

Early full donor myeloid chimerism after reduced-intensity stem cell transplantation using a combination of fludarabine and busulfan

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Background and Objectives. The aim of this study was to evaluate lineage-specific chimerism reconstitution after reduced-intensity allogeneic stem cell transplantation (RIST) using a combination of fludarabine (30 mg/m² for 6 days) and busulfan (4 mg/kg for 2 days).

Design and Methods. We prospectively enrolled 8 consecutive patients with hematologic malignancies who were not candidates for conventional transplantation because of either high age or organ dysfunction. Host-donor chimerism was evaluated using polymerase chain reaction-based amplification of a polymorphic short tandem repeat region.

Results. All of our patients achieved engraftment within a median of 11 days after transplantation. On day 30, full donor myeloid cell chimerism (>90%) was achieved in 7 patients whereas full donor T-cell chimerism was achieved in only one patient. Thus, in contrast to other reported results, full donor chimerism was achieved earlier in the myeloid lineage than the T-cell lineage. On day 60, however, T-cell chimerism caught up with myeloid chimerism. Two patients developed grade II-IV acute graft-versus-host disease (GVHD) before the detection of full donor T-cell chimerism.

Interpretation and Conclusions. Our findings suggest that the kinetics of lineage-specific chimerism depend on the agents used in the conditioning regimen, and may provide insight into the chimerism kinetics and pathogenesis of GVHD. Thus, the strategy for controlling immunosuppression after RIST

should be modified according to the type of conditioning regimen applied.

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Key words: reduced-intensity stem cell transplantation, chimerism, short tandem repeat, fludarabine, busulfan.

To extend the application of allogeneic hematopoietic stem cell transplantation (HSCT), non-myeloablative or reduced-intensity stem cell transplantation (NST/RIST) has been investigated as a less toxic modality.¹⁻⁴ The anti-tumor activity of the procedure mainly depends on the immune-mediated graft-versus-tumor (GVT) effect. Childs *et al.* reported that full donor T-cell chimerism preceded the appearance of a GVT effect.⁵ Therefore, monitoring the lineage-specific kinetics of chimerism after NST/RIST may be important for optimizing the immune modulation. However, T-cell and myeloid cell reconstitution after a combination of fludarabine and busulfan, one of the most popular RIST regimens,^{2,4} has not yet been reported. Hence, we evaluated serial lineage-specific chimerism after Slavin-type RIST without anti-thymocyte globulin.

Design and Methods

Between October 2000 and February 2001, eight patients (5 men and 3 women) underwent RIST from an HLA-matched sibling donor after a conditioning regimen that included fludarabine (30

mg/m²/day on days -8 to -3) and busulfan (4 mg/kg/day on days -4 and -3). The patients' characteristics and transplant outcomes are summarized in Table 1. All of the patients had hematologic malignancies and their median age was 50 years (range, 29-55 years). Those who were below 50 years old (n=4) had organ dysfunction and were ineligible for conventional transplantation. Continuous intravenous administration of cyclosporin A (3 mg/kg/day) was started from day -1 and tapered off before day 100. Acute GVHD was graded as described by Glucksberg *et al.*⁶

We assessed donor-recipient chimerism by polymerase chain reaction (PCR)-based amplification of a polymorphic short tandem repeat (STR) region. Chimerism was evaluated using heparinized peripheral blood samples taken 30, 60, and 90 days after transplantation. Samples were separated using Ficoll-hypaque into mononuclear cells and a precipitate that included red blood cells and myeloid cells. Mononuclear cells were further separated into CD3-positive and -negative fractions with immunomagnetic beads, using anti-CD3 monoclonal antibody (Miltenyi Biotec, Germany). Myeloid cells were collected by lysing the red blood cells in the precipitate. The PCR-STR assay was performed as previously described by Thiede *et al.*⁷ In brief, DNA was extracted from selected cells using a commercially available kit (Wizard Genomic DNA purification kit, Promega, Madison, WI, USA). Multiplex PCR was performed using primer sets (AmpFISTR, Applied Biosystems, Foster City, CA, USA). Four-color fluorescence detection was performed on an ABI 310 automated DNA sequencer (Applied Biosystems). For each STR allele, the area under the curve for the corresponding signal was automatically processed

using GeneScan 3.1 software (Applied Biosystems). The percentage of donor cells was calculated as (area signal donor) / (area signal donor + area signal recipient).

Results

The median number of infused CD34⁺ cells was 3.7×10⁶/kg (range, 2.9 to 6.5×10⁶/kg). All the patients achieved a neutrophil count > 0.5×10⁹/L within a median duration of 11 days after transplantation (range, 10-15 days). A platelet count > 20×10⁹/L without transfusion was achieved within a median of 11.5 days (range, 10 to 19). Regimen-related toxicities were minimal, but grade II-IV acute GVHD developed in 3 patients, one of whom died. The remaining 7 patients were alive in remission 135-230 days after RIST (median, 204 days). None required donor lymphocyte infusion.

Chimerism analyses of donor T-cells and myeloid cells on days 30, 60 and 90 are shown in Figure 1. On day 30, donor chimerism of myeloid cells was up to 94% (S.D. 10.4), whereas that of T-cells was significantly lower (79%, S.D. 11.1, *p*=0.01 by paired-t test). While 7 of the 8 patients had achieved full donor myeloid chimerism (> 90%) by day 30, only one had > 90% donor T-cells. By day 60, however, T-cell chimerism had caught up with myeloid chimerism, and there was no significant difference between the two lineages.

Discussion

NST/RIST has been investigated in several independent institutions. Therefore, various conditioning regimens have been reported to date and the intensities of these regimens have varied from truly non-myeloablative to a more intensive regi-

Table 1. Patients' characteristics and outcome.

No.	Age/Sex	Diagnosis	Status at transplantation	CD34 ⁺ dose (10 ⁶ /kg)	Days to ANC > 0.5×10 ⁹ /L	Days to Plts > 20×10 ⁹ /L	Acute GvHD	Survival
1	53/F	NHL	1 st CRu	4.7	+11	+11	(-)	CR +230
2	55/M	AML	1 st relapse	2.9	+12	+12	(+) grade II	CR +224
3	29/M	NHL	1 st relapse after APBSCT	3.0	+15	+19	(-)	CR +205
4	55/F	MDS (RAEB)	1 st CR	3.1	+10	+10	(+) grade II	CR +204
5	49/M	ALL	3 rd CR	3.8	+11	+13	(+) grade III	Died of GVHD +72
6	45/F	AML	2 nd CR	6.5	+10	+11	(-)	CR +162
7	46/M	NHL	2 nd CR after APBSCT	4.6	+10	+14	(-)	CR +137
8	51/M	CML	1 st CP	3.6	+13	+11	(-)	CR +135

NHL, non-Hodgkin's lymphoma; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; RAEB, refractory anemia with excess of blasts; CR, complete remission; CRu, uncertain CR; CP, chronic phase; APBSCT, autologous peripheral blood stem cell transplantation; ANC, absolute neutrophil count.

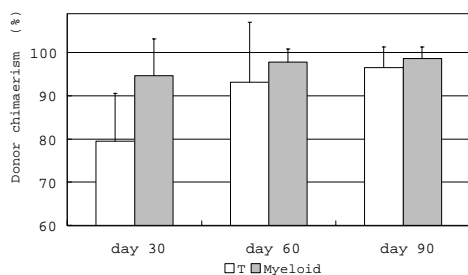


Figure 1. Mean percentages (with standard deviations) of donor T and myeloid cells on days 30, 60 and 90 after reduced-intensity stem cell transplantation. The number of patients studied was 8, 8, and 7 at each time point, respectively.

men that is close to that used in conventional HSCT.^{1-4,8} In addition to fludarabine, which is a mainstay of NST/RIST regimens, some centers use relatively myelosuppressive agents such as busulfan or melphalan, whereas others use immunosuppressive agents including cyclophosphamide.

Although it has been reported that full donor chimerism was achieved early after RIST using a combination of fludarabine and busulfan without anti-thymocyte globulin,⁴ no detailed data are available regarding lineage-specific chimerism after this regimen. In this study, we found that full donor chimerism was achieved earlier in the myeloid lineage than in the T-cell lineage. In contrast, Childs *et al.* showed that full donor T-cell engraftment preceded myeloid engraftment after a conditioning regimen consisting of fludarabine and cyclophosphamide.⁵ This discrepancy may have resulted not from the delay in T-cell engraftment, but from the rapid achievement of donor myeloid engraftment in our series, since 7 of the 8 patients had > 90% donor-derived myeloid cells by day 30 in this study, whereas this was seen in only 4 of the 14 patients reported by Childs *et al.*⁵ Although the method used to collect myeloid cells differed somewhat between the two studies, we suspect that the strong activity of busulfan against myeloid cells suppressed recipient-derived myeloid hematopoiesis,⁹ resulting in the early full donor myeloid chimerism. Moreover, McSweeney *et al.* recently reported an early full donor myeloid chimerism after NST using low-

dose TBI combined with cyclosporin A and mycophenolate mofetil (MMF).⁸ Their conditioning regimen was truly *non-myeloablative* and weaker than that used in this study. Therefore, post-transplant immunosuppression also appeared to affect the induction of chimerism after NST/RIST.

Interestingly, two patients developed grade II-IV acute GVHD before achieving full donor T-cell chimerism, which also conflicts with the observation by Childs *et al.* that the development of acute GVHD was always preceded by full donor T-cell chimerism.⁵ Hence, our observation may provide insight into the chimerism kinetics and pathogenesis of GVHD. Myelo/immuno-reconstitution after NST/RIST largely depends on the agents used in the conditioning regimen. Thus, the strategy used to control GVHD/GVT after NST/RIST should be carefully modified according to the specific type of conditioning regimen applied.

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HN, YK, YT, and SM were primarily responsible for this work, from conception to submitted manuscript. The remaining authors qualified for authorship according to the World Association of Medical Editors criteria, and have taken specific responsibility for the following parts of the content: TS, TO, KY, KU, AC, KI, MO, KN, collection of clinical data; SK, YK, KK, AI, AM, RT, KT, HW, laboratory work.

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Potential implications for clinical practice

As reduced intensity conditioning regimens are increasingly used for allogeneic stem cell transplantation¹⁰⁻¹² evaluation of lineage-specific chimerism may be relevant to understand and control GVHD.

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