 4 Gy total body irradiation (TBI) in two fractions on day −1 (*7*). GVHD prophylaxis was continuous intravenous infusion of tacrolimus 0.03 mg/kg, starting on day −1. Once oral intake could be tolerated, patients were administered oral tacrolimus at a dose ratio of 1:2–3, in two divided doses based on the latest intravenous dose. In the absence of GVHD, it was tapered from day 100 until day 150. Tacrolimus was reduced when serum creatinine levels were elevated above 1.5 times baseline or other serious adverse effects occurred. If grade II–IV acute GVHD developed, methylprednisolone at a dose of 1 to 2 mg/kg was added to tacrolimus.

Supportive Cares

All of the patients were managed in reverse isolation in laminar airflow-equipped rooms and received trimethoprim/sulfamethoxazole for *Pneumocystis carinii* prophylaxis. Fluoroquinolone and fluconazole were administered for prophylaxis of bacterial and fungal infections, respectively. Prophylaxis of herpes virus infection with acyclovir was also given. Neutropenic fever was managed according to the guidelines (*13*). Cytomegalovirus (CMV) pp65 antigenemia was monitored once a week. If positive results were identified, preemptive therapy with foscarnet was initiated. Hemoglobin and platelet counts were maintained at >7 g/dL and 10×10E9/L, respectively, with in-line filtered and irradiated blood transfusions. All the patients received granulocyte colony stimulating factor (G-CSF) at a dose of 5 μg/kg intravenously, starting on day 1 until durable neutrophil recovery was achieved.

Assessment of Engraftment, GVHD, Regimen-Related Toxicity (RRT) and Survival

Engraftment was defined as the first of two consecutive days in which white blood cell counts >1.0×10E9/L or the absolute neutrophil counts (ANC) >0.5×10E9/L. The date of platelet recovery was defined as the first of seven consecutive days during which the nontransfused platelet count was at least 20×10E9/L.

Chimerism was assessed using fluorescent in situ hybridization in sex-mismatched donor-recipient pairs. In sex-matched pairs, PCR for variable number of tandem repeats was used with donor cells detected at a sensitivity of 10% (*14*). Whole blood and CD3-positive cell chimerism was assessed at the time of granulocyte engraftment. When engraftment was delayed, chimerism was assessed at least once during life.

Primary graft failure was defined as peripheral cytopenia and marrow hypoplasia occurring later than day 60, without detection of donor markers by cytogenetic and/or molecular techniques (*15*). Late graft failure was defined among the patients who attained neutrophil engraftment as a decline of ANC to less than 0.5×10E9/L for at least seven consecutive days with evidence of severe hypocellularity of bone marrow confirmed by histopathological examination. PIR was diagnosed as reported previously (*9*). When febrile patients (body temperature ≥38 °C) with no evidence of infection or adverse effects of medication exhibited skin eruption, diarrhea, jaundice (serum total bilirubin >2.0 mg/dL) or body weight gain >10% of baseline, these changes were defined as immune reactions. Immune reactions developing ≥6 days before engraftment were defined as PIR. Acute and chronic GVHD were diagnosed and graded according to standard criteria (*16*, *17*). GVHD was clinically diagnosed in combination with skin or gut biopsies after engraftment or attainment of 100% donor chimerism. All the patients who had evidence of donor cell engraftment were considered to be evaluable for acute GVHD. Response to corticosteroid was evaluated according to the report by Martin et al. (*18*). Chronic GVHD was evaluated in patients who survived without relapse or disease progression for at least 100 days after transplantation. Patients with hematologic malignancies in complete remission (CR) at the time of transplant, in chronic phase of chronic myelogenous leukemia (CML), with refractory anemia (RA), or refractory anemia with ringed sideroblasts (RARS) of myelodysplastic syndrome (MDS) and with nonmalignant disease were defined as being at standard risk. The other patients were defined as being at high risk. Chemotherapy resistance was defined as relapse after initial cytotoxic chemotherapy or failure to achieve remission.

Overall survival (OS) was applied to all the patients and

measured from the date of transplantation (day 0) to the date of death from any cause. Event-free survival (EFS) was evaluated in patients who survived in remission for at least 30 days after transplantation and measured from the date of transplantation to the date of relapse or death. Patients who have never achieved CR after transplant were considered to have had a recurrence on day 0. Surviving patients were censored at the time of the last follow-up. RRT was defined as any nonhematologic organ dysfunction from day 0 to day 28 and was graded according to the Bearman's criteria (*19*). Toxicities of foscarnet were evaluated using the Common Terminology Criteria for Adverse Events version 3.0 (*20*). TRM was defined as death without the primary disease progression.

Endpoints and Statistical Analysis

Data were analyzed as of December 2005. The primary end point of this study was to evaluate the feasibility of GVHD prophylaxis using tacrolimus in the setting of RI-CBT. The secondary end points were to assess the incidences of RRT, acute and chronic GVHD, infections, EFS and OS. Cumulative incidences were estimated for engraftment, achievement of donor chimerism, acute GVHD and cytomegalovirus reactivation to take account of a competing event (e.g., death) (*21*). Gray's test was used to compare different cumulative incidence curves (*21*). The probabilities of EFS and OS were estimated from the time of transplantation, according to the Kaplan-Meier product limit method. Log-rank test was used to compare these two outcomes. The following patient or transplant characteristics (baseline factors) were analyzed using a Cox regression model for their prognostic value on EFS and OS: patient age, sex, performance status, disease risk, previous history of autologous stem-cell transplantation (yes/no), disparity of HLA-A, -B, -DR antigen (one/two antigen mismatched), number of infused nuclear cells and number of CD34-positive cell dose. Stat View 5.0, Statistical Analysis System (SAS; SAS Institute Inc., Cary, NC) and S Plus 2000 (Mathsoft, Seattle, WA) were used for all statistical analyses.

RESULTS

Characteristics of the Study Patients

Patient characteristics and cord blood grafts are summarized in Table 1. Median age was 56.5 years (range, 22–68), and median weight was 57 kg (range, 40–75 kg). Twenty-two patients were refractory to cytotoxic chemotherapies. Another nine patients had chemosensitive diseases including acute myeloid leukemia (AML) in second CR (n=4), CML in second chronic phase (n=2), adult T-cell leukemia in partial remission (n=2), and malignant lymphoma in second CR (n=1). Two patients who had AML with prior MDS (n=1) had not received pretransplantation chemotherapy. The remaining patient had transfusion-dependent severe aplastic anemia.

TABLE 1. Patient characteristics

Variables	N or median (range)
Total patients	34
Sex (male/female)	
Male	11
Female	23
Age, median years (range)	56.5 (22–68)
Weight, mean kg (range)	57 (40–75)
Underlying diseases (n)	
Acute myeloid leukemia	13[a,b]
Myelodysplastic syndrome	3[c,d]
Acute lymphoblastic leukemia	3[e,f]
Adult T-cell leukemia	6[g]
Chronic myeloid leukemia	3[h]
Malignant lymphoma	5[i]
Severe aplastic anemia	1
Risk of underlying diseases	
High	26
Low	8
Previous history of autologous stem-cell transplantation	
Yes	2
No	32
HLA mismatches	
One	40
Two	30
Number of infused nuclear cells/kg, median (range)	2.4 (1.6–4.8)

Patients with hematologic malignancies in complete remission at the time of transplant, in chronic phase of chronic myelogenous leukemia, with refractory anemia or refractory anemia with ringed sideroblasts of myelodysplastic syndrome, and with nonmalignant diseases were defined as being at standard risk. The other patients were defined as being at high risk.

[a] Three patients were in the second complete remission. The primary diseases were refractory to chemotherapy in 8 patients. The remaining 2 patients had not received prior chemotherapy.

[b] Data of Chromosomal abnormalities were available in 10 patients. Those revealed normal karyotype (n=8) and complex karyotype (n=2).

[c] The primary diseases were refractory to chemotherapy in 2 patients. The remaining 1 patient had not received prior chemotherapy.

[d] Data of chromosomal abnormality was available in 1 patient. It revealed 47XY, +21 [1], 46XY [29].

[e] The primary diseases were refractory to chemotherapy in all the 3 patients.

[f] Chromosomal abnormalities reveals t (9; 22) (q34; q11) and 45 X, -Y [4], 69, XXY [1], 46, XY [14] (n=1).

[g] The primary diseases were refractory to chemotherapy in 5 patients. The remaining 1 patient received retransplantation due to graft failure of reduced-intensity cord blood transplantation.

[h] The disease status of those patients were the second chronic phase (n=1), accelerated phase (n=1), and blast crisis (n=1).

[i] One patient was in complete remission. The primary diseases were refractory to chemotherapy in the remaining 4 patients.

Engraftment

Thirty-one patients achieved neutrophil engraftment at a median of day 20 (range, 12–33). Platelet engraftment was achieved in 27 patients, at a median of day 38 (range, 24–216). All of the three patients without primary engraftment died at a median of day 26 (range, 20–34) due to sepsis (n=2) and intracranial hemorrhage (n=1). Neither primary nor late graft failure was diagnosed in any of the 34 patients.

TABLE 2. Prognostic factors of neutrophil engraftment and 100% donor chimerism

	N	Percent (95% CI)	*P* value
Neutrophil engraftment			
Variable			
Total cell dose			0.010
≥3×10E7/kg	7	100 (68–100)	
<3×10E7/kg	27	89 (69–100)	
HLA disparities			0.0097
HLA 5/6 match	4	100 (64–100)	
HAL 4/6 match	30	90 (71–100)	
100% donor chimerism			
Total cell dose			0.25
≥3×10E7/kg	7	100 (68–100)	
<3×10E7/kg	27	93 (72–100)	
HLA disparities			0.96
HLA 5/6 match	4	100 (64–100)	
HAL 4/6 match	30	93 (75–100)	

TABLE 3. Regimen-related toxicity according to Bearman's criteria

Grade	0	I	II	III	IV
Central nervous system	32	0	1	0	1
Lung	33	0	0	1	0
Kidney	28	4[a]	2[a]	0	0
Liver	22	10[b]	2[b]	0	0
Heart	34	0	0	0	0
Gut	10	19	5	0	0

[a] Median serum creatinine level: 1.2 mg/dL (range, 0.9–1.8).
[b] Median serum aspartate aminotransferase and alanine aminotransferase levels of those patients were 29 IV/L (range 18–274) and 24 IV/L (range 12–593), respectively.

Chimerism Analysis

Chimerism data were obtained from all the 34 patients. Thirty-two patients (94%) achieved complete donor chimerism at day 60. Median time to complete donor chimerism was 22 days (range, 13–38). One patient who died of TRM within 28 days of RI-CBT had complete donor chimerism before neutrophil engraftment. All the surviving patients were monitored for chimerism every 3 months, showing complete donor chimerism during the follow-up even after the discontinuation of immunosuppressants.

No significant association was identified between complete donor chimerism and either infused cell dose or HLA disparity (Table 2).

Cause of Death

Nine patients died during follow-up. Four patients died due to TRM at a median day of 30 (range, 20–46). The remaining five patients died due to the primary disease progression at a median of day 171 (range, 103–203).

Regimen-Related Toxicity and Transplantation-Related Mortality

RRT was shown in Table 3 Grade III–IV RRT developed in two patients. One patient developed fatal intracranial hemorrhage on day 26. TRM within 100 days of RI-CBT was 12% (95% confidence interval [CI], 1–23%). Primary causes of death were sepsis (n=2), encephalitis (n=1), and intracranial hemorrhage (n=1). Three patients developed creatinine level abnormality associated with tacrolimus: grade 1 in one, grade 2 in one, and grade 3 in one patient. Those patients required dose modifications of tacrolimus for toxicities. No patients developed tacrolimus-associated hypertension, diabetes, neurotoxicity, or microangiopathy.

Pre-Engraftment Immune Reactions

Fifteen of the 34 patients (44%; 95% CI, 27–61%) developed PIR. PIR was treated supportively without corticosteroid in all the patients.

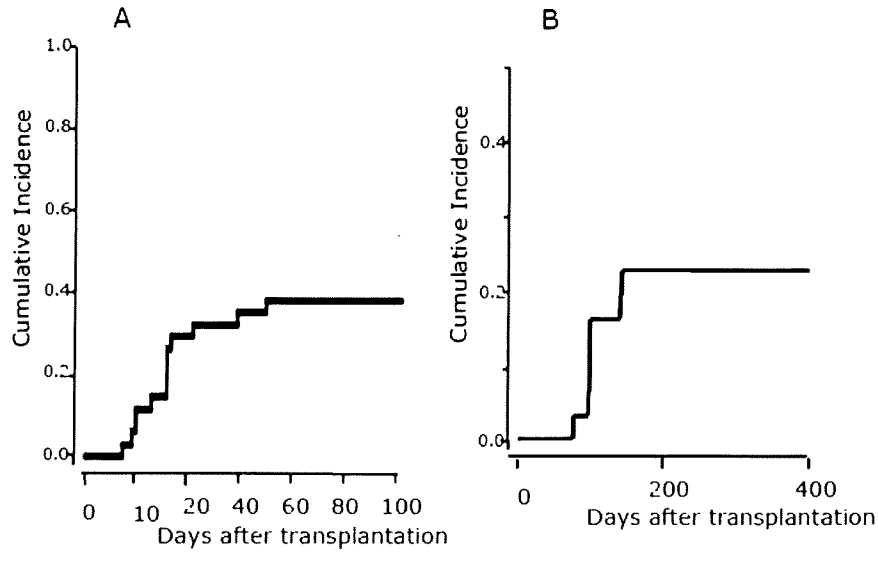

FIGURE 1. (A) Cumulative incidence of grade II–IV acute GVHD. Fourteen of 31 patients (45%; 95% CI, 28%–63%) who achieved primary engraftment developed grade II–IV acute GVHD. (B) Cumulative incidence of chronic GVHD. Seven of the evaluable 26 patients (27%) developed chronic GVHD.