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Unmanipulated bone marrow transplantation from one-HLA locus mismatched siblings carries high transplant-related mortality in Chinese patients

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Background and Objectives. We compared the outcome of bone marrow transplantation (BMT) from HLA-identical siblings (MSD) and one HLA-locus mismatched siblings (PMSD) in Chinese patients with hematologic malignancies in terms of transplant-related mortality (TRM) and disease relapse to see whether PMSD can feasibly increase the availability of donors in our population.

Design and Methods. Medical records of patients who had received a BMT from sibling donors in the Queen Mary Hospital, Hong Kong, from March 1990 to Februrary 2000 were reviewed (MSD 326, PMSD 20). Patients and their donors were matched for HLA-A, -B and DRB1 loci using standard serologic methods as well as polymerase chain reaction-sequence specific primers. All patients received standard anti-microbials and graft -versus host disease (GVHD) prophylaxis including cyclosporin A and a short course of methotrexate.

Results. A total of 346 BMT patients were analyzed of whom 326 and 20 patients had received transplants from matched and one locus mismatched siblings, respectively. Patients receiving BMT from PMSD had a significantly higher TRM than those receiving their BMT from MSD (p=0.0016). Six patients received BMT from HLA-DR PMSD: one died 2 months post-BMT as a result of post-transplantation-related lymphoproliferative disease. Fourteen patients received BMT from HLA-A or –B PMSD: 11 of these patients died after a median of 5.6 months (range 0.6-13.7 months) due to severe GVHD (n=5), graft failure (n=2), bleeding (n=1), leukemic relapse (n=2) and thrombotic thrombocytopenic purpura (n=1). Two out of the three survivors had primary graft failure: one of these two required infusion of back-up marrow and the other had autologous regeneration. Patients in the PMSD group were at greater risk of developing severe GVHD than their MSD-recipient counterparts (p<0.001). There was no significant difference in the probability of disease relapse between patients who received BMT from MSD or PMSD.

Interpretation and Conclusions. BMT from PMSD (especially those with mismatches at HLA class I loci) carried a higher risk of TRM and morbidity than BMT from MSD in our population.

Key words: high dose chemotherapy, inflammatory breast cancer, cardiotoxicityKey words:

Allogeneic bone marrow transplantation (BMT) has improved the long-term survival of patients with hematologic malignancies. However, patients who may benefit from transplantation may not have an HLA-matched sibling donor (MSD) and in this situation, HLA-matched unrelated donor (MUD) is an alternative source of hematopoietic stem cells. The probability of finding a suitable MUD among ethnic Chinese is about 40%[1] but the procedure carries high transplant-related mortality (TRM) due to severe graft-versus-host disease (GVHD) and graft failure.

To overcome these limitations, transplantation from partially mismatched sibling donors (PMSD) who share one HLA haplotype and are matched for various HLA-A, B or DRB1 loci on the unshared haplotype has been considered as an alternative.[2,3] BMT from PMSD with two or more mismatched loci have been associated with high TRM due to severe GVHD and a high chance of graft failure. *Ex vivo* donor T-cell depletion from the graft marrow may alleviate the problem but its survival benefit is offset by a higher incidence of graft failure and disease relapse. Studies from Seattle[2,3] showed that patients who receive BMT from siblings with one mismatched HLA-locus have an overall survival comparable to those who receive a BMT from an HLA identical sibling. On the other hand, data from the International Bone Marrow Transplant Registry (IBMTR) showed, based on serologic definition of HLA specificities, that patients receiving BMT from PMSD with one mismatched antigen had a higher TRM than those receiving BMT from HLA-identical siblings.[4] It remains unclear whether these data can be extended to the Chinese population in whom ethnic differences in HLA frequencies and linkage disequilibrium are expected.[5] We, therefore, compared the clinical outcome of patients who received BMT from HLA-identical siblings (MSD) and from one HLA-locus mismatched siblings (PMSD) in Chinese patients in terms

of TRM and disease relapse to see whether PMSD can feasibly increase the availability of donors in our population.

Design and Methods

Patients

The medical records of 352 Chinese patients who received related allogeneic BMT in Queen Mary Hospital, Hong Kong, from March 1990 to February 2000, were reviewed. The patients were analyzed according to the source of hematopoietic stem cells. In this period 326 and 20 patients received BMT from MSD and PMSD, respectively. Five patients, two of whom died of primary graft failure, received transplants from parents. Among the three survivors, one patient did not engraft requiring infusion of his own back-up marrow. The remaining two patients remain alive and disease-free at nine years and one year after transplantation. One patient received a transplant from a child but died of primary graft failure one month after the BMT. These patients (6 in total) were excluded from subsequent analysis to avoid any possible confounding effects.

Characterization of HLA in patients and donors

Donor-recipient pairs transplanted in the early years of the study period were typed for HLA-A, -B, and –DR specificities using standard serologic techniques employing antisera standardized for use in a Chinese population. In the latter part of the study period, patients and donors were tested for a minimum of HLA-A, HLA-B and HLA-DRB1 alleles using a polymerase chain reaction (PCR) method with sequence-specific primers (SSP) employing commercially prepared primer kits.[6] Twenty-two primer mixes were used to identify 19 HLA-DR specificities. DRB1 allelic typing was performed using 11 primer mixes for DR4 subtyping, 12 primer mixes for DR15/DR16 subtyping and 12 primer mixes for DR8/DR 12 subtyping.

Bone marrow conditioning and antimicrobial prophylaxis

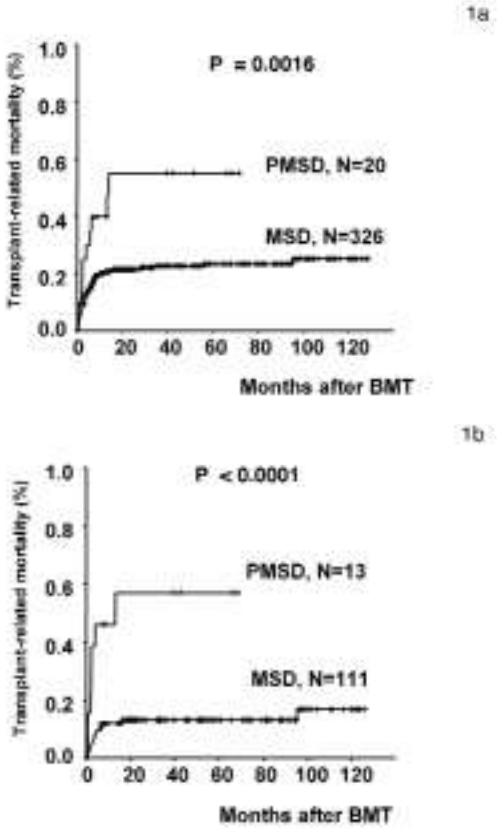
The conditioning regimens are shown in Table 1. All patients were given standard antibacterial (ciprofloxacin 500 mg twice daily) and antifungal prophylaxis (fluconazole 200 mg daily) from the commencement of conditioning until donor marrow engraftment.[7,8] Patients receiving BMT from MSD were given acyclovir (200 mg oral thrice daily) as prophylaxis against herpes simplex infection and those from PMSD were given high dose acyclovir (10 mg/kg intravenously every 8 hours) until engraftment, followed by ganciclovir (5 mg/kg) three times a week until day 120, for prophylaxis against cytomegalovirus (CMV) infection. Additional antimicrobial therapies were given according to the discretion of the attending physicians.

Table 1. Baseline characteristics of patients receiving BMT from HLA-matched sibling donors (MSD) and partially mismatched (one-HLA locus) sibling donors (PMSD). CML-CP, chronic myeloid leukemia in chronic phase; AP, accelerated phase; BT, blastic transformation; AML, acute myeloblastic leukemia; CR, complete remission; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia; Bu, busulfan; Cy, Cyclophosphamide; TBI, total body irradiation. MNC, mononucleated cells; ANC, absolute neutrophil count. NA, not applicable due to graft failure and early transplant-related mortality. Engraftment is defined as the time (day post-BMT) when ANC $\geq 0.5\times10^9$/L. Patients in the PMSD group were at a significantly greater risk of severe GVHD than their MSD-recipient counterparts. ψ $p< 0.001$.

	MSD	*PMSD*
N 326	20	
Male:Female	194:132	13:7
Median Age (Range)	34 (15-56)	34 (19-53)
Underlying diseases		
CML		
CP	101	10
AP	6	2
BT	4	1
AML		
CR1	37	0
CR2 or 3	35	0
At relapse	37	2
ALL		
CR1	25	0
CR2 or 3	12	1
At relapse	6	0
MDS	16	2
Others	47	2
Conditioning		
Bu-Cy	140	8
Cy-TBI	61	7
Bu-Cy-TBI	70	4
Others	55	1
MNC (10^8/kg)	3.2 (0.7-10.6)	3.1 (1.7-4.8)
ANC engraftment (day)	20 (10-54)	22.5 (11-47)
GVHD (overall)		
≤ Grade 1	212	7
Grade 2-3	78	3
Grade 4	17	6 y
NA	19	4

Prophylaxis against GVHD

All patients (MSD- or PMSD-recipients) received cyclosporin A (3 mg/kg intravenously or 8 mg/kg orally day 1 – 50, tailed off at 6 months) and a short course of methotrexate (15 mg/m^2 on day 1, 10 mg/m^2 on days 3, 6 and 11). The severity of GVHD was graded according to the criteris described by Glucksberg *et al*.[9] Patients developing GVHD received additional immunosuppression according to the discretion of the attending physicians.



Figures 1a,b. Transplant-related mortality (TRM) of patients after BMT from HLA-matched sibling donors (MSD) and partially mismatched (one-HLA locus) sibling donors (PMSD). Figure 1a includes patients with all disease categories and figure 1b includes only patients with chronic myeloid leukemia. In both figures, TRM of patients in the PMSD group was significantly greater than that in the MSD group.

Statistical analysis

Transplant-related mortality (TRM) was estimated by the method of Kaplan and Meier from the time of BMT to the time of death or last follow-up. Baseline characteristics in different patient categories (MSD- or PMSD-recipients) were compared using the Mann-Whitney test and TRM was compared using the log-rank test. Comparison of the occurrence of severe GVHD in different categories was done using Fisher's exact test. *p* values less than 0.05 are considered statistically significant.

Results

Baseline characteristics at transplantation

The clinical features of the patients are shown in Table 1. There was no significant differences in baseline characteristics, quantity of hemopoietic stem cells infused or donor marrow engraftment between the three groups of patients.

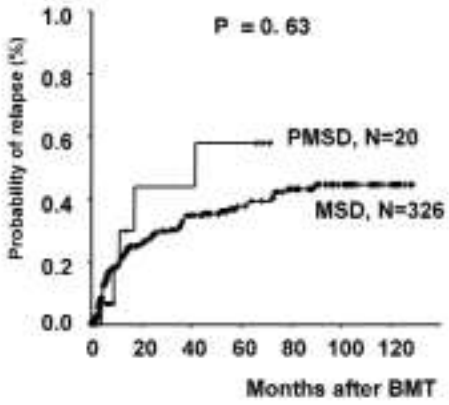


Figure 2. The probability of disease relapse after transplantation in patients (all disease categories) receiving BMT from HLA-matched sibling donors (MSD) and partially mismatched (one-HLA locus) sibling donors (PMSD). There was no significant difference between the two groups of patients (log-rank test, $p>0.05$). Similar findings were noted in patients with chronic myeloid leukemia (data not shown). Disease relapse was defined by hematologic, cytogenetic or molecular studies and could be medullary or extramedullary in location.

Survival outcomes of patients

As shown in Figure 1a, TRM of patients receiving BMT from MSD and PMSD were 24% (at 120 months) and 55% (at 60 months) (p=0.0016). As a difference in underlying diseases could be a confounding factor, a subgroup analysis was performed confined to patients with chronic myeloid leukemia (CML) (Figure 1b). TRM of patients in the MSD and PMSD groups were 18% (120 months) and 58% (60 months) (p<0.0001). Patients in the PMSD group had a greater risk of developing severe GVHD (overall grade 4) than their MSD-recipient counterparts (p<0.001) (Table 1). There was no significant difference in the probability of disease relapse between patients who received BMT from MSD or PMSD (Figure 2). Similar findings were noted in patients with CML (data not shown).

Clinical characteristics of the PMSD group

Table 2 shows the characteristics of the 20 patients receiving BMT from a PMSD. Six patients had one mismatch at the HLA class II region (DRB1): one of these patients died as a result of post-transplant lymphoproliferative disease 2 months after transplantation. Fourteen patients received BMT from HLA-A or –B PMSD; 11 of these patients died after a median of 5.6 months (range 0.6-13.7 months). The causes of mortality were severe GVHD (n=5), graft failure (n=2), bleeding (n=1), leukemic relapse (n=2) and throm-

Table 2. Clinical characteristics and HLA typings of 20 patients who received BMT from PMSD. For the donors, the mismatched HLA loci are shown. CML-CP, chronic myeloid leukemia in chronic phase; AP, accelerated phase; BT, blastic transformation; AML, acute myeloblastic leukemia; MDS, myelodysplastic syndrome; ALL-CR2, acute lymphoblastic leukemia in second complete remission; SAA, severe aplastic anemia; MM, multiple myeloma; GVHD, graft-versus-host disease; GF, graft failure, TTP, thrombotic thrombocytopenic purpura; ICH, intracerebral hemorrhage; PTLD, post-transplantation lymphoproliferative disease.

Patient	Sex/Age	Diagnosis	Patient HLA						Donor locus (mismatch)	Dead (cause)	Time (Months)	Remarks
			A		B		DR					
1	M/46	MDS	2	33	17	27	0301	14	A11,33	Yes (GVHD)	13.7	
2	M/30	SAA	2	11	15	56	2	12	A11,11	Yes (GVHD)	6.0	
3	M/26	CML-CP	11	24	46	51	09	12	A2,24	Yes (GVHD)	2.3	
4	M/43	CML-CP	2	33	13	61	8	15	B61,75	Yes (GVHD)	2.3	
5	M/39	CML-AP	2	2	46	62	4	11	B46,46	Yes (GVHD)	0.6	
6	F/48	MDS	2	29	7	75	2	9	A11,29	Yes (Relapse)	12.7	
7	M/22	CML-CP	11	11	46	75	0901	12	A2,11	Yes (Relapse)	13.2	
8	F/22	CML-BT	2	9	7	22	2	4	B7,B15	Yes (GF)	4.1	
9	M/19	AML-Relapse	3	11	38	46	7	14	A2,3	Yes (GF)	5.6	
10	M/37	CML-CP	2	31	58	60	11	1302	A2,33	Yes (TTP)	7.1	
11	F/53	CML-CP	11	24	51	60	12	16	B13,51	Yes (ICH)	0.7	
12	M/39	CML-CP	24	24	58	60	8	13	A2,24	No	60.1	
13	M/46	CML-CP	2	2	13	27	04	15	B13,22	No	33.5	Graft failure
14	F/33	CML-CP	24	11	13	61	12	12	A2, 24	No	2.5	Graft failure
15	F/25	CML-AP	24	24	13	51	09	12	DR4,12	Yes (PTLD)	2.0	
16	F/35	ALL-CR2	2	24	22	46	8	12	DR8,9	No	45.6	Relapse
17	M/32	CML-CP	24	33	13	17	1302	16	DR7,16	No	36.3	Relapse
18	F/36	MM	24	11	75	76	0901	15	DR15,15	No	0.9	
19	M/28	AML-Relapse	2	24	17	46	7	14	DR7,9	No	65.2	
20	M/27	CML-CP	24	31	17	60	9	13	DR11,13	No	62.5	

botic thrombocytopenic purpura.[1] The difference in mortality between the two groups of patients (class I vs class II mismatches) was statistically significant (p=0.018). Among the three survivors with HLA class I loci mismatches, two patients had primary graft failure: one required infusion of back-up marrow and the other had autologous regeneration.

Discussion

In this study we demonstrated that in Chinese patients, BMT from siblings with one mismatched HLA locus were associated with higher transplant-related mortality and morbidity than those from HLA-identical siblings. The worse TRM was not related to the heterogeneity of the underlying diseases, patients' age, conditioning regimens, cell dose in the donor marrow or differences in donor marrow engraftment.

The mechanisms underlying the worse outcomes of BMT from PMSD in our population were not apparent. One possible explanation is related to the resolution of the HLA typing methodology. Studies from Seattle, using both serologic and DNA typing, showed that patients receiving BMT from PMSD had an overall survival identical to those receiving a BMT from a MSD.[2,3] An inferior survival of patients receiving BMT from PMSD was reported from the IBMTR study, which was based exclusively on serologic data.[4] It has been suggested that patients and donors who were serologically compatible at -DR in the IBMTR study could have been mismatched for DRB1 subtypes. Therefore, patient-donor pairs who are mismatched at one HLA-locus as defined by serologic methods may be genetically more disparate than those defined by DNA typing.[10] In the present study, both serologic and DNA typing were used to define HLA specificities (see Methods). Whether the poorer survival and greater morbidity of BMT recipients from PMSD were associated with HLA disparity at sequence level would have to be tested by further studies. An alternative explanation is the ethnic difference in linkage disequilibrium of HLA alleles.[5] In particular, the possible role of mismatches in the HLA-C locus should not be overlooked. In Caucasians, mismatches for HLA-C are associated with higher transplant-related mortality.[11,12] Testing for HLA-C was not performed in our donor-recipient pairs and it is possible that a known mismatch at the HLA-A or –B loci could also reflect a mismatched HLA-C allele in linkage disequilibrium with the known mismatch.

We also demonstrated that mismatches at class I loci were associated with higher risk of transplant-related mortality and graft failure than those at class II loci. The results contrast with those reported in the West showing inferior outcomes of BMT from sib-

lings with one locus mismatched at HLA-DR.[13] The discrepant results in BMT outcome will have to be confirmed in a large cohort of BMT patients.

In conclusion, the present study showed that, in Chinese patients, allogeneic BMT from siblings with one mismatched HLA locus were associated with significantly higher transplant-related mortality than those from HLA identical siblings. Further studies are needed to define permissible mismatches in this population and to evaluate modification of immunosuppression during transplantation for the prevention of graft failure and GVHD.

Contributions and Acknowledgments

AYH Leung : data analysis (clinical data) and drafting of the article; AKW Lie : design of the study and critical review of the draft; WY Au: data interpretation and critical review of the draft; BR Hawkins: data analysis (HLA typing data) and critical review of the draft; YL Kwong: design of the study and critical review of the draft; R Liang : data interpretation and critical review of the draft. All the above authors have approved the final version of the manuscript to be submitted to Haematologica.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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Potential implications for clinical practice

The results of the present study lead immediately to a revision (currently in progress) of the definition of permissible mismatches in the Chinese population and of the need to modify the current immunosuppression protocols for patients receiving BMT from HLA mismatched siblings.

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