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The feasibility of reduced-intensity allogeneic hematopoietic stem cell transplantation from a related donor with HLA one-antigen with or without one-allele mismatch

It is still unclear whether reduced-intensity stem cell transplantation (RIST) from an HLA-mismatched related donor is feasible for hematologic malignancies. In the current study on the use of antithymocyte globulin (ATG) in 13 patients, we focused on this issue by evaluating regimen-related toxicities, engraftment, graft-versus-host disease (GVHD), infection, and overall survival. Our results suggest that this procedure may be acceptable for patients without a matched related donor.

Haematologica 2003;88:115-117
(*http://www.haematologica.org/2003_01/88115.htm*)

A total of 13 patients underwent RIST from a serologically HLA one-locus mismatched related donor between March 2000 and September 2002. The characteristics of these patients are shown in Tables 1 and 2. Both HLA antigen and allele matching were generally evaluated, since any disparity in HLA allele typing was considered to be a risk factor in allogeneic hematopoietic stem cell transplantation from an unrelated donor.[1-3] We defined *HLA one-locus mismatch* as any mismatch of one HLA -antigen, with or without a one-allele mismatch.

The conditioning regimen consisted of cladribine (0.66 mg/kg) or fludarabine (180 mg/m^2), busulfan (8 mg/kg), and rabbit anti-thymocyte globulin (ATG: 5 mg/kg in 2 patients, and 10 mg/kg in 16 patients). Infectious prophylaxis procedures have been described previously.[4] Prophylaxis against GVHD was performed with cyclosporine (CSP) alone in the initial 7 patients. Thereafter, short-term methotrexate (MTX) was added to CSP in the subsequent 6 patients because of the observation of severe acute GVHD in the earlier group. Patients who developed grade II-IV acute GVHD were treated with methylprednisolone at a dose of 1-2 mg/kg/day *iv*. Infectious disease was defined as an illness associated with symptoms and signs consistent with an infection, with microbiological documentation of a pathogen. Microbiological documentation consisted of the isolation of a pathogen by culture from a sterile or non-sterile site, or by histologic or immunohistologic evidence. The primary endpoint of this study was the evaluation of engraftment, defined as >0.5×10^9/L absolute neutrophil count (ANC) or >1.0×10^9/L white blood cell count (WBC), and the toxicities associated with the procedure. The secondary end-points included evaluation of the extent of GVHD and infectious episodes. Differences in incidence were evaluated using Fisher's exact test. Actuarial overall survival was estimated by the Kaplan-Meier method.

We found that all of the patients tolerated our RIST regimen and organ toxicities were limited to less than grade II hepatic and stomatitis/gastrointestinal toxicity, except in one patient (UPN 389) who developed a subdural hematoma. The median number of CD 34$^+$ cells infused was 3.6×10^6/kg (range, 2.2 to 7.3×10^6/kg, Table 1) and the median duration of neutropenia was 12 days (range, 7-20, Table 1). Chimerism analysis was performed on days 30, 60, 90, 120, 180, 240, 300 and 360, and we confirmed that 11 of the 13 patients achieved engraftment from this HLA-mismatched transplantation. This result further suggests that our regimen, incorporating ATG, enables successful engraftment by overcoming the HLA barrier that is limited to HLA one-antigen with or without one-allele, as recently reported by Gajewski *et al*.[5] One patient developed primary graft failure and the rapid emergence of recipient-type hematopoiesis on day 17, suggesting that our regimen is not truly myeloablative, and that the RIST procedure, relative to conventional transplantation with a myeloablative regimen, saves patients by retaining the ability of the marrow space to be repopulated by the recipient's own cells.

Table 1. Patients' characteristics.

UPN	*Sex*	*Age*	*Disease*	*Status at transplant*	*Regimen*	*HLA mismatched locus*	*RRT grade*	*CD34*	*Duration of neutr.*
267	M	29	AML	CR	2CdA/Bu/ATG	DRB1 (antigen)	0	2.23	15
295	M	20	RMS	NR	2CdA/Bu/ATG	DRB1 (antigen)	1(hepatic)	7.29	11
312	M	27	RMS	NR	2CdA/Bu/ATG	B (antigen) + A (allele)	1(hepatic)/2 (stomatitis)	2.8	7
326	F	19	MDS	CR	2CdA/Bu/ATG	B (antigen)	2 (stomatitis)	4.38	11
332	M	15	AML	CR	2CdA/Bu/ATG	B (antigen) + A (allele)	0	2.39	–
333	M	66	MDS	CR	2CdA/Bu/ATG	B (antigen)	0	5.09	–
449	M	54	MDS	NR	Flu/Bu/ATG	B (antigen)	1(hepatic)	2.58	12
384	M	53	MDS	CR	Flu/Bu/ATG	DRB1 (antigen)	0	2.43	11
389	M	58	ALL	CR	Flu/Bu/ATG	B (antigen) + A (allele)	2(CNS)	3.39	13
426	F	54	MDS	NR	Flu/Bu/ATG	B (antigen) + DRB1 (allele)	0	6.98	14
434	M	47	MM	NR	Flu/Bu/ATG	B (antigen) +A (allele) + DRB1 (allele)	1(hepatic)	4.03	11
446	F	57	AML	CR	Flu/Bu/ATG	B (antigen)	0	4.04	20
496	F	24	ARCC	NR	Flu/Bu/ATG	A (antigen)	2 (stomatitis)/1 (gastrointestinal)	3.57	13

UPN: unique patient number; RMS: rhabdomyo sarcoma; MDS: myelodysplastic syndrome; ARCC: adrenal cell carcinoma; CR: complete remission; NR: no remission; CD34: CD34 cell dose×10^6/kg; neutr: neutropenia.

Table 2. Complications and clinical course.

UPN	bacteria	fungus	virus	CMV antigenemia	GVHD proph	aGVHD grade	cGVHD	Rel/Prog	actual status	Cause of death
267	bacteremia	–	–	+	CSP	2 (skin)	limited	471	relapse +471	–
295	–	disseminated candidiasis	–	+	CSP	3 (skin/gut)	NA	35	died +56	GVHD/Infection
312	bacteremia	aspergillus pneumonia	CMV pneumonia/enteritis	+	CSP	4 (skin/gut/liver)	NA	–	died +118	GVHD/Infection
326	bacteremia	cutaneous aspergillosis	–	+	CSP	4 (skin/gut/liver)	NA	–	died +68	GVHD/Infection
332	–	–	–	–	CSP	–	NA	–	graft failure	–
333	septic shock	–	–	–	CSP	–	NA	–	died +8	Infection
449	–	–	–	+	CSP	2 (skin)	extensive	–	CR +329	–
384	bacteremia	–	VZV	+	CSP/MTX	0	none	–	CR +553	–
389	bacteremia	–	–	+	CSP/MTX	2 (skin)	NA	236	died +236	disease
426	–	–	CMV retinitis	+	CSP/MTX	0	limited	–	CR +399	–
434	–	–	–	+	CSP/MTX	0	none	–	CR +367	–
446	–	disseminated candidiasis	–	–	CSP/MTX	0	NA	–	died +29	Infection
496	enterocolitis	–	–	–	CSP/MTX	1 (skin)	limited	–	PD +193	–

proph: prophylaxis; CSP: cyclosporine; aGVHD: acute GVHD; cGVHD: chronic GVHD; NA: not applicable; Rel/Prog: relapse or progression; CR: complete remission.

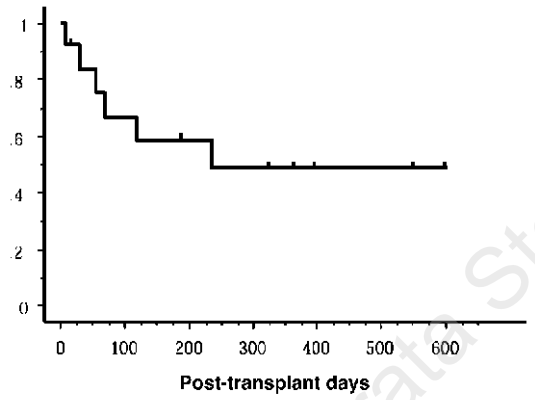

Figure 1. Estimated overall survival.

Another interesting finding of our study was that the incidence of grade II-IV acute GVHD depended on the type of GVHD prophylaxis (CSP alone vs. CSP/MTX;p=0.015, Table 2), but not on additional HLA allele disparity (p=0.99). Furthermore, grade III-IV acute GVHD developed in 3 patients in the CSP-alone group, and was directly related to their mortality. Currently, most GVHD prophylaxis in RIST procedures involves CSP alone when using ATG or CAMPATH-1H.[6,7] However, our observation further supports the recently published notion that GVHD prophylaxis should be intensified in RIST procedures.[8] Although the data are still limited due to the small number of patients and short follow-up period, our results suggest that MTX did not suppress engraftment and was essential for preventing GVHD in RIST, at least from a related donor with HLA one-antigen with or without one-allele mismatch.

Profound immune suppression by the conditioning regimen could contribute to the high incidence of infection (10/13, 77%), and particularly CMV antigenemia (Table 2).

However, this could still be managed by response-oriented pre-emptive therapy guided by the level of CMV antigenemia.[4] Four patients developed serious fungal diseases and subsequently died, and in most of these cases death was attributed to the use of steroid therapy for the treatment of acute GVHD.

Four patients, including 3 who were not in remission at transplantation, are currently alive in CR (Table 2). Overall, the early transplant-related mortality (TRM) on days +100 and +200 was 31% (4/13) and 39% (5/13), respectively. The estimated overall survival was 46%, with a median follow-up of 193 days (range: 8-553 days, Figure 1).

In summary, the results of this study suggest that RIST from a related donor with HLA one-antigen with or without one-allele mismatch could achieve allogeneic engraftment and that GVHD could be adequately prevented by using MTX in addition to CSP. The development of severe acute GVHD led to a high mortality rate due to complicated infections which were induced by the intense use of steroids for the treatment of GVHD.

Kunihisa Nakai, Shin Mineishi, Masahiro Kami, Yoshinobu Kanda, Ryuji Tanosaki, Yoichi Takaue

Stem Cell Transplantation Unit, National Cancer Center Hospital, Tokyo, Japan

Key words: mismatched reduced-intensity stem cell transplantation, engraftment, GVHD.

Funding: this research was supported by a Grant-in-Aid for Scientific Research from the Ministry of Health, Labor and Welfare, Japan.

Correspondence: Yoichi Takaue, M.D., Stem Cell Transplant Unit, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. Phone: international +81.3.35422511. Fax: international +81.3.35423815. E-mail: ytakaue@ncc.go.jp

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received September 26, 2002; accepted November 4, 2002.

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Chimerism-directed adoptive immunotherapy in the prevention and treatment of post-transplant relapse of leukemia in childhood

We present the role of frequent monitoring of hematopoietic chimerism in the prediction of post-transplant relapse and our initial experience with adoptive immunotherapy in the prevention and treatment of hematologic relapse in children after allogeneic hematopoietic stem cell transplantation.

Haematologica 2003;88:117-118
(*http://www.haematologica.org/2003_01/88117.htm*)

Relapse of leukemia remains the major cause of treatment failure in allogeneic hematopoietic stem cell transplantation (HSCT) in children. In a prospective study we used frequent monitoring of hematopoietic chimerism (HC)[1,2] to identify patients with a high risk of post-transplant relapse and thus indicated for adoptive immunotherapy (AI).[3-6] Between January 1997 and June 2001 we performed a total of 54 unmanipulated allogeneic HSCT from HLA-identical siblings (28) or matched unrelated donors (26) in 50 consecutive children with hematologic malignancies in the University Hospital Motol, Prague. Fifty-two evaluable follow-ups from forty-eight patients at a median age of 10 years (2-18 years) with acute lymphoblastic leukemia (ALL; 18/17), acute myelogenous leukemia (AML; 17/14), chronic myelogenous leukemia (CML; 8), myelodysplastic syndrome (MDS; 6) and juvenile myelomonocytic leukemia (JMML; 3) were included in this prospective chimerism study. Written informed consent was obtained from the parents. We analyzed HC in peripheral blood samples using polymerase chain reaction of variable number of tandem repeats (ApoBII, CoI2A1, YNZ22, D1S80, HVR-Ig, TPO) with a maximum sensitivity of 0.5%.[7] Peripheral blood samples were taken weekly from day +8 until day +100, then once a month during the first year following HSCT and thereafter according to clinical and laboratory outcome.[1] Complete donor chimerism (CC), characterized by the disappearance of recipient cells until day +28 and sustained emergence of donor cells was documented in 29/52 follow-ups. Mixed chimerism (MC), characterized by the re-emergence or persistence of recipient cells after day +28, was found in 23/52 follow-ups. Transient MC (trMC) was seen in 9 follow-ups and increasing MC (inMC) in 14 follow-ups. Considering the transplant-related mortality until day +100, 44 follow-ups were evaluable for relapse-free survival (RFS). At a median follow-up of 16.5 months RFS for the CC group was 20/22, while that for the MC group was 11/22. RFS for the trMC group was 7/9 as compared to 4/13 for the inMC group (Figure 1). Two patients with CC (1 ALL, 1 AML) relapsed after transplantation without prior detection of MC; in both extramedullary relapse occurred.

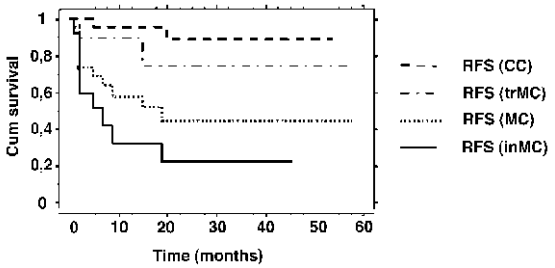

Figure 1. Kaplan-Meier estimates of relapse-free survival for the CC and the MC groups. Forty-four patients were evaluable for RFS. RFS for the CC group was 20/22, whilst that for the MC group was 11/22. RFS for the MC group was 7/9 as compared to 4/13 for that in the MC group.

AI was used in the prevention and treatment of post-transplant relapse in 13 patients/14 follow-ups (ALL 4, AML 5, CML 3, JMML 1/2). Treatment was started on the basis of inMC (9), in molecular relapse[8] (1) or in hematologic relapse (3/4). Withdrawal of post-transplant immunosuppression (IS) was performed in 11 patients, 5 patients with no or only transient response to withdrawal of IS received second-line therapy by donor lymphocyte infusion (DLI). In 3 follow-ups without IS, DLI was applied as a front-line therapy.[4] Doses of $CD3^+$ cells varied between 1×10^5 and 2.4×10^8/kg body weight according to type of donor and indication for DLI. Complete response to AI, defined as sustained recurrence of CC and continuous complete remission (CCR), was documented in 6/14 follow-ups (second post-transplant relapse in a patient with JMML, 3/3 patients with CML, and in only 2/9 patients with acute leukemia) at a median follow-up of 28 months (range 6 to 46 months). One patient with ALL achieved CC but died soon after of severe graft-versus-host disease (GVHD). Only temporary responses (transient decrease or disappearance of MC) were seen in 3 follow-ups (2 AML, 1 JMML) with subsequent hematologic relapse 8, 9 and 20 months after the initiation of AI. No response to AI was seen in 4 follow-ups (2 ALL, 2 AML). Overall survival of the patients treated with AI was 8/13 (ALL 1/4, AML 3/5, CML 3/3, JMML 1/1). Out of 3 patients/4 follow-ups treated in hematologic relapse only 1 (JMML) is alive in CCR. Pre-emptive AI was performed in 10 patients. Initial response was documented in