Unrelated or mismatched related donor stem cell transplantation for myeloid malignancies with a fludarabine- and busulfan-based reduced-intensity conditioning regimen

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SUMMARY

Many patients with myeloid malignancies are ineligible for conventional allogeneic hematopoietic stem cell transplantation (allo-HSCT) with myeloablative conditioning because of their age, medical problems, or the lack of a suitable HLA-identical related donor. The outcome of 9 patients with myeloid malignancies who underwent allo-HSCT following fludarabine- and busulfan-based reduced intensity conditioning (RIC) from unrelated or mismatched related donor is herein described. Six patients received bone marrow from an unrelated donor, two received a serologically mismatched related allograft, and one received serologically mismatched unrelated cord blood. All patients demonstrated successfully engraftment at a median of 15 days and showed complete donor chimerism of 95% on day 28. One patient developed grade 3 regimen-related toxicity. Grade II to IV acute graft-versus-host disease (GVHD) and extensive type chronic GVHD developed in 6 and 4 patients, respectively. Of the two patients who received the second HSCT, one died of relapse and one died of acute GVHD. The remaining 7 patients are currently alive in remission. The overall survival at 4 years after allo-HSCT was 77.8%. These observations suggest that these RIC regimens enable rapid engraftment of stem cells from alternative donors with tolerable toxicities in patients who are not eligible for conventional HSCT.

Key words: reduced-intensity conditioning, myeloid malignancies, fludarabine, busulfan, TBI

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伊勢美樹子\(^2\)、中世古知昭\(^1\)、阿部大二郎\(^1\)、小田佳世\(^1\)、大和田千恵子\(^1\)、小澤真一\(^1\)、武内正博\(^1\)、堤田恵美子\(^1\)、清水直美\(^1\)、増田真一\(^1\)、原 龍恒\(^1\)、西村幸樹\(^1\)、齋藤 康\(^1\)；骨髄系悪性腫瘍に対する用量減量前処置による非血球者間及びHLA不適合血縁者間間種造血幹細胞移植.

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I. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) has become established as one of the curative therapies for hematologic malignancies and other hematologic or immunologic disorders, especially for myeloid malignancies due to their graft-versus-leukemia effect[1]. The best results have been obtained in younger recipients who underwent allo-HSCT from HLA-identical sibling donors because of the increased risk of regimen-related toxicities (RRT) that occurs with increasing age, and graft-versus host disease (GVHD) that depends on HLA disparity. Older patients, however, constitute a large group of patients with an extremely poor prognosis if treated with conventional chemotherapy. Most of these patients are ineligible for conventional allo-HSCT with myeloablative conditioning because of age, medical problems other than leukemia, or the lack of a suitable HLA-identical related donor.

The development of nonmyeloablative or reduced-intensity conditioning (RIC) regimens has enabled older or medically infirm patients with hematologic malignancies to be treated with allo-HSCT[2-6]. These regimens rely largely on graft-versus-tumor effects rather than high-dose therapy to eliminate residual malignant cells. Several studies have demonstrated a sustained engraftment with a relatively low risk of RRT and non-relapse mortality. The most widely applied combinations of the RIC regimens consist of fludarabine (Flu) plus alkylating agents (busulfan (BU), cyclophosphamide (CY), or melphalan)[2,3,5], which are chosen for their immunosuppressive and anti-neoplastic capacities, respectively, or fludarabine plus low-dose total body irradiation (TBI)[4,7].

Recently, allo-HSCT from an alternative donor has been explored for patients who lack a suitable HLA-identical sibling donor. It involves allo-HSCT from HLA-matched unrelated donor (UR-BMT), HLA-mismatched related donor, or cord blood transplantation (CBT). Some reports indicate that the outcomes from alternative donors are comparable to that from HLA-identical siblings using a myeloablative regimen [8,9]. However, few data are presently available for allo-HSCT from alternative donors with RIC regimens for elderly or infirm patients.

This study reports the outcome of 9 patients with myeloid malignancies who underwent either unrelated or mismatched related donor allo-HSCT following a Flu and BU-based RIC regimen between July 2003 and May 2007.

II. Patients and Methods

Patient Characteristics

Between July 2003 and May 2007, 9 patients with myeloid malignancies underwent allogeneic HSCT with a Flu and BU-based RIC regimen. Transplantation candidates with RIC regimen were required to have at least one of the following risk factors: (1) an age older than 55 years, (2) comorbidity, and (3) treatment failure after intensive therapies including allo-HSCT with a myeloablative conditioning regimen. The characteristics of the patients at the time of transplantation are summarized in Table 1. The median age of the patients was 55 years (range, 45 to 60 years) with a sex ratio (M/F) of 6/3. The follow-up period ranged from 106 to 1626 days (median, 758 days). Six patients received bone marrow from an unrelated donor, 1 patient received unrelated cord blood, and 2 patients received an allograft from a mismatched related donor (bone marrow in one patient and peripheral blood stem cells in the other). Two patients received the second HSCT with RIC regimen. At the time of transplantation, 3 patients with acute myelogenous leukemia (AML) and 2 with myelodysplastic syndrome...
(MDS) were in complete remission (CR). One patient with chronic myelogenous leukemia (CML) was in first chronic phase (CP). Of the remaining 3 patients, two had refractory MDS, and one had CML in an accelerated phase (AP). Both patients with CML were refractory to imatinib.

**HLA compatibility**

Donor-recipient pairs were selected on the basis of serologic matching for HLA-A, B, and DR antigens. A search for unrelated donors was made through the Japan Marrow Donor Program (JMDP) for patients without suitable HLA-matched related donors. If no appropriate related or unrelated donor was identified, then the Japanese Cord Blood Bank Network (JCBBN) was searched. A cord blood graft was selected on the basis of serologic matching for 4 to 6 antigens at HLA-A, B, and DR loci with a total cell dose of greater than 2x10^7/kg. For DRB1 and some A and B loci, high-resolution allele-specific DNA typing was performed. Six patients had an unrelated donor selected through the JMDP. 3 donor-recipient pairs of them had an allele-level single mismatch (HLA-A locus, two pairs; DRB1 locus, one pair). One patient had an unrelated donor selected through the JCBBN, which was mismatched for two antigens (HLA-A and B loci). The remaining two patients had a related donor; one patient received a single antigen (HLA-B locus) mismatched bone marrow and the other received two antigens (HLA-B and DR loci) mismatched peripheral blood stem cells.

**Conditioning regimens**

The conditioning regimen consisted of 30 mg/m^2 Flu intravenously daily from days -7 to day -2 (total dose 180 mg/m^2), 4 mg/kg BU
orally daily on days -5 and -4 (total dose 8 mg/kg), and 4 Gy TBI on day -1 (single dose or 2 divided doses). Two patients (Nos. 8 and 9), who had undergone their first allo-HSCT with a TBI-containing myeloablative regimen, received the following non-TBI regimen, respectively: (1) 30 mg/m² Flu intravenously daily from days -8 to day -4 (total dose 150 mg/m²) and 4 mg/kg BU orally daily on days -5 and -4 (total dose 8 mg/kg), (2) 30 mg/m² Flu intravenously daily from day -7 to day -2 (total dose 180 mg/m²), 4 mg/kg BU orally daily on days -5 and -4 (total dose 8 mg/kg), and 80 mg/m² melphalan intravenously on day -2.

Prophylaxis and treatment of GVHD

The patients received a combination of cyclosporine A (CsA) and short course of methotrexate (MTX) as prophylaxis for GVHD. MTX was administered intravenously at a dose of 10 mg/m² on day 1 and a dose of 7 mg/m² on days 3 and 6. CsA was administered at a dose of 3 mg/kg/day from day -1 as a continuous intravenous infusion adjusted to maintain a trough level of 350 to 400 ng/ml. Two patients received a combination of tacrolimus and the same dose and schedule of MTX because of an allograft from a serologically single antigen mismatched related donor or an allele-level single mismatched unrelated donor. Tacrolimus was administered at a dose of 0.025 mg/kg/day from day -1 as a continuous intravenous infusion adjusted to maintain a trough level of 15 to 20 ng/ml. One patient (No. 8) who underwent the second HSCT from the same donor in a previous allo-HSCT, received CsA alone. The intravenous administration of CsA or tacrolimus was substituted by the oral administration when tolerated. CsA or tacrolimus was tapered 5% per week after day 50 if the patient had no GVHD. Acute and chronic GVHD were evaluated in patients who had survived at least 100 days with sustained engraftment. Acute GVHD was treated primarily with 1-2 mg/kg of prednisolone (PSL). Extensive chronic GVHD was treated primarily with PSL and CsA/tacrolimus.

Engraftment, a chimerism analysis and survival

The day on which granulocyte engraftment was achieved was defined as the first of 3 consecutive days on which the patient achieved a granulocyte count of greater than 0.5 x 10⁹/L. The day on which platelet engraftment was achieved was defined as the first of 7 consecutive days with a platelet count of greater than 50 x 10⁹/L without platelet infusion. A chimerism analysis was performed on the bone marrow at four weeks interval by variable number of tandem repeat (VNTR) single nucleotide polymorphism analysis using the polymerase chain reaction. The disease-free survival (DFS) and overall survival (OS) of the patients after allo-HSCT was analyzed by the Kaplan-Meier method.

Supportive Care

Antimicrobial prophylaxis included levofloxacin for bacterial infection, fluconazole for fungal infection, trimethoprim/sulfamethoxazole for Pneumocystis carinii infection, and acyclovir for Herpes virus infection according to the institutional protocol. Monitoring for cytomegalovirus (CMV) antigenemia was performed once a week after the engraftment, with ganciclovir therapy started if positive results were obtained.

III. Results

Engraftment and Chimerism analysis

The clinical outcomes for all patients are summarized in Table 2. All patients demonstrated a successful sustained
engraftment. Granulocyte engraftment was achieved at a median of 15 days (range, 14 to 22 days), and platelet engraftment was achieved at a median of 25 days (range, 13 to 35 days) after the HSCT. A chimerism analysis confirmed more than 95% donor-type engraftment by day 28 in all patients. In addition, no patient developed either primary or secondary graft failure.

**Acute and chronic GVHD**

Five patients developed grade II to III acute GVHD (related marrow transplantation, \( n = 1 \); unrelated marrow transplantation, \( n = 4 \)). One patient (No. 9) who underwent the second HSCT for secondary MDS after the previous allo-HSCT developed grade IV acute GVHD. All patients survived at least 100 days after the HSCT. Among them, five patients developed chronic GVHD (one patient with limited and four patients with extensive GVHD). The affected organs included the skin (\( n = 4 \)), mouth (\( n = 3 \)), liver (\( n = 2 \)), and eye (\( n = 1 \)).

**Regimen-related Toxicities and Infections**

RRT was defined as any non-hematologic organ dysfunction from day 0 to day 28 according to Bearman et al.'s criteria[12].
The RIC regimens were well tolerated, and the degree of toxicity was mild. Hemorrhagic cystitis by Adenovirus type 11 was observed in one patient. One patient (No. 9) developed grade 3 thrombotic microangiopathy (TMA) [13]. CMV antigenemia was detected in 3 patients, of whom one patient developed CMV pneumonia which was successfully treated with ganciclovir. Enterocolitis due to Clostridium difficile developed in two patients.

**Relapse, non-relapse mortality and survival**

One patient (No. 8) with MDS who underwent the second HSCT from the same donor in previous allo-HSCT relapsed on day 125 and died. One patient (No. 9) died of acute GVHD on day 106. As of December 2007, all other seven patients are alive in CR with full donor chimerism at a median follow-up of 807 days (range, 124 to 1626 days). Both the DFS and OS of the patients at 4 years after allo-HSCT were 77.8% (Fig. 1).

### IV. Discussion

Graft failure and non-relapse mortality are major problems after allo-HSCT with an RIC regimen, especially for transplantation from unrelated or mismatched related donors[14]. In the present series, rapid and sustained engraftment with complete donor chimerism was demonstrated in all patients, including one patient who received unrelated cord blood mismatched at 2 HLA-antigens. Previous heavy treatment and a relatively intensive RIC regimen may contribute, in part, to successful engraftment. The low RRT using this RIC regimens is also remarkable. Only one patient developed grade 3 TMA. This patient developed TMA concomitant with acute GVHD after achieving complete donor chimerism, thus suggesting that TMA was primarily due to acute GVHD rather than RRT from the conditioning treatment. No veno-occlusive disease was observed.

Relapse and progressive disease continues to be the major cause of treatment failure after the RIC regimen, particularly in patients with large tumor burdens at time of transplantation [6,15,16]. Refractory or rapidly progressing disease (i.e., high-risk AML or MDS) may be best-controlled using a conditioning regimen with a greater anti-neoplastic intensity[7]. Previous studies have demonstrated that allo-HSCT with RIC regimen can benefit patients with high-risk AML or MDS[15-17]. Despite the fact that the patients in this study were mostly high-risk; two underwent a second HSCT, three had adverse cytogenetics, two were in beyond

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**Fig. 1** Survival of the patients after allo-HSCT with the RIC regimen. 
(A) Disease-free survival (DFS). (B) Overall survival (OS). Both DFS and OS at 4 years post transplantation are 77.8%.
second CR or non-remission; only one patient with refractory MDS (No. 8) relapsed. Because this patient had many anti-HLA antibodies and no HLA-matched related or unrelated donor, she underwent a second HSCT with RIC from the same donor (2-HLA-antigen mismatched daughter) of the initial myeloablative HSCT in complete donor chimerism. She did not experience either acute or chronic GVHD and relapsed on day 125.

Baron et al. demonstrated in their analysis of HLA-matched related or unrelated nonmyeloablative HSCT that chronic extensive GVHD was highly associated with a decreased risk of relapse/progression and significantly better progression-free survival[18]. On the other hand, grade II to IV acute GVHD was not associated with decreased risk of relapse but, rather, caused increased non-relapse mortality and therefore, significantly decreased progression-free survival[18]. In the current series, six out of 9 patients developed grade II to IV acute GVHD, which mainly manifested as skin rashes. One patient (No. 7) who received one allele-mismatched unrelated BMT developed grade III acute GVHD which manifested as gastrointestinal symptoms and Clostridium difficile enterocolitis simultaneously. One patient (No. 9), who underwent the second HSCT with RIC for secondary MDS after the initial myeloablative HSCT, developed severe acute GVHD. Although TMA was successfully managed by plasma exchange, she progressed to grade IV acute GVHD and eventually died from multi-organ failure on day 106. Five out of 9 patients developed chronic GVHD, of whom four had extensive disease. These patients were all successfully managed and did not show a fatal outcome.

Except for 2 patients with a second transplantation, seven patients with their first transplantation are currently alive in remission. This result is very promising for patients with myeloid malignancies who could not receive myeloablative conditioning because of higher age or medical problems and lack of family donor. Despite the small number of patients and the short follow-up period, these observations suggest that Flu and BU-based RIC regimens reduce the risk of graft failure and enable rapid engraftment of complete donor chimerism with tolerable RRT even in unrelated or mismatched related donor HSCT setting. However, these regimens might be inappropriate and not widely applicable for the second HSCT. Further investigations are thus called for.

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要 旨
同種造血幹細胞移植療法の最近の進歩は著しいが，
高齢や器障害，合併症などの理由より，通常の骨
髄破壊的前処置による同種造血幹細胞移植の適応にな
らない患者は未だ多く，さらにHLA適合血縁ドナーを
有する患者は30%以下に限られる。我々は，HLA適合
血縁ドナーを有しない骨髄系悪性腫瘍9症例に対して，
フルラビンとフルスラファンを基本とする用量減量前
処置により，非骨髄者間またはHLA不適合血縁者間
同種造血幹細胞移植を行った。血縁者間骨髄移植が6
例，HLA血清型不適合血縁者間移植が2例，HLA血清
型不適合非血縁者間骨髄移植が1例，全例で移植後
中央値15日日に生者が得られ，28日目にはドナー型が
95%以上の完全キメラとなった。1例でグレード3の
移植関連毒性がみられた。2度以上の急性GVHDを6
例に認めた，全身型の慢性GVHDを4例に認めた。移植
後100日以内の早期死亡はなく，2回目の造血幹細胞移
植であった2症例のうち，1例が再発にて，1例が急
性GVHDにて死亡した。残る7症例は現在も寛解生存
中であり，同種造血幹細胞移植後の4年無病生存率は
77.8%であった。これらの結果から，通常の骨髄破壊的
移植が適応にならず，HLA適合血縁ドナーを有しない
症例に対しても，今回の用量減量前処置を用いた同種
移植によりドナー幹細胞の速やかな生着が得られ，毒
References


