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European results of matched unrelated donor bone marrow transplantation for chronic myeloid leukemia. Impact of HLA class II matching

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Summary:

We have retrospectively analyzed the impact of prognostic factors on the outcome of serologically HLA-matched unrelated donor (UD) BMT for CML. For this purpose, we have studied a cohort of 366 patients transplanted in Europe between January 1985 and December 1994. The median age of the 211 males and 155 females was 34 years; 238 patients were transplanted in first chronic phase and 116 in advanced phases. The median interval from diagnosis to BMT was 827 days. GVHD prophylaxis consisted of CsA and MTX in 202 patients or of *ex vivo* or *in vivo* T cell depletion (TCD) in 129. Recently, DNA-based methods of HLA-class II typing have been used to improve donor selection. We obtained complete data on 300 donor/recipient (D/R) pairs. Among them, we have identified three groups of patients, according to specific HLA-DRB1 D/R compatibility. Two hundred and ten patients received marrow from donors identical for HLA-DRB1 (group 1). Thirty-one patients received BMT from a donor who was HLA-DRB1 mismatched (group 2) and 59 from a donor in whom specific HLA-DRB1 typing was not performed (group 3). The overall survival was 37 ± 3% at 2 years and leukemia-free survival (LFS) was 31 ± 3%. In univariate analysis, five variables had a favorable effect on LFS: transplant in first chronic phase ($P = 0.0001$), time interval from diagnosis to BMT shorter than the median ($P = 0.01$), prophylaxis of GVHD without TCD ($P = 0.001$), acute GVHD <grade III ($P = 0.0009$) and HLA-DRB1 D/R matching ($P = 0.0001$). Transplant-related mortality (TRM) was 49 ± 4% in group 1, 79 ± 8% in group 2 and 80 ± 6% in group 3 ($P = 0.0001$). Multivariate analysis confirmed that HLA-DRB1 matching was the most significant factor influencing survival ($P = 0.04$), LFS ($P = 0.013$) and TRM ($P = 0.0049$). From these results, we have defined a 'good risk' group, ie patients transplanted in first chronic phase, from an HLA-DRB1 matched donor, without TCD as prophylaxis against GVHD. The 2 year LFS, TRM and relapse incidence for this group were 51 ± 5%, 47 ± 5% and 2 ± 2%, respectively. This suggests that the long-term outcome of patients with favorable prognostic features can approach that of patients transplanted from geno-identical siblings. In contrast, the TRM for patients transplanted for advanced disease from non HLA-DRB1-identical donors was 94%. Such a high TRM clearly indicates that UD BMT is not justifiable for these individuals.

Keywords: chronic myeloid leukemia; unrelated donor bone marrow transplant

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The centers participating in this study from the chronic leukemia working party of the European Group for Blood and Marrow Transplantation are listed in the Appendix.
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For patients with CML, allogeneic BMT from an HLA-identical sibling is the treatment of choice.[1–4] However, most patients do not have a suitable sibling donor and, for these individuals, there are a number of treatment options. Most are now treated with α-IFN, as this therapy can induce a major or a complete cytogenetic response in some patients[5] and improve the median survival, compared to those treated with hydroxyurea alone.[6,7] Nevertheless, fewer than 50% of patients have a useful cytogenetic response and blast transformation is observed even in those patients who have responded. Finally, there is no evidence, as yet, that this treatment can cure any patient. Similarly, autologous transplantation may offer prolongation of survival in some patients,[8] but is also unlikely to result in cure.

Unrelated donor (UD) BMT is a possible alternative treatment. In general, the results using unrelated donors are poorer than those obtained using genetically identical related donors.[9–13] This may be due to a number of factors including modifications in the conditioning regimen, the

method of GVHD prophylaxis, and a tendency to delay the transplant so that the interval from diagnosis to BMT is greater than that in an equivalent cohort of sibling transplant patients. Furthermore, there is an increased incidence of acute and chronic GVHD and an increased incidence of life-threatening viral infections, both of which might reflect the degree of HLA disparity.[9–13] Selection of suitable unrelated donors has previously relied upon serological identification of HLA-A, -B and -DR alleles. Sequencing of the HLA genes has now revealed a far greater degree of polymorphism at these loci than that detected by serology.[14] As a consequence, many unrelated serologically matched pairs have subsequently been shown to have undisclosed mismatches. In order to analyze the impact of improved resolution of class II typing on UD BMT outcome, we have conducted a retrospective analysis of 366 patients reported to the chronic leukemia registry of the European Group for Blood and Marrow Transplantation (EBMT) who were transplanted for CML using unrelated donor marrow. DNA-based typing of HLA-DRB1 was available for the majority of patients allowing us to analyze the effects of accurate matching on transplant outcome.

Patients and methods

Patients

The registry of the chronic leukemia working party (CLWP) of the EBMT contains data on 374 patients who were transplanted for CML from donors matched serologically for HLA-A, HLA-B and HLA-DR. Three hundred and sixty-six of these patients are evaluable for analysis of survival, leukemia-free survival (LFS), transplant-related mortality (TRM) and relapse incidence (RI). The patients were transplanted between January 1985 and December 1994. Analysis was conducted after February 1995, giving a median follow-up of 32 months (range 2–110 months). Patient details are provided in Table 1.

Table 1 Clinical details of 366 patients

Median age in years (range)	34 (1–58)
Nos of patients	366
Male	211
Female	155
Disease status transplant	
First chronic phase	238
Accelerated phase	79
2nd or subsequent chronic phase	21
Blast transformation	16
Status not given	12
Median interval from diagnosis to BMT in days (range)	827 (70–3719)
No. transplanted <1 year from diagnosis	37
No. transplanted 1–2 years from diagnosis	117
No. transplanted 2–3 years from diagnosis	78
No. transplanted >3 years from diagnosis	123
Not given	11
Donor/recipient sex match (absolute Nos)	
MM	109
FM	99
MF	58
FF	80
Not given	20
Prophylaxis for GVHD (absolute Nos)	
CsA and MTX	202
In vivo TCD (ATG ± antibody)	71
Ex vivo TCD	58
CsA alone	19
Other/not given	16
Total body irradiation (absolute Nos)	
Single dose	114
Fractionated	208
Occurrence of acute GVHD	
Grade 0	107
Grade I	81
Grade II	71
Grade III	58
Grade IV	49

Conditioning regimen

Of the 366 patients, 322 (88%) received TBI and chemotherapy. The irradiation protocols differed between centers. One hundred and fourteen (35%) patients received single dose (SD) irradiation and 208 (65%) patients were treated with various schemes of fractionated (F) TBI.

Prophylaxis of GVHD

There was considerable variation between centers in the measures used to prevent GVHD. Two hundred and two (55%) patients received CsA and short course MTX.[15] Fifty-eight (16%) received marrow which had been depleted of T cells *ex vivo* (*ex vivo* TCD). An additional 71 (19%) patients received *in vivo* T cell depletion (*in vivo* TCD) with either monoclonal antibodies or anti-thymocyte globulin (ATG). Nineteen (5%) patients were treated with CsA alone and information relating to the method of GVHD prophylaxis was unavailable for 16 patients.

Donor and recipient compatibility

All donor/recipient (D/R) pairs were HLA-A, -B and -DR matched using serological methods. The class II loci were typed either by serological methods or by more definitive DNA-based methods. The latter, ie RFLP, PCR sequence-specific oligonucleotide probing (PCR-SSOP) and PCR sequence-specific priming (PCR-SSP), have varying powers of resolution of class II specificities.[16] Recently these methods have been used to improve donor selection. In order to analyze the impact of more accurate HLA class II typing on the clinical outcome of transplant, we required additional information on the nature and results of the matching techniques employed for individual patients. We sent a further detailed questionnaire to all BMT centers concerned. Forty-seven centers replied, giving complete data on 300 D/R pairs, who are evaluable for survival, LFS, TRM and RI. HLA-DRB1-specific alleles were identical for 210 D/R pairs. There were one or two HLA-DRB1 mismatches for 31 D/R pairs and specific HLA-DRB1 typing had not been done in 59. HLA-DQB1 alleles were identical for 164 D/R pairs, mismatched for 14 and not done in 122 pairs. HLA-DPB1 alleles were identical for 36 D/R pairs, mismatched for 81, and not performed in 183 donors and their recipients.

We have identified three different groups of patients, according to the HLA-DRB1 D/R compatibility (Table 2). Two hundred and ten patients who received marrow from an HLA-A, -B and DRB1 identical donor were referred to as group 1 (matched group). Thirty-one patients who received bone marrow from a donor who was HLA-DRB1 mismatched were referred to as group 2 (mismatched group). Fifty-nine patients in whom specific HLA-DRB1 typing was not performed were referred to as group 3. There were no differences between the three groups of patients with respect to age, interval from diagnosis to transplant, and the method of TBI. However, a higher proportion of patients in group 1 were transplanted in first chronic phase than in group 2 ($P = 0.03$) and in group 3 ($P = 0.024$). There was no difference between groups 1 and 2 with respect to the prophylaxis of GVHD. In contrast, patients in group 3 were more likely to receive *in vitro* or *in vivo* T cell-depleted marrow than in group 1 ($P = 0.02$).

Statistical analysis

All analyses were performed using the BMDP statistical package. Leukemia-free survival (LFS) was defined as survival without evidence of hematological relapse. To evaluate the probability of relapse (RI), patients dying either from the toxicity of the procedure or from any other cause not related to leukemia were censored at the time of death. The transplant-related mortality (TRM) was defined as death while in complete remission and patients were censored at time of relapse or last follow-up. LFS, RI, TRM and overall survival were estimated by the product-limit method.[17]

In the entire population of patients who received an UD BMT ($n = 366$), a series of characteristics were studied for a possible effect on outcome using the log rank test (Mantel–Cox),[18] ie patient age at transplant, patient gender, donor gender, gender matching, TBI method, disease stage at time of transplant, time interval between diagnosis and BMT, method of prophylaxis of GVHD, incidence and grade of acute GVHD and graft failure and rejection.

In a second analysis, a sub-group of 300 patients for whom information was available concerning specific HLA-DRB1 typing was divided into three groups, ie matched, mismatched and not known. Patient-, disease- and treatment-related variables were compared between the three cohorts using the χ^2 statistical method for qualitative variables. All variables recognized as possible prognostic factors or differing significantly between the three groups in univariate analysis were studied using the proportional hazard model.[19] In addition to HLA-DRB1 typing, we studied patient age at transplant, patient gender, donor gender, year of transplant, disease status at time of transplant (first chronic phase *vs* all other stages), time interval between diagnosis and transplant, method of prophylaxis of GVHD (CsA and MTX *vs in vivo* or *ex vivo* TCD) and TBI (fractionated *vs* single dose).

Results

All patients

Engraftment: Engraftment was defined as the attainment of a peripheral blood ANC $> 0.5 \times 10^9$/l for 3 consecutive days. Three hundred and thirteen patients were evaluable for engraftment. Two hundred and seventy-nine (89%) patients engrafted and 34 (11%) patients experienced graft rejection.

Survival and leukemia-free survival: Of 366 evaluable patients, 230 have died. The overall survival was $37 \pm 3\%$

Table 2 Characteristics of the three groups

	Group 1 DRB1 matched		*Group 2 DRB1 mismatched*		*Group 3 DRB1 not done*	
Patient numbers	210		31		59	
Median follow-up in months (range)	29.5	(2–94)	47	(1–89)	55	(22–110)
Median age in years (range)	34	(1–59)	31	(15–47)	35	(11–58)
Interval diagnosis to BMT in days (range)	816	(70–3719)	887	(354–3494)	846	(216–8573)
Disease status at transplant						
First chronic phase	153		17		34	
Advanced phase	54		14[a]		24[b]	
Not known	3				1	
Total body irradiation						
Single dose	63		10		21	
Fractionated	128		18		34	
No TBI	19		3		4	
Prophylaxis against GVHD						
CsA	11		2		4	
CsA and MTX	124		22		24	
In vivo TCD	26		2		14[c]	
In vitro TCD	45		5		16	
Other	4				1	

[a]Group 1 *vs* group 2: $P = 0.03$.
[b]Group 2 *vs* group 3: $P = 0.024$.
[c]Group 1 *vs* group 3: $P = 0.02$.

at 2 years and 35 ± 3% at 5 years. Three factors were identified in univariate analysis which significantly affected actuarial survival (Table 3): disease status at transplant (44 ± 3% (first chronic phase) *vs* 23 ± 4% (advanced phase) ($P = 0.0002$); time interval from diagnosis to BMT (40 ± 4% for patients transplanted before the median of 827 days from diagnosis and 33 ± 4% for those transplanted beyond 827 days from diagnosis ($P = 0.04$)); and the grade of acute GVHD (respectively 49 ± 4% (grade 0–I), 41 ± 6% (grade II) and 13 ± 3% (grade III–IV) ($P < 0.0001$)).

The LFS was 33 ± 2% and 31 ± 3%, 2 years and 5 years after transplant, respectively. Of the variables analyzed for their influence on LFS, four had a significant effect: disease status at transplant (40 ± 3% *vs* 20 ± 4% ($P = 0.0001$)); time interval from diagnosis to BMT (38 ± 4% compared to 28 ± 3% ($P = 0.01$)); the method of prophylaxis of GVHD (41 ± 3% (CsA and MTX), 28 ± 5% (*in vivo* TCD) and 20 ± 5% (*ex vivo* TCD) respectively ($P = 0.001$)); and the grade of acute GVHD (43 ± 4%, 37 ± 6% and 13 ± 3% respectively ($P = 0.0009$)).

Transplant-related mortality: Death due to transplant-related toxicity occurred in 210 patients, giving a 2 year probability of TRM of 61 ± 3%. Factors which significantly affected the risk of TRM in univariate analysis were: status at transplant (55 ± 3% (in first chronic phase) *vs* 72 ± 5% (in advanced phase) ($P = 0.002$)); time interval from diagnosis to BMT (56 ± 4% *vs* 65 ± 4% ($P = 0.04$)); and the grade of acute GVHD (46 ± 4%, 57 ± 6% and 87 ± 3% respectively ($P < 0.0001$)).

Relapse incidence: Thirty-four patients had an hematological relapse, of whom 20 have subsequently died. The actuarial risk of relapse was 16 ± 3% at 2 years and 20 ± 3% at 5 years. In univariate analysis, four factors had significant effects on the risk of relapse: status at transplant (12 ± 3% for patients transplanted in first chronic phase and 29 ± 5% for those with more advanced disease ($P = 0.009$)); the method of prophylaxis of GVHD (5 ± 2%, 29 ± 9% and 36 ± 8% respectively ($P < 0.0001$)); the grade of acute GVHD (20 ± 4%, 14 ± 5%, and 5 ± 4% respectively ($P = 0.02$)); and the technique of administering TBI (8 ± 3% (SD) *vs* 23 ± 4% (F) ($P = 0.04$)).

Table 3 Univariate analysis of 366 patients: 2 years leukemia-free survival, relapse incidence, survival and transplant-related mortality

Variables	*LFS %*	*RI %*	*Survival %*	*TRM %*
Interval diagnosis-BMT				
<Median of 827 days	38 ± 4	13 ± 3	40 ± 4	56 ± 4
>Median of 827 days	28 ± 3	22 ± 5	33 ± 4	65 ± 4
P value	**0.01**	0.11	**0.04**	**0.04**
Total body irradiation				
Single dose	36 ± 4	8 ± 3	36 ± 4	61 ± 5
Fractionated	30 ± 3	23 ± 4	37 ± 3	60 ± 4
P value	0.36	**0.04**	0.94	0.89
Disease status at BMT				
First chronic phase	40 ± 3	12 ± 3	44 ± 3	55 ± 3
Advanced phase	20 ± 4	29 ± 5	23 ± 4	72 ± 5
P value	**0.0001**	**0.009**	**0.0002**	**0.002**
GVHD prophylaxis				
CsA and MTX	41 ± 3	5 ± 2	41 ± 3	57 ± 3
In vivo TCD	28 ± 5	36 ± 8	38 ± 6	56 ± 6
Ex vivo TCD	20 ± 5	29 ± 9	29 ± 6	72 ± 7
P value	**0.001**	**<0.0001**	0.1	0.06
Grade of GVHD				
0–I ($n = 188$)	43 ± 4	20 ± 4	49 ± 4	46 ± 4
II ($n = 71$)	37 ± 6	14 ± 5	41 ± 6	57 ± 6
III–IV ($n = 107$)	13 ± 3	5 ± 4	13 ± 3	87 ± 3
P value	**0.0009**	**0.02**	**<0.0001**	**<0.0001**

Significant P values are indicated in bold font.

Influence of HLA-DRB1 matching on outcome

Three hundred donor and recipient pairs were evaluable for the determination of the influence of HLA-DRB1 matching on outcome. Patients were defined as identically matched (group 1) or mismatched (group 2) according to the criteria set out in the Methods section. Patients in whom HLA-DRB1 typing results were not known were defined as group 3.

Engraftment: Details of engraftment were provided for 258 of the 300 D/R pairs. Graft rejection occurred in 16 (8%) of the 187 patients in group 1, in three of the 24 patients in group 2 (12.5%) and in 6 of the 47 (12.7%) in group 3 (P = NS).

Leukemia-free survival: HLA-DRB1 matching is the most important factor influencing LFS. The 210 patients in the matched group (group 1) had an LFS at 2 years of 44 ± 3% compared to 17 ± 7% for the 31 patients in the mismatched group (group 2) ($P = 0.0004$) and to 17 ± 5% for the 59 patients in group 3 ($P < 0.0001$) (Figure 1). The influence of HLA-DQB1 and HLA-DPB1 were studied independently in the HLA-DRB1 matched group (group 1) and in groups 2 and 3 (Table 4). The LFS did not appear to be significantly affected by the HLA-DQ or -DP disparity. However, the influence of HLA-DQB1 or -DPB1 cannot be adequately assessed due to the small number of patients for whom adequate data are available.

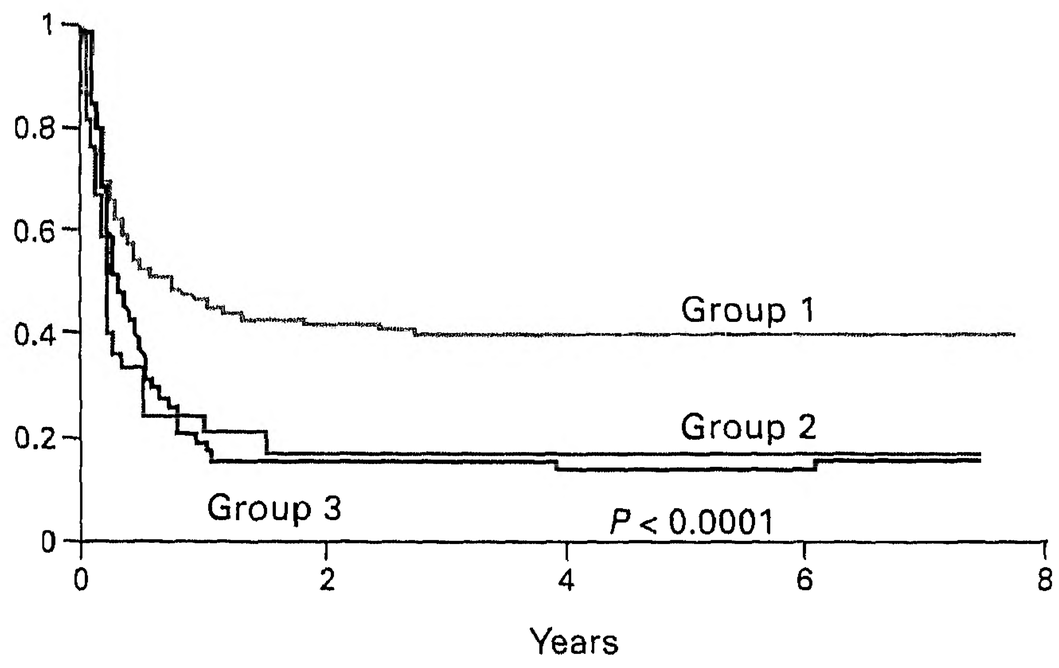

Figure 1 Kaplan–Meier probability of leukemia-free survival in patients who received DRB1-matched transplants (group 1) compared with those who received DRB1-mismatched transplants (group 2) and with those for whom HLA-DRB1 typing was not done (group 3). The probability of leukemia-free survival at 2 years was 44 ± 3% for 210 DRB1-matched transplants, 17 ± 7% for 31 mismatched transplants and 17 ± 5% when DRB1 typing was not done ($P < 0.0001$).

Table 4 HLA class II compatibility. Impact on 2 years LFS

	Group I (DB1 id)		*Group II (DRB1 MM)*		*Group III (DRB1 ND)*		*Total*
	n	*LFS%*	*n*	*LFS%*	*n*	*LFS%*	*n*
	210	44 ± 3	31	17 ± 7	59	17 ± 5[a]	300
DQB1							
Identical (id)	135	51 ± 4	15	20 ± 10	14	7 ± 7	164
Mismatched (MM)	6	50 ± 20	7	28 ± 17	1	0	14
Not done (ND)	69	47 ± 6	9	25 ± 15	44	20 ± 6	122
		$P = 0.85$		$P = 0.98$		$P = 0.25$	
DPB1							
Identical (id)	31	53 ± 9	4	0	1	0	36
Mismatched (MM)	68	50 ± 6	12	33 ± 14	1	0	81
Not done (ND)	111	44 ± 5	15	21 ± 11	57	23 ± 6	183
		$P = 0.13$		$P = 0.25$		$P = 0.65$	

[a] Group 1 *vs* group 2: $P = 0.0004$; group 1 *vs* group 3: $P = 0.0001$.

The matched and mismatched groups were also studied for the influence of other prognostic factors. Two hundred and four evaluable patients were transplanted in first chronic phase, 153 in group 1, 17 in group 2 and 34 in group 3. The LFS was improved if the donor was HLA-DRB1 matched, being 47 ± 4% in group 1 compared to 23 ± 10% in group 2 ($P = 0.014$). This was largely due to a reduced TRM of 48 ± 4% for patients in group 1 compared to 71 ± 11% in group 2 ($P = 0.013$). The LFS was 29 ± 8% for the 34 patients in group 3, and the difference with group 1 was also statistically significant ($P = 0.05$), with a TRM of 66 ± 9%. Similar differences between the three groups were seen for patients who were transplanted in advanced phases of the disease.

Transplant-related mortality: HLA-DRB1 matching was also the most important factor influencing the risk of TRM (Figure 2). The TRM was 49 ± 4% in group 1 (matched group) and 79 ± 8% in group 2 (mismatched group) ($P = 0.0002$). This was largely related to an increased incidence of acute GVHD of grade II–IV in the mismatched group (23 of 31 patients) (74%) compared to the matched group (93 of 210 patients) (44%) ($P = 0.002$). The TRM was also higher for the patients of group 3 (80 ± 6%) when compared to group 1 ($P < 0.0001$). Grade II–IV acute GVHD occurred in 32 of the 59 patients (54%) (NS).

Relapse incidence: The relapse incidence was not affected by HLA-DRB1 disparity.

Multivariate analysis: Sufficient data were available for 286 patients to be analyzed in a Cox proportional hazard model[19] using the covariates described in the Methods section. The results are shown in Table 5. Three factors were associated with LFS and relapse risk: there was a significant reduction in relapse risk with an improved LFS for patients transplanted in the first chronic phase of the disease, who were conditioned with SD TBI, and received CsA and MTX as prophylaxis against GVHD. Two factors were associated with improved survival and reduced TRM: younger age when age was analyzed as a continuous variable, and HLA compatibility. Indeed, HLA-DRB1 match-

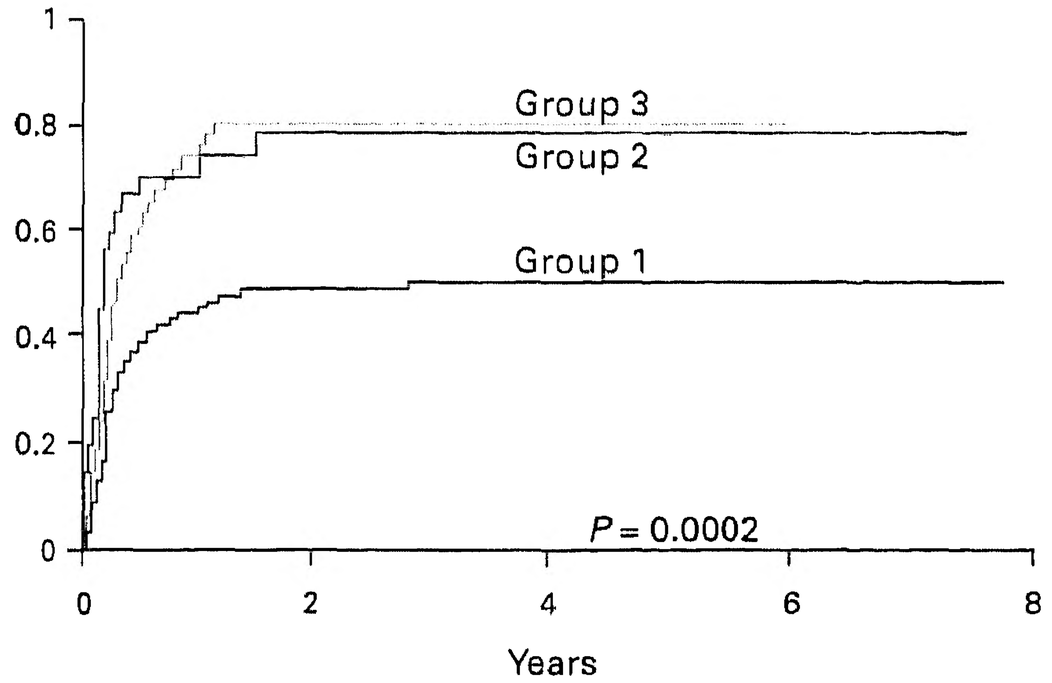

Figure 2 Transplant-related mortality in patients who received matched transplants (group 1) compared to those who received mismatched transplants (group 2) and to those for whom HLA-DRB1 typing was not done (group 3). At 2 years after transplantation, the transplant-related mortality was 49 ± 4% for the 210 DRB1-matched patients, and respectively 79 ± 8% and 80 ± 6% for 31 HLA-DRB1-mismatched transplants or when HLA-DRB1 typing was not done ($P = 0.0002$).

Table 5 Results of multivariate analysis for 286 patients

	Unfavorable prognostic factor	*Relative risk*	*P value*
Survival	Older age	1.02 (1.01–1.04)	0.0052
	Advanced phase disease	1.59 (1.15–2.22)	0.005
	DRB1 mismatched	2.07 (1.26–3.38)	0.004
	DRB1 not done	1.89 (1.29–2.78)	0.0012
LFS	Advanced phase disease	1.69 (1.23–2.3)	0.012
	In vivo/ex vivo TCD	1.48 (1.07–2.04)	0.017
	DRB1 mismatched	1.85 (1.14–3.02)	0.013
	DRB1 not done	1.77 (1.22–2.58)	0.0028
	Fractionated TBI	1.4 (1.02–1.94)	0.04
RI	Advanced phase disease	5.5 (2.27–14.3)	0.0001
	In vivo/ex vivo TCD	10.6 (3.64–30.8)	<0.0001
	Fractionated TBI	7.45 (2.35–23.6)	0.0006
TRM	Older age	1.02 (1.005–1.04)	0.012
	DRB1 mismatched	2.07 (1.25–3.44)	0.0049
	DRB1 not done	1.87 (1.24–2.8)	0.0026
	Advanced phase	1.47 (1.03–2.08)	0.03

ing was the most significant factor influencing survival ($P = 0.004$), LFS ($P = 0.013$) and TRM ($P = 0.0049$). The outcome for patients without HLA-DRB1 typing results was comparable to that for patients with an HLA-DRB1 mismatched donor and there was a significant difference when compared to HLA-DRB1 matched transplants for survival ($P = 0.0012$), LFS ($P = 0.0028$) and TRM ($P = 0.0026$).

From the results of the multivariate analysis we have defined a 'good risk' group, ie patients transplanted in first chronic phase from a matched donor and treated with CsA and MTX as prophylaxis against GVHD. The survival, LFS, TRM and RI for this group are $51 \pm 5\%$, $51 \pm 5\%$ (Figure 3), $47 \pm 5\%$ and $2 \pm 2\%$ respectively. In contrast, the TRM for patients transplanted for advanced disease from an HLA-mismatched donor is 94%.

Discussion

HLA-identical sibling donors can be found for approximately 30–40% of patients with chronic myeloid leukemia. Over the last decade, the development of numerous registries has facilitated the identification of HLA-A, -B and -DR identical volunteer unrelated donors for an additional 25–45% of patients.[20,21] However, despite a successful outcome for some patients, UD BMT is associated with a higher incidence of complications, specifically primary or secondary graft failure, severe acute GVHD, and viral infections, than HLA-identical sibling transplant.[22–24] This, together with recent publications confirming improved survival times with α-interferon and possibly with autologous transplantation,[6–8] has made the role of UD BMT in CML increasingly difficult to define. Better selection of the recipient and donor, and/or improved transplant technique may reduce the incidence of post-BMT complications and result in improved clinical outcome. In this study, we have retrospectively analyzed the impact of prognostic factors on the outcome of UD BMT in a large cohort of patients transplanted for CML in Europe, and have identified a number of variables which may be used to improve patient and donor selection.

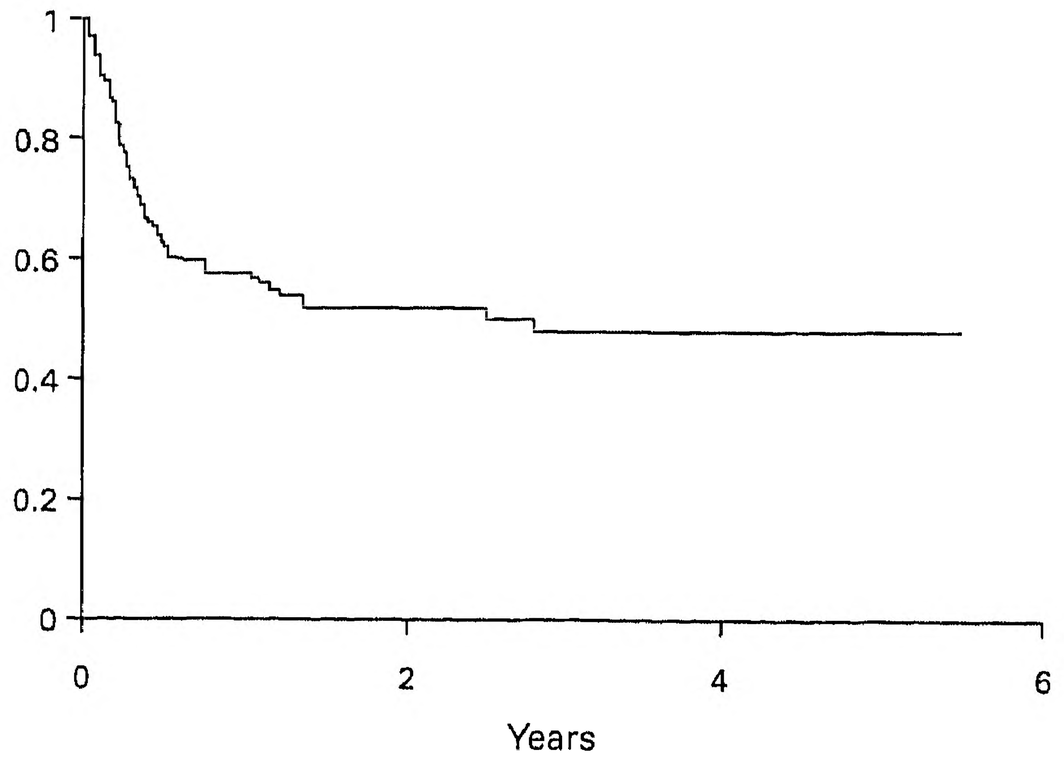

Figure 3 Kaplan–Meier probability of survival for 'good-risk' patients. At 2 years after transplantation, the probability of survival was 51% for 99 patients transplanted in first chronic phase from a matched donor and treated with CsA and MTX as prophylaxis against GVHD.

We have demonstrated that the most influential factor for survival, leukemia-free survival and transplant-related mortality is the presence of HLA disparity. This in turn is associated with an increased risk of severe acute GVHD. The selection of donors who are more precisely matched with their recipients is essential if we are to be able to reduce the incidence of GVHD-related mortality and morbidity. Due to the extreme polymorphism of certain HLA loci and limited resolution of serological typing, such mismatches have not previously been exposed. However, DNA-based typing methods for HLA-DRB1 are now available[16] and we have clearly shown that identity at this locus as defined by molecular methods strongly correlates with improved outcome compared with either serological DR matching only or HLA-DRB1 mismatching. This suggests that these mismatches are immunogenic to allo-specific HLA-DRB1 restricted T cells.

Despite the obvious advantages that HLA-DRB1 matching seems to offer, the actual benefits may have been underestimated in this study. This is because the exact definition of the alleles present may only be achieved by sequencing. DNA-based techniques to identify HLA-DRB1 alleles have been developed only within the last decade and have undergone modifications and improvements for the relatively low resolution RFLP to higher resolution PCR-SSOP and PCR-SSP. Furthermore, these latter techniques are dependent on knowledge of the sequence to optimize probe and primer design. These too have been improving as sequence information becomes available. It is likely therefore, that a higher proportion of HLA-DRB1 'matched' pairs are truly matched in patients typed recently than in those typed earlier.

The impact of HLA-DQB1 matching on the outcome of UD BMT cannot be assessed accurately as so few pairs matched at HLA-DRB1 were mismatched at HLA-DQB1. Due to the proximity of the loci (100 kb)[25] and a lack of recombination between them, strong associations have developed between HLA-DRB1 and HLA-DQB1. In practice, matching for HLA-DRB1 means matching for HLA-DQB1. Similarly those mismatched for HLA-DRB1 are likely to be mismatched for HLA-DQB1.

Linkage between HLA-DRB1 and HLA-DPB1 is not evident. A high level of recombination occurs between these loci[26] and as a result, most unrelated pairs matched at HLA-A, -B and -DRB1 are mismatched at HLA-DPB1.[27,28] However, mismatching at HLA-DPB1 did not seem to significantly affect transplant outcome in HLA-DRB1 matched pairs as has been previously reported.[29]

The role of class I mismatching cannot be estimated from this study as all the evaluable patients were class I matched by serological methods. It is probable that some degree of class I mismatching would have been apparent if in all D/R pairs the testing had included isoelectric focusing (IEF). A recent publication addressing the role of assays for cytotoxic T cell precursors (CTLp) in the selection of donors for UD BMT reported an association of high frequency CTLp and class I mismatching as determined by IEF.[30]

Several variables which are known to influence the outcome of HLA-identical sibling transplants were also found

to have a prognostic role of UD BMT. Older age was an unfavorable risk factor for survival and transplant-related mortality. Several studies have found a significant effect for recipient age.[4,24,31] More recent studies have suggested that the adverse effect of patient age may be largely related to HLA compatibility. In a series of 33 patients aged 50 years or older, who received transplants from HLA-identical family members for CML in chronic phase within 1 year of diagnosis, the probability of survival at 4 years was in excess of 80%.[32] In contrast, in a group of patients aged less than 18 years who underwent UD BMT for acute and chronic leukemias, there was no significant difference in the LFS between patients who were serologically matched with their donors and those who were mismatched.[33]

Patients transplanted for advanced disease had significantly decreased survival, LFS and increased RI. This too confirms previously reported results for both sibling and unrelated donor transplants.[4,24,31] Early transplant, ie prior to the median duration between diagnosis and BMT, conferred a benefit for survival, LFS and TRM in univariate, and RI in multivariate analysis. It is possible that there is a delay in proceeding to UD BMT compared to sibling transplant, partly because of the time restraints imposed by the need to identify a donor[34] and partly because of a natural reluctance on the part of patient and/or physician to undertake a high-risk procedure. This delay may contribute to disease progression which itself has an adverse effect on transplant outcome. Major cytogenetic responses to α-IFN are rarely seen within 9–12 months of initiation of therapy[5] and patients are understandably advised to persist with α-IFN for prolonged periods of time before considering UD BMT. However, a multivariate analysis of patients undergoing allogeneic transplants for CML has recently demonstrated an adverse effect of prolonged (ie >12 months) treatment with α-IFN. This effect was mainly attributable to an increased risk of graft failure and fatal infections. In this study, primary or secondary graft failure occurred exclusively in patients with donors other than HLA-identical family members and was further restricted to patients who had been previously exposed to α-IFN.[35] This observation requires confirmation but if correct adds further complexity to decisions relating to the optimal management of CML.

Of the 286 patients who received TBI as part of their conditioning regimen and were evaluable in multivariate analysis, one-third received SD TBI and two-thirds were treated with fractionated TBI. Fractionated TBI has an adverse influence on the relapse rate. This observation confirms that of a retrospective study from the French Registry which reported a significant increase in relapse incidence for patients treated with fractionated TBI compared to those who received SD TBI[36] but is in contrast to a number of previously published studies. In a review of the literature pertaining to TBI techniques for both acute and chronic leukemias, identical relapse rates were found for SD and fractionated TBI in patients who received T cell non-depleted bone marrow.[37] Similarly a report from the International Bone Marrow Transplant Registry did not identify fractionated TBI as a risk factor for relapse after allogeneic transplant for CML.[31] It is likely that the differences between these reports reflect the variables associated with irradiation (ie number of fractions, total dose, dose rate, radiation source, use of shielding etc) and will only be resolved when the radiobiological effects of these parameters can be accurately predicted.

The method of GVHD prophylaxis influenced both leukemia-free survival and relapse risk. The increased incidence of acute GVHD has encouraged many investigators to employ *ex vivo* or *in vivo* T cell depletion in order to reduce the associated morbidity and mortality. In this analysis, it has not been possible to investigate the effects of different methods of *ex vivo* and *in vivo* T cell depletion, due to the small number of patients in each category, but it is entirely possible that the methodology itself may affect outcome. In HLA-identical sibling marrow transplants for CML, T cell depletion has been associated with a reduction in the incidence and severity of GVHD but at the expense of a higher frequency of graft failure and leukemic relapse.[38] In a series of 462 patients (of whom 196 had CML), who received UD BMT facilitated by the National Marrow Donor Panel (NMDP), 70 received T cell-depleted grafts. They did not appear to have an increased risk of relapse when compared to patients who received unmanipulated marrow.[34] Similarly, in a series of 48 consecutive patients who received T cell-depleted unrelated marrow at a single center, the 2 year probability of relapse was low at 8.8%, suggesting an apparent preservation of graft-versus-leukemia activity.[39] However, in our large retrospective study, we have found that T cell depletion was an independent risk factor for relapse and was also associated with a decreased LFS in both univariate and multivariate analysis. It is now possible to overcome some of the deleterious effects of T cell depletion in the sibling transplant setting by the use of donor leukocyte infusions at the time of relapse.[40,41] In the future it may be appropriate to exploit the beneficial effects of T cell depletion (ie a reduced incidence and severity of acute and chronic GVHD) by administering T cell-depleted marrow and then replacing limited numbers of lymphocytes at various time points post-transplant.[42]

In summary we have identified a number of prognostic variables for UD BMT for CML. Some of these (ie age, disease status and interval from diagnosis to transplant) were expected. We have also shown the adverse effect of T cell depletion as it was performed during the years of this study. More importantly we have demonstrated the importance of accurate HLA-DRB1 matching on the outcome of transplant. Other parameters, in particular the CMV serostatus of the donor and recipient, HLA-C matching[43,44] and functional studies such as CTLp assays[30] could not be analyzed in this particular study but are likely to play a role in determining transplant outcome. In the meantime, accurate DNA-based methodology for HLA-DRB1 matching is now available and should be used to facilitate the identification of the optimal donor. Our data suggest that the long-term outcome of patients with CML with favorable prognostic features can approach that of patients transplanted from HLA-identical siblings. In contrast, the transplant-related mortality of patients transplanted in advanced phases of the disease from non-HLA-DRB1-identical donors is such that alternative therapies should be found for these individuals.

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Appendix

The following transplantation centers participated in the CLWP registry of the EBMT: Hopital Saint Louis, Paris, France; Hammersmith Hospital, London, UK; Rigshospitalet, Copenhagen, Denmark; Hospital Clinic, Barcelona, Spain; Royal Free Hospital, London, UK; Hopital Claude Hurez, Lille, France; Klinikum Großhadern, München, Germany; Abt. Innere MedizinIII, Ulm, Germany; Ospedale San Martino, Genova, Italy; Rikshospitalet, Oslo, Norway; Huddinge Hospital, Huddinge, Sweden; University Hospital St Radboud, Nijmegen, The Netherlands; Hopital du Haut Leveque, Pessac, France; Hadassah University Hospital, Jerusalem, Israel; Royal Liverpool University Hospital, Liverpool, UK; University Hospital, Leuven, Belgium; Medical School of Hannover, Hannover, Germany; Hopital Edouard Herriot, Lyon, France; Kantonspital, Basel, Switzerland; Cliniques universitaires St Luc, Brussels, Belgium; University Hospital, Innsbruck, Austria; University Hospital, Leiden, The Netherlands; Institut of Hematology Seragnoli, Bologna, Italy; Hopital Henri Mondor, Creteil, France; Hopital Michallon, Grenoble, France; Hopital Cimiez, Nice, France; Hotel-Dieu, Nantes, France; University Hospital Utrecht, The Netherlands; Glasgow Royal Infirmary, Glasgow, UK; Hopital de Brabois, Nancy, France; Hopital Cantonnal Universitaire, Geneva, Switzerland; Ospedale di Niguarda, Milano, Italy; Policlinico San Matteo, Pavia, Italy; Addenbrookes Hospital, Cambridge, UK; Hopital Jean Minjoz, Besançon, France; Hopital Necker, Paris, France; Ospedale di Carregi, Firenze, Italy; Heinrich Heine Universitat, Dusseldorf, Germany; Hopital de Enfants Malades, Paris, France; CHRU, Angers, France; Royal Infirmary, Edinburgh, UK; CHRU, Saint Etienne, France; Ospedale Regina Margherita, Torino, Italy; Charing Cross Hospital, London, UK; Institut Gustave Roussy, Villejuif, France.