total bilirubin>2.0 mg/dL), or body weight gain greater than 10% of baseline, these parameters were defined as immune reactions. Reactions were classified into the following three subtypes according to timing: pre-engraftment, peri-engraftment, and postengraftment. Immune reactions which developed 6 or more days before engraftment were defined as pre-engraftment immune reactions (PIR). Those within 5 days of engraftment were defined as ES. Others were defined as postES, which generally corresponded to acute GvHD. Acute and chronic GvHD were graded according to the consensus criteria (*25*, *28*). In the treatment of PIR, ES, and GvHD, response to corticosteroid was evaluated as reported previously (*29*).

Primary Endpoints and Statistical Analysis

The primary endpoint of this study was to investigate clinical characteristics of immune reactions after RI-UCBT. Immune reactions were divided into three categories: PIR, ES, and acute GvHD. The following variables were assessed: fever, serum levels of CRP, skin eruption, diarrhea, jaundice, central nervous system complications, weight gain greater than 10% of baseline, documented infections, and response to corticosteroid. The secondary endpoint was to investigate whether these reactions had a prognostic impact.

Overall survival (OS) and relapse-free survival (RFS) were determined using the Kaplan-Meier method. Final follow-up was conducted in July 2004, with a median follow-up of surviving patients being 16.0 (range 13.8–32.4) months. Surviving patients were censored on the last day of follow-up. ES and GvHD were analyzed in patients who achieved initial engraftment. Cumulative incidence of PIR, ES, GvHD, relapse, and nonrelapse-related mortality (NRM) were calculated using Gray's method (*30*), treating death without each type of immune reaction as a competing risk. A multivariate Cox proportional hazards model was used to identify independent and significant prognostic factors for OS and RFS. The variables entered in each analysis were patient age, sex, primary diseases, risks, number of transfused mononuclear cells, HLA-disparity, and dose of TBI. PIR and acute GvHD were included as a time-dependent covariate. A significance level of 5% was set as the limit for inclusion in the model. Prognostic factors that were significant at $P<0.05$ in the stepwise proportional model analysis were considered to be important in influencing survival.

RESULTS

Engraftment and Chimerism Analysis

Forty-five patients achieved engraftment. Median day of engraftment was day 19 (range 11–55). Cumulative incidences of engraftment and death without engraftment at day 100 were 79% and 18%, respectively (Fig. 1). Rescue of primary graft failure occurred in two patients after second RI-UCBT. The remaining 10 patients died before engraftment after a median of 24.5 (range 15–45) days. Causes of death included regimen-related toxicity (n=2), infection (n=6), progression of underlying disease (n=1), and multiple organ failure caused by pre-ES (n=1).

Chimerism data were obtained from 52 patients. Cumulative incidence of complete donor chimerism at day 60 was 97%, and median time to complete donor chimerism was 22 (range 8–54) days. Complete donor chimerism was documented in the seven patients who died of NRM before engraftment.

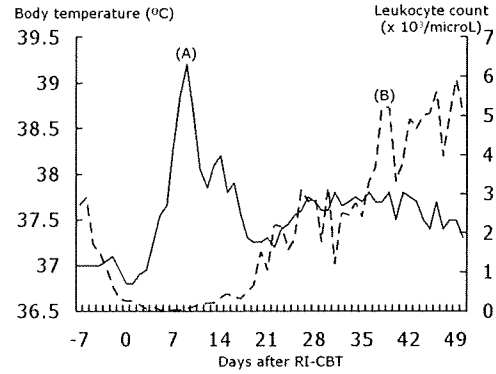

FIGURE 1. Typical clinical course of pre-engraftment immune reaction (PIR). High-grade fever developed on a median of day 9, during severe neutropenia. (A) Body temperature; (B) leukocyte count. RI-CBT, reduced-intensity cord blood transplantation.

Pre-engraftment Immune Reaction

Twelve patients who developed documented infection before engraftment were excluded from the analysis of PIR. Thirty-five of the remaining 45 (78%) patients developed PIR on a median of day 9 (range 6–13). Typical clinical courses of PIR are shown in Figure 2. PIR was observed in three patients who had never engrafted as well as those who had achieved engraftment.

Compared with ES and GvHD, body weight gain, high-grade fever, and elevation of serum levels of CRP were more frequent in PIR. In contrast, jaundice was more common in ES and GvHD than in PIR (Table 2). Histologic examination of the skin was conducted in six patients. Infiltration of mononuclear cells was not prominent in any of the six patients. Common findings were vascular dilatation (n=4) and intercellular edema in the dermis (n=4).

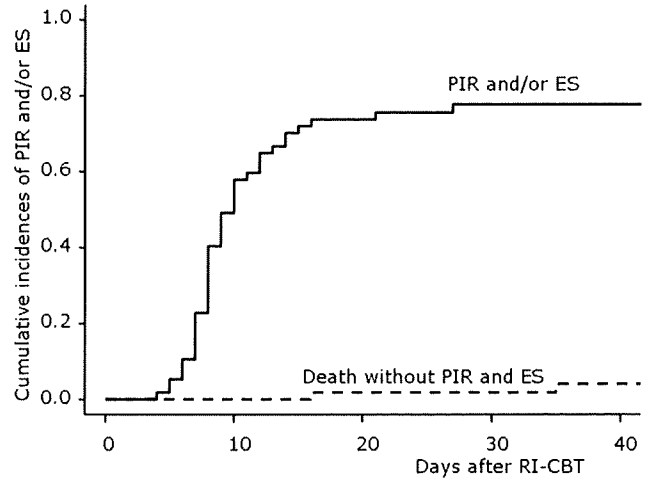

FIGURE 2. Days to PIR or engraftment syndrome (ES), treating death without these complications as a competing risk. Cumulative incidence of PIR or ES was 78%.

TABLE 2. Clinical characteristics of immune responses after reduced-intensity cord-blood transplantation

Type of immune responses	PIR	ES	GvHD
Number of patients with immune responses/number of evaluable patients	35/45	36/44	29/44
Fever (median, range)	39.8 (37.5–41.2)	39.0 (36.8–40.4)	39.2 (37.2–40.7)
Skin rash	16	15	21
Diarrhea	19	20	23
T-Bil >2.0 mg/dL	10	21	18
Body weight gain >10% of baseline	14	3	0
Central nervous system complications	0	5	0
Serum levels of C-reactive protein (mg/dL) (median, range)	14.1 (2.3–25.6)	6.5 (0.2–23)	8.3 (0.9–38.6)
Use of corticosteroid	23	25	25
Response to cortisosteroid (CR/PR/MR/NC)	14/5/3/1	4/4/1/16	15/7/1/2

PIR, preengraftment immune reactions; ES, engraftment syndrome; GvHD, graft-versus-host disease; CR, complete response; PR, partial response; MR, minimal response; NC, no change.

Among the 23 patients given corticosteroid, response was as follows: complete response (CR) (n=14), partial response (PR) (n=5), minimal response (MR) (n=3), and no change (NC) (n=1). PIR subsided spontaneously in the remaining 12 patients in whom corticosteroid had not been administered.

Engraftment Syndrome

Of the 45 patients who achieved engraftment, 44 were included in the analyses of ES, with the remaining patient being excluded because of documented *P. aeruginosa* septicemia. ES developed in 36 patients. Cumulative incidence of PIR or ES was 78% (Fig. 3). Clinical characteristics of ES are shown in Table 2. Five patients with ES developed central nervous system toxicity: cyclosporine neurotoxicity (n=1), limbic encephalopathy (n=2), and metabolic encephalopathy (n=2). No pathogens including bacteria, fungi, or viruses were cultured from cerebrospinal fluid in the five patients. Corticosteroid was given to 25 patients with ES, with the following response: CR (n=4), PR (n=4), MR (n=1), and NC (n=16). Corticosteroid was more frequently required in the patients with preceding PIR and ES than in those with de novo ES (21/27 vs. 4/9), and in the 14 patients with preceding PIR, ES was refractory to corticosteroid.

Postengraftment Immune Reactions (Acute GvHD)

Of the 45 patients who survived longer than 6 days after engraftment, 44 patients were included in the analysis of GvHD. The other patient was excluded because of *E. fecalis* bacteremia. Thirty patients developed acute GvHD: grade I (n=1), II (n=9), III (n=13), and IV (n=7). Skin or gastrointestinal biopsy was conducted in 25 patients. GvHD was histopathologically confirmed in all of these patients. Histopathologic examination was not conducted in the remaining five patients. Cumulative incidence of grade I to IV acute GvHD, treating death without GvHD as a competing risk, was 51% (Fig. 4). It was not possible to differentiate GvHD from

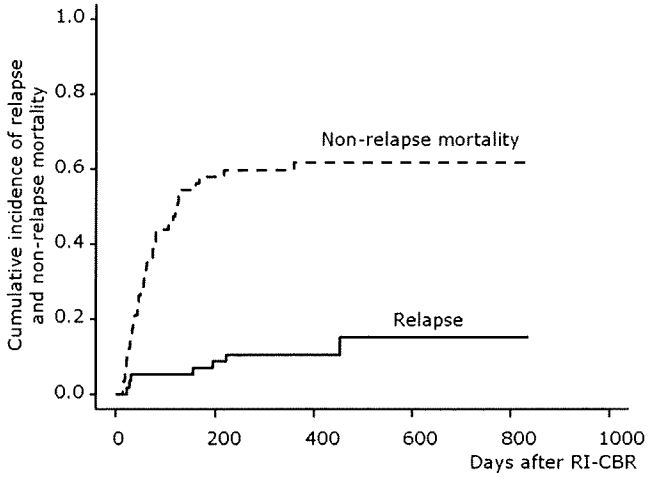

FIGURE 3. Cumulative incidences of relapse and nonrelapse mortality at day 180 were 62% and 15%, respectively.

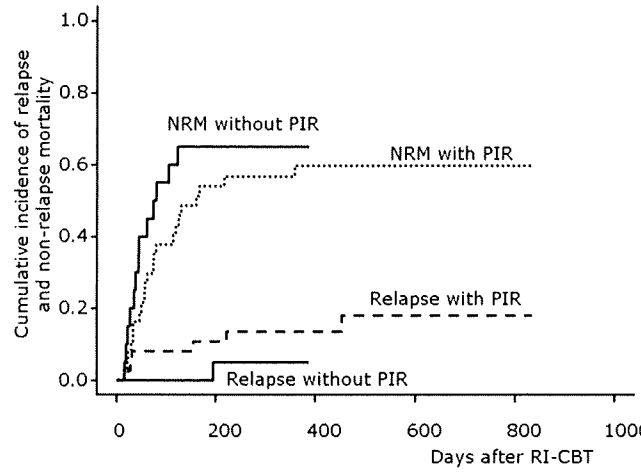

FIGURE 4. Cumulative incidences of relapse and nonrelapse mortality (NRM) for patients grouped by the presence or absence of PIR. Cumulative incidences of relapse were 18% in patients with PIR and 5% in those without it (P=0.32). Cumulative incidences of nonrelapse mortality were 60% in patients with PIR and 65% in those without it (P=0.35).

preceding PIR or ES in 15 patients who displayed continuous symptoms of immune reactions. Acute GvHD developed after resolution of PIR or ES in 12 patients. Cumulative incidences of grade I to IV acute GvHD grouped by the presence or absence of PIR were 57% and 40%, respectively ($P=0.16$).

Organ involvement was as follows: skin stage 1 (n=6), stage 2 (n=6), stage 3 (n=9), and stage 4 (n=1); liver stage 1 (n=6), stage 2 (n=5), stage 3 (n=8), and stage 4 (n=7); gut stage 1 (n=3), stage 2 (n=13), and stage 3 (n=5). Among the 25 patients given corticosteroid to treat GvHD, responses were CR (n=15), PR (n=7), MR (n=1), and NC (n=2).

Nonrelapse Mortality

Thirty-two patients died without disease progression. Cumulative incidences of relapse and NRM at day 180 were 15% and 62%, respectively. Causes of NRM comprised acute GvHD (n=5), interstitial pneumonitis (n=2), thrombotic microangiopathy (n=3), heart failure (n=2), cytomegalovirus infection (n=2), invasive aspergillosis (n=2), miliary tuberculosis (n=1), cerebral hemorrhage (n=2), bacteremia (n=7), pneumonia (n=3), multiple organ failure caused by PIR (n=1), alveolar hemorrhage (n=1), and gastrointestinal bleeding (n=1).

Effect of PIR, ES, and GvHD on Relapse and NRM

Cumulative incidences of relapse were 18% and 5% in patients with and without PIR, respectively ($P=0.32$). Cumulative incidences of NRM were 60% and 65% in patients with and without PIR, respectively ($P=0.35$). Because development of ES was closely associated with PIR, these two reactions could not be separated from each other in evaluation of their effect on relapse and NRM. Development of GvHD was not a significant prognostic factor for relapse or NRM when PIR was treated as a time-dependent covariate.

DISCUSSION

We have demonstrated that most patients exhibited some immune reactions, whereas a certain proportion of findings were accounted for by infection and regimen-related toxicity. In particular, it is likely that diarrhea was in some cases caused by melphalan, which has dose-limiting gastrointestinal toxicity (*31*). However, the development of similar reactions in most patients suggests that these reactions are characteristic of RI-CBT. PIR developed during posttransplant myelosuppression. When compared with ES and GvHD, the higher CRP levels and fever observed in PIR suggest that the inflammation occurring in this reaction is intense. Although optimal treatment remains unknown, corticosteroid was administered at the discretion of the primary physician. Most patients responded to corticosteroid, although PIR occasionally progressed and merged with ES and GvHD despite immunosuppressive treatments. Because cytokine storm associated with PIR might trigger the development of ES or GvHD, suppression of PIR could be effective in reducing NRM. This small-sized study failed to show a prognostic impact of PIR, and the clinical significance of this reaction awaits further investigation.

The mechanism of PIR remains unknown. Pathologic examination of the skin obtained from six patients showed edema and vascular dilatation without lymphocytic infiltration. Interestingly, PIR occurred in patients who had not achieved engraftment, suggesting that the mechanism of PIR differs from that of ES/GvHD. The reaction is probably related to the response of adult recipients to transplanted cord blood rather than to the cord-blood engraftment. Antithymocyte globulin (ATG) and corticosteroid, which have strong immunosuppressive properties, were commonly used in CBT (*6, 8–11, 14, 17, 32–34*), whereas neither was used in this study. Immune reactions after CBT might therefore have manifested more easily with the present regimen. PIR could be attributed to a cytokine storm induced by massive proliferation of cells with a unique cytokine profile. Another possibility is homeostasis-driven proliferation of naive T cells in highly immunosuppressed individuals, as demonstrated in murine models (*35*). This reaction is associated with cytotoxic cytokines (*35*). However, fever as a transient response to contamination with maternal blood or cells during cord-blood collection cannot be excluded (*36*), and reactivation of virus such as human herpesvirus 6 might be associated with PIR (*37*).

The reaction at engraftment was similar to the reaction known as ES after autologous transplantation (*27*). The inflammation occurring in ES was less intense than that observed in PIR, as evidenced by less marked fever, weight gain, and CRP elevation (Table 2). In this regard, corticosteroid, which was given for PIR and continued during the manifestation of ES, might have masked the inflammatory reaction of ES. Surprisingly, five patients with ES developed central nervous system complications, with two diagnosed as having limbic encephalopathy. This type of neurologic complication has not been emphasized in allo-SCT using marrow or peripheral blood and might be characteristic of CBT (*38*). Fluid accumulation during this period might have accentuated the tendency for brain edema. Engraftment processes may differ between CBT and conventional allo-SCT.

Postengraftment reaction was characterized by a higher incidence of jaundice and a lower incidence of edema when compared with PIR and ES. Clinical manifestation was consistent with the immune reaction conventionally known as acute GvHD. Although the incidence of GvHD after CBT for adult patients has been reported to be low, the incidence of grade II to IV acute GvHD varies from 25% to 72% (*9–12, 21–24, 39*) and has not been thoroughly investigated. In the present study, the incidence of grade I to IV acute GvHD was 51%. GvHD is a significant problem in RI-CBT as well as in conventional myeloablative CBT. Cord blood might have the potential to elicit an intense graft-versus-host effect, creating a niche for early engraftment and GvL effects.

Few studies have described the immune reaction after CBT, and none have characterized PIR and ES in CBT. In the present study, there are several possible reasons for these immune reactions being distinct. First, we only enrolled adult patients because children develop GvHD less frequently than do adults (*5, 6, 8*). Second, the median nucleated cell dose in our study (2.9×10^7 /kg) was greater than that reported in certain studies performed in Western countries (*9–12*). The low median body weight (53.8 kg) among the Japanese patients in this study might have favored engraftment and immune reactions. Third, 84% of our patients received cord blood from donors mismatched at tow to three HLA loci. The

association between HLA disparity and the risk of GvHD remains unclear in CBT. Although most studies have failed to show a significant relationship between HLA disparity and the risk of GvHD (*5*, *8*, *14*, *33*), a recent multivariate analysis of the largest series showed a significant association between acute GvHD and HLA disparity (*40*). Fourth, our conditioning regimen, which did not include ATG and used cyclosporine alone for GvHD prophylaxis, was mild, allowing the manifestation of immune reactions.

Although the present study provides an important description of the immune reaction after RI-UCBT, it contains certain limitations. This was a small retrospective study, and unrecognized bias caused by heterogeneous patient background might have influenced the results. Furthermore, the diagnostic criteria for immune reactions based on clinical and pathologic findings could not exclude infection or toxicity from various drugs including conditioning regimens. Therefore, it is possible that incidence of immune reactions was overestimated, particularly for PIR developing during neutropenia. In contrast, immunosuppressive treatments (mostly corticosteroid) for preceding complications could have masked the incidence and severity of ES and GvHD. ES has some similarities to acute GvHD, and it is sometimes difficult to make an accurate diagnosis of these complications. Further investigations are warranted to reveal the mechanism of immune reactions after RI-CBT and to develop a strategy of their control without reducing GVL effects.

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bjh research paper

Impact of human leucocyte antigen mismatch on graft-*versus*-host disease and graft failure after reduced intensity conditioning allogeneic haematopoietic stem cell transplantation from related donors

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Summary

The impact of human leucocyte antigen (HLA) incompatibility between donor and recipient on graft-*versus*-host disease (GVHD) and graft failure after reduced-intensity conditioning stem cell transplantation (RICT) remains to be elucidated. We retrospectively analysed outcome in 341 patients who underwent RICT from related donors for haematological malignancies. The overall cumulative incidence of grade II–IV acute GVHD (aGVHD) was 40% for all subjects; 39% in recipients with HLA-matched donors, 44% in those with one-locus-mismatched donors, and 50% in those with two- to three-loci-mismatched donors. In a Cox regression model adjusted for potential confounders, the tendency for grade II–IV aGVHD ($P = 0{\cdot}01$), chronic GVHD (cGVHD) ($P = 0{\cdot}05$) and graft failure ($P = 0{\cdot}033$) increased with HLA disparity. Use of peripheral blood grafts instead of marrow was a risk factor for cGVHD. Use of antithymocyte globulin was associated with reduced aGVHD and cGVHD. Overall survival (OS) in recipients of two- to three-loci-mismatched RICT at 2 years (18%) was significantly worse than that in patients who received one-locus-mismatched RICT (51%) and HLA-matched RICT (48%) ($P < 0{\cdot}0001$). A two- to three-loci mismatch was identified as an independent risk factor for OS ($P < 0{\cdot}001$), but there was no significant difference in OS between HLA-matched and one-locus-mismatched RICT. HLA incompatibility between the donor and recipient is an important risk factor for graft failure, aGVHD, cGVHD and OS after RICT. RICT from a one-locus-mismatched donor may represent an effective alternative approach in patients with high-risk malignancies who lack HLA-matched related donors.

Keywords: human leucocyte antigen, graft-*versus*-host disease, rejection, reduced intensity conditioning, antithymocyte globulin.

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Allogeneic haematopoietic stem cell transplantation (SCT) is a potentially curative treatment for haematologic malignancies. A growing body of evidence suggests that allogeneic SCT is also useful for the treatment of bone marrow failure, congenital metabolic disorders, non-haematologic disorders including solid tumours and autoimmune diseases (Burt *et al*, 2003; Slavin *et al*, 2004). However, high transplant-related mortality (TRM) precludes the wider application of allogeneic SCT for these diseases. Recently, several investigators have reported encouraging results with allogeneic SCT using a reduced-intensity conditioning (RIC) transplant regimen (RICT) (Bacigalupo, 2004). These regimens have been designed to reduce TRM and provide a platform for durable donor cell engraftment to exploit a graft-*versus*-tumour effect.

Graft-*versus*-host disease (GVHD) is still a major obstacle to allogeneic SCT. Human leucocyte antigen (HLA) disparity between the SCT donor and recipient is the most critical factor that governs the severity of GVHD after conventional allogeneic SCT. Studies in patients who have been transplanted from a related donor other than an HLA-identical sibling after myeloablative conditioning have shown that HLA incompatibility increases the incidence and severity of acute GVHD (aGVHD), as well as the incidence of graft failure (Beatty *et al*, 1985). Although it was initially assumed that RIC may reduce the incidence of GVHD, GVHD appears to be a significant clinical problem following RICT (Khouri *et al*, 1998; Slavin *et al*, 1998; Nagler *et al*, 2000; Schetelig *et al*, 2002; Mielcarek *et al*, 2003; Bacigalupo, 2004; Diaconescu *et al*, 2004). While most studies on RICT have been performed in an HLA-matched related setting, alternative donor grafts are increasingly used in RICT (Kottaridis *et al*, 2000; Giralt *et al*, 2001; Maris *et al*, 2003; Niederwieser *et al*, 2003; Wong *et al*, 2003; Bacigalupo, 2004; Goggins & Rizzieri, 2004). Our current knowledge regarding the association of HLA incompatibility with GVHD, graft failure and survival is based primarily on results obtained in the setting of conventional and myeloablative allogeneic SCT. However, the risk factors that affect the transplant outcome after RICT, including engraftment, GVHD and survival, are still poorly defined. The present study was performed to analyse the impact of HLA incompatibility on graft failure, aGVHD, chronic GVHD (cGVHD) and survival in patients with haematological malignancies who received RICT from a related donor.

Patients and methods

Patients

We retrospectively analysed data from patients with haematological malignancies who underwent RICT from a related donor at 21 transplant centres in Japan. This study was approved by Institutional Review Board of each individual centre. All patients were treated with RIC regimens before allogeneic SCT because of high-risk clinical features that made them ineligible for conventional myeloablative allogeneic SCT. The stem cell source was either bone marrow or granulocyte-colony stimulating factor (G-CSF)-mobilised peripheral blood stem cells (PBSC) from related donors. Patients who received a manipulated graft and those who received cord blood were excluded from the analyses. Patients who received a graft from an HLA-matched non-sibling donor were also excluded because the numerous secondary factors and minor histocompatibility antigens present were significantly different to those in full-sibling matches. A total of 341 patients who underwent allogeneic RICT from related donors for haematological malignancies between 1998 and 2004 were evaluated in this study.

Transplantation procedure

Serologic typing for HLA-A, -B and -DR antigens of the donor and recipient was performed with a standard two-stage complement-dependent test of microcytotoxicity. Serologically HLA-matched sibling pairs were considered to be genotypically HLA-identical based on the results of family analysis. In pairs other than serologically identical sibling pairs, alleles at the HLA-A, -B and -DRB1 loci were identified by middle-resolution DNA typing as described previously (Sasazuki *et al*, 1998). HLA-mismatch in the graft-*versus*-host (GVH) vector was defined when the recipient's antigens or alleles were not shared by the donor, while mismatch in the host *versus* donor (HVG) vector was defined as when the donor's antigens or alleles were not shared by the recipients. The conditioning regimen and GVHD prophylaxis were conducted according to the guidelines of each institution. RIC regimens were defined as reported previously (Bacigalupo, 2002, 2004; Champlin *et al*, 2000). The most frequently used RIC regimens were fludarabine-based (fludarabine 150–180 mg/m^2 with either cyclophosphamide 60 mg/kg, busulphan 8 mg/kg or melphalan 80–140 mg/m^2) with or without either total body irradiation (TBI) 2–4 Gy or antithymocyte globulin (ATG) 5–10 mg/kg. Patients conditioned with >6 Gy TBI and those conditioned with >8 mg/kg of busulphan were excluded from the study. The most frequently used prophylaxis regimens for GVHD were ciclosporin (CSP) alone or CSP plus methotrexate (MTX).

Definitions

Risk status at transplantation was categorised as either standard risk or high risk. Standard-risk diseases included acute leukaemia in first complete remission, chronic myeloid leukaemia in first chronic phase and refractory anaemia of myelodysplastic syndrome. Other diseases were categorised as high-risk disease. Graft failure was analysed in patients who survived more than 28 d post-transplant according to the criteria reported previously (Petersdorf *et al*, 2001); graft failure was defined as failure of the absolute neutrophil count (ANC) to surpass 0·5 × 10^9/l before relapse, death or second

transplantation, as well as a decrease in the ANC to $<0{\cdot}1 \times 10^9$/l on at least three consecutive determinations with a finding of severe hypoplastic marrow. The aGVHD, graded according to the standard criteria (Przepiorka *et al*, 1995), was defined as moderate to severe (grade II–IV) disease. All patients who had no evidence of graft failure were considered to be evaluable for aGVHD. GVHD persisting beyond day +100 or *de novo* GVHD occurring after day +100 was classified as cGVHD. Biopsy-proven cGVHD occurring between days 80 and 100 was also included. The incidence of cGVHD was calculated in patients followed for at least 100 d and was classified as none, limited or extensive as well as none, *de novo*, quiescent or progressive (Sullivan *et al*, 1991). Overall survival (OS) was defined as the duration of survival between transplant and either death or the last follow-up.

Statistical analysis

The primary endpoint of this study was the incidence of grade II–IV aGVHD and graft failure. The secondary endpoint was the incidence of cGVHD and OS among the patients. The cumulative incidence of aGVHD was calculated using a method described by Gooley *et al* (1999) to eliminate the effect of competing risks. The competing event for aGVHD was defined as death without aGVHD II–IV. For each endpoint, a Cox proportional hazard model was used for uni- and multivariate analyses. The factors included in the analysis were HLA disparity (one-locus mismatch, two- to three-loci mismatch *versus* identical), type of graft (bone marrow *versus* PBSC), previous history of SCT (yes *versus* no), type of donor (family *versus* sibling), recipient age (age 60 years or more *versus* less than 60 years), use of TBI (yes *versus* no), use of ATG (yes *versus* no), GVHD prophylaxis (CSP with MTX, tacrolimus with MTX, and others *versus* CSP alone), and risk status (standard *versus* high). To evaluate the association between CD34 cell counts and the development of aGVHD, subjects were categorised into three groups by tertile and linearity was assessed by score test in a proportional hazard model. We defined statistical significance as a P-value $<0{\cdot}05$. All the statistical analyses were performed using STATA version 8 (STATA Corp., College Station, TX, USA).

Results

Patient characteristics

The numbers of patients who received a graft from an HLA-matched, one-locus-mismatched and two- to three-loci-mismatched donor were 250, 57 and 34 respectively (Table I). The respective median age of these patients were 54, 50·5 and 46·5 years. Among 341 patients, 286 received a graft from a sibling donor and 55 received a graft from a family member other than a sibling. Family donors included 22 sons, 17 daughters, three fathers, 10 mothers, one uncle and two unknown. A total of 110 patients had malignant lymphoma, 106 had acute leukaemia, 74 had mylodysplastic syndrome, 30 multiple myeloma and 21 had chronic myeloid leukaemia. A total of 323 patients received PBSC, whereas the remaining 18 were given bone marrow. The HLA-matched group included significantly higher proportions of patients who did not receive ATG ($P < 0{\cdot}001$) and those who were given CSP alone for GVHD prophylaxis ($P < 0{\cdot}001$) compared with the HLA-mismatched group. Gender, disease, risk status at transplant, previous history of SCT, stem cell source, use of a TBI-containing conditioning regimen and year of transplant were evenly distributed between the groups (Table I).

Acute GVHD

The cumulative incidence of grade II–IV aGVHD in this study population was 40% (95% CI, 35–46%) (Fig 1A). It was 39% (95% CI, 33–45%) in recipients with HLA-matched donors, 44% (95% CI, 30–57%) in those with one-locus-mismatched donors, and 50% (95% CI, 29–68%) in those with two- to three-loci-mismatched donors (Fig 1B); there was a marginally significant difference between two- to three-loci-mismatched RICT and HLA-matched RICT [hazard ratio (HR), 1·72; 95% CI, 0·94–3·14; $P = 0{\cdot}079$]. Similar results were obtained when the incidence of grade III–IV severe aGVHD was analysed (data not shown). A relationship between multiple incompatibility for HLA and a risk of aGVHD was further supported by a Cox regression model adjusted for potential confounders (Table II). Patients who received a graft from a one-locus-mismatched donor and a two- to three-loci-mismatched donor had a HR for aGVHD of 1·83 (95% CI, 1·04–3·22; $P = 0{\cdot}035$) and 2·44 (95% CI, $1{\cdot}14$–$5{\cdot}21_3$; $P = 0{\cdot}021$), respectively, when compared with those from an HLA-matched donor. A greater incidence of grade II–IV aGVHD was observed with increased HLA disparity ($P = 0{\cdot}010$). Thus, the number of mismatched HLA loci between the donor and recipient was thus a continuous variable with respect to the incidence of grade II–IV aGVHD. No other variables significantly influenced the development of aGVHD after RICT. In patients receiving PBSC grafts, there was no association between the numbers of $CD34^+$ cells and the development of aGVHD ($P = 0{\cdot}904$).

Of note, the development of aGVHD did not reach a plateau within 3 months after RICT, with a median onset on day 30 (Fig 1). The onset of aGVHD was earlier after two- to three-loci-mismatched RICT compared with HLA-matched RICT, and reached a plateau within 40 d post-transplant. The median number of days before the onset of aGVHD after HLA-matched RICT, one-locus-mismatched RICT and two- to three-loci-mismatched RICT was 39, 18 and 24 respectively.

Human leucocyte antigen-C typing is not routinely performed in haemopoietic stem cell transplantation (HSCT) from a related donor in Japan. In this study, HLA-C typing data were available in 75 donor–recipient pairs. Acute GVHD developed in 32% (95% CI, 21–44%) of patients who received a graft from an HLA-C matched donor, and in 56% (95% CI,

Table I. Characteristics of subjects according to human leucocyte antigen-matching status.

	Identical ($n = 250$)	One-mismatched ($n = 57$)	Two or more mismatched ($n = 34$)	P-value
Recipient age (range, median)	16–70, 54	25–61, 50·5	21–60, 46·5	0·03
Recipient sex, female:male (unknown)	109:141	43:14	20:14	0·007
Previous history of HCT, no:yes	205:45	27:9	36:19	0·64
Disease				
Acute leukaemia	73	17	16	
Chronic myeloid leukaemia	16	3	2	
Myelodysplastic syndrome	48	18	8	
Malignant lymphoma	90	15	5	
Multiple myeloma	23	4	3	0·163
Risk status				
Standard	52	8	3	
High	198	49	31	0·154
HCT type				
PBSC	236	55	32	
BM	14	2	2	0·805
Donor				
Sibling	250	28	8	
Family	0	29	26	
Year of transplant				
<2000	5	1	1	
2000+	245	56	33	0·922
TBI				
No	213	44	25	
Yes	37	13	9	0·117
ATG				
No	236	36	20	
Yes	14	21	14	<0·001
GVHD prophylaxis				
CSP alone	99	8	10	
CSP + MTX	122	30	10	
FK + MTX	5	13	12	
Other	24	6	2	<0·001

HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.

21–86%) of those who received a graft from an HLA-C mismatched donor. Although this difference was not statistically significant ($P = 0·261$), the impact of HLA-C mismatch on the incidence of aGVHD remains to be elucidated because of limited numbers of subjects for evaluation.

CGVHD

Recipients of HLA-matched RICT ($n = 156$) and 42 recipients of HLA-mismatched RICT (one-locus mismatch, 32; two- to three-loci mismatch, 10) were evaluable for cGVHD. The cumulative incidence of cGVHD was 61% (95% CI, 52–67%), 69% (95% CI, 44–77%) and 67% (95% CI, 28–88%) after HLA-matched RICT, HLA one-locus-mismatched RICT and two- to three-loci-mismatched RICT respectively (Fig 2). The cumulative incidence of extensive cGVHD was 38%, 34% and 60% after HLA-matched RICT, HLA one-locus and two- to three-loci-mismatched RICT respectively. There was no significant difference between the three groups regarding the incidence of cGVHD or extensive cGVHD. Similarly, there was no significant difference between the groups in the incidence of *de novo*, quiescent and progressive cGVHD. As shown in Table III, a tendency for cGVHD was observed with increased HLA disparity in multivariate analysis ($P = 0·05$). In addition, use of PBSC grafts, no use of ATG, and high-risk disease were identified as independent risk factors for cGVHD. In patients receiving PBSC grafts, there was no association between the numbers of $CD34^+$ cells and the development of cGVHD ($P = 0·613$; HR, 0·95; 95% CI, 0·76–118).

Graft failure

The incidence of graft failure was 3·7% (95% CI, 1·7–6·9%) in recipients with an HLA-matched donor, 5·7% (95% CI,

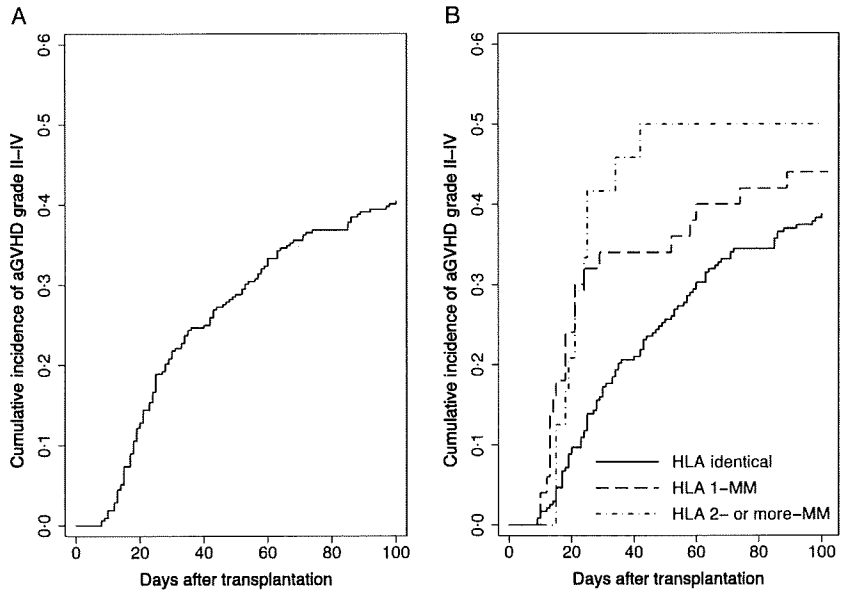

Fig 1. Incidence of grade II–IV acute graft-*versus*-host disease (aGVHD). The curves represent the cumulative incidence of grade II–IV aGVHD in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation (A) for all available subjects ($n = 312$) and (B) in relation to the extent of human leucocyte antigen mismatch (identical, $n = 238$; one-locus mismatch, $n = 22$ and two to three-loci mismatch, $n = 7$).

1·2–15·7%) in those with a one-locus-mismatched donor, and 10·3% (95% CI, 2·2–27·4%) in those with a two- to three-loci-mismatched donor. Multivariate analysis revealed a significant increase of graft failure in patients who received a graft from a two- to three-loci-mismatched donor (HR, 8·58; 95% CI, 1·37–53·9; $P = 0·022$, Table IV), and the extent of HLA mismatch between the donor and recipient was a continuous variable with respect to the incidence of graft failure ($P = 0·033$). Use of ATG did not significantly influence the incidence of rejection after RICT ($P = 0·166$).

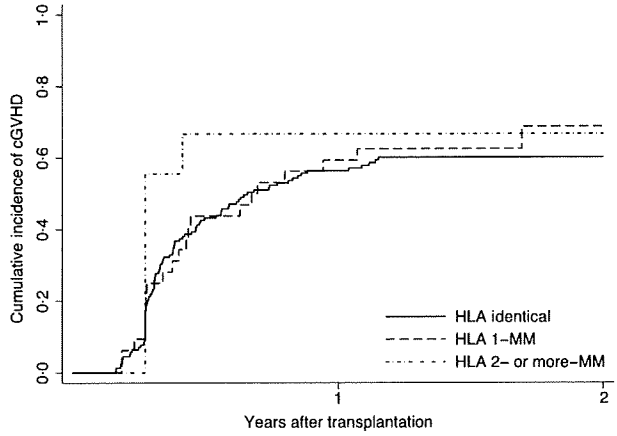

Fig 2. Incidence of chronic graft-*versus*-host disease (cGVHD). The curves represent the cumulative incidence of cGVHD in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation in relation to the extent of human leucocyte antigen mismatch (identical, $n = 156$; one-locus mismatch, $n = 32$ and two-to three-loci mismatch, $n = 10$).

Survival

To elucidate the impact of HLA mismatch on transplant outcome, OS was analysed. With a median follow-up of 347 d, OS in patients who received a graft from an HLA-matched donor, a one-locus-mismatched donor and a two-to three-loci-mismatched donor was 48% (95% CI, 42–54%), 51% (95% CI, 39–61%) and 18% (95% CI, 7–32%), respectively, at 2 years after RICT. OS after HLA one-locus-mismatched RICT was comparable with that after HLA-matched RICT (Fig 3). However, OS after two- to three-loci-mismatched RICT was significantly worse post-transplant compared with that after HLA-matched RICT. Multivariate analysis identified two- to three-loci HLA mismatch (HR, 3·41; 95% CI, 2·03–5·73; $P < 0·001$), previous history of haematopoietic cell transplantation (HR, 1·42; 95% CI, 1·00–2·02; $P = 0·052$), and high-risk disease (HR, 2·06; 95% CI, 1·33–3·30; $P = 0·002$) as independent risk factors for shorter survival (Table V). As only three patients with standard-risk disease received RICT from a two- to three-loci-mismatched donor, the relationship between multiple HLA incompatibility and OS was further evaluated in patients with high-risk diseases and those with standard diseases separately. In high-risk patients, the 2-year OS was 15% (95% CI, 5–30%), 52% (95% CI, 40–63%) and 43% (95% CI, 36–49%) in recipients with a two- to three-loci-mismatched donor, one-locus-mismatched donor and matched donor respectively. HLA

Table II. Uni- and multivariate analyses for possible risk factors for acute GVHD of grade II or more.

	Evaluable (n = 313)					
	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
HLA						
Identical	1·00			1·00		
1-MM	1·29	0·81–2·05	0·282	1·83	1·04–3·22	0·035
2 or more MM	1·72	0·94–3·14	0·079	2·44	1·14–5·21	0·021
		Trend	0·068		Trend	0·010
HCT type						
PBSC	1·00			1·00		
BM	0·65	0·26–1·59	0·342	0·70	0·28–1·73	0·441
Previous history of HCT						
No	1·00			1·00		
Yes	1·15	0·75–1·77	0·52	0·90	0·57–1·44	0·663
Recipient age (years)						
<60	1·00			1·00		
≥60	0·90	0·57–1·42	0·656	0·94	0·59–1·50	0·808
Recipient sex						
Female	1·00			1·00		
Male	1·16	0·82–1·66	0·401	1·28	0·88–1·84	0·196
TBI						
No	1·00			1·00		
Yes	0·96	0·60–1·55	0·881	0·77	0·43–1·38	0·386
ATG						
No	1·00			1·00		
Yes	0·85	0·50–1·44	0·543	0·55	0·29–1·02	0·057
GVHD prophylaxis						
CSP alone	1·00			1·00		
CSP + MTX	0·74	0·50–1·09	0·124	0·70	0·46–1·04	0·079
FK + MTX	1·07	0·57–2·02	0·826	0·70	0·32–1·50	0·355
Other	1·12	0·61–2·06	0·724	1·37	0·65–2·90	0·410
Risk						
Standard	1·00			1·00		
High	1·52	0·93–2·47	0·095	1·43	0·87–2·36	0·159

GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; MM, mismatched; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

two- to three-loci mismatch, but not one-locus mismatch, was again a risk factor for shorter survival ($P < 0·0001$, Fig 4A). In standard-risk patients, the 2-year OS was 73% (95% CI, 58–84%), 40% (95% CI, 12–67%) and 38% (95% CI, 1–81%) in recipients with a matched, one-locus-mismatched donor and two- to three-loci-mismatched donor respectively (Fig 4B). In contrast to high-risk disease, one-locus mismatch was a risk factor for shorter survival in patients with standard-risk disease ($P = 0·079$).

Of the 178 patients who died following RICT, 90 deaths were directly attributed to disease progression or relapse. Non-relapse mortality was 49%, including 60 deaths (34%) because of infection and/or GVHD, 22 deaths (12%) from other transplant-related toxicities and six deaths (3%) from other diseases. There was no difference in the cause of death between HLA-matched RICT and HLA-mismatched RICT (data not shown).

Discussion

In this study, we found that HLA disparity was a continuous and independent risk factor for grade II–IV aGVHD and cGVHD as well as graft failure following RICT. Furthermore, two- to three-loci HLA mismatch was a risk factor for survival, and one-locus mismatch was a risk factor for survival in patients with standard-risk disease, but not in those with high-risk disease. Our finding, that HLA disparity impacts on grade II–IV aGVHD in patients receiving RICT, was quite consistent

Table III. Uni- and multivariate analyses for possible risk factors for chronic GVHD.

	Evaluable (n = 198)					
	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
HLA						
Identical	1·00			1·00		
1-MM	1·09	0·68–1·76	0·709	1·57	0·89–2·75	0·116
2 or more MM	1·25	0·55–2·85	0·6	2·20	0·84–5·75	0·108
		Trend	0·543		Trend	0·050
HCT type						
PBSC	1·00			1·00		
BM	0·31	0·10–0·97	0·044	0·28	0·88–0·90	0·032
Previous history of HCT						
No	1·00			1·00		
Yes	0·90	0·56–1·42	0·641	0·75	0·45–1·26	0·274
Recipient age (years)						
<60	1·00			1·00		
≥60	1·31	0·86–1·99	0·214	1·16	0·74–1·81	0·512
Recipient sex						
Female	1·00			1·00		
Male	1·18	0·82–1·69	0·375	1·27	0·87–1·87	0·221
TBI						
No	1·00			1·00		
Yes	0·76	0·46–1·24	0·265	0·61	0·33–1·11	0·104
ATG						
No	1·00			1·00		
Yes	0·66	0·36–1·23	0·194	0·42	0·20–0·85	0·016
GVHD prophylaxis						
CSP alone	1·00			1·00		
CSP + MTX	1·10	0·72–1·68	0·67	1·00	0·63–1·56	0·966
FK + MTX	0·76	0·37–1·55	0·444	0·53	0·23–1·24	0·143
Other	0·98	0·51–1·88	0·961	1·36	0·63–2·96	0·435
Risk						
Standard	1·00			1·00		
High	1·67	1·06–2·64	0·027	1·74	1·09–2·79	0·021

GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; MM, mismatch; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, antithymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

Table IV. Uni- and multivariate analyses for host-*versus*-graft human leucocyte antigen mismatching on graft failure risk.

	Evaluable (n = 325)							
			Univariate			Multivariate		
	Rejected	Not-rejected	HR	95% CI	P-value	HR	95% CI	P-value
Identical	9	234	1·00	Reference		1·00	Reference	
1-MM	3	50	1·6	0·42–6·12	0·337	1·18	0·19–7·26	0·855
2 or more MM	3	26	3·01	0·77–11·8	0·114	8·58	1·37–53·9	0·022
				Trend	0·035		Trend	0·033

HR, hazard ratio; MM, mismatch.
Multivariate analysis adjusted for age, sex, donor type, previous history of haematopoietic cell transplantation, total body irradiation conditioning, antithymocyte globulin conditioning, graft-*versus*-host disease prophylaxis and risk status.

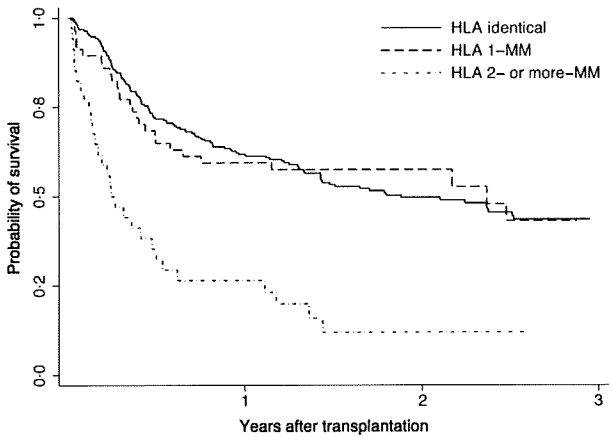

Fig 3. Overall survival (OS) based on the extent of human leucocyte antigen (HLA) mismatch. The curves represent OS in patients with haematological malignancies following reduced-intensity conditioning transplant regimen from a related donor as a function of time after transplantation in relation to the extent of HLA mismatch (identical, $n = 250$; one-locus mismatch, $n = 56$ and two to three-loci mismatch, $n = 34$).

with earlier findings in conventional myeloablative SCT (Beatty *et al*, 1985; Ringden & Nilsson, 1985; Anasetti *et al*, 1990; Anasetti & Hansen, 1994; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003), but has not been well described in the setting of RICT.

The cumulative incidence of grade II–IV aGVHD after HLA-matched RICT was 39% in this study population, which is similar to that in recent reports from other groups following RICT (Levine *et al*, 2003; Martino *et al*, 2003; Wong *et al*, 2003; Bacigalupo, 2004; Diaconescu *et al*, 2004; Goggins & Rizzieri, 2004), although a recent retrospective comparison of myeloablative SCT with RICT conditioned with 2 Gy TBI and fludarabine showed less aGVHD after RICT in matched unrelated donor transplants (Sorror *et al*, 2004).

It was initially assumed that the incidence and severity of aGVHD might decrease after RICT compared with conventional SCT. Pretransplant conditioning can activate host tissues to secrete inflammatory cytokines and amplify GVHD (Xun *et al*, 1994). The relationship between conditioning intensity, inflammatory cytokine and GVHD severity was further supported by animal models (Hill *et al*, 1997) and clinical observation (Gale *et al*, 1987; Clift *et al*, 1990; Deeg *et al*, 1991). In experimental models, the development of mixed donor–host chimaerism may facilitate the establishment of anti-host tolerance (Colson *et al*, 1996; Manilay *et al*, 1998). In contrast, minimally cytotoxic conditioning may enable the persistence of host antigen-presenting cells, which would enhance presentation of host alloantigens to donor T cells (Shlomchik *et al*, 1999; Teshima *et al*, 2002; Duffner *et al*, 2004).

There are several possible explanations that can account for this unexpectedly high incidence of aGVHD after RICT. First, the median age of patients who underwent RICT in the current study was 53 years, which was much higher than that among patients who received conventional SCT. A greater age has been associated with an increased risk for GVHD after conventional SCT (Ringden & Nilsson, 1985; Gale *et al*, 1987; Anasetti *et al*, 1990; Weisdorf *et al*, 1991; Nash *et al*, 1992). Secondly, CSP alone was administered for GVHD prophylaxis in one-third of the patients in this study, whereas a combination of two agents was exclusively used in conventional SCT. Thirdly, the RIC regimens used in our study were more intensive than the non-myeloablative conditioning regimens used by the Seattle group (Sorror *et al*, 2004). These differences may counterbalance the potential beneficial aspects of reducing the intensity of conditioning. Nonetheless, the onset of GVHD was delayed in RICT; 15% of aGVHD developed between days 60 and 100 in our study, as previously reported (Mielcarek *et al*, 2003). Following conventional SCT, most of aGVHD develops within 50 d post-transplant (Snover, 1984; Beatty *et al*, 1985; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003).

The incidence of aGVHD rose with increasing HLA mismatch, from match through multi-loci mismatch, following RICT. In addition, the onset of aGVHD was earlier with increasing HLA mismatch. These findings are consistent with data from the myeloablative setting (Beatty *et al*, 1985; Ringden & Nilsson, 1985; Anasetti *et al*, 1990; Anasetti & Hansen, 1994; Petersdorf *et al*, 1998; Sasazuki *et al*, 1998; Morishima *et al*, 2002; Kanda *et al*, 2003). Studies from the Japan Marrow Donor Programme and others have demonstrated the importance of HLA-C mismatching in rejection, GVHD and mortality in myeloablative SCT from unrelated donors (Petersdorf *et al*, 2001, 2004; Morishima *et al*, 2002; Flomenberg *et al*, 2004; Sasazuki *et al*, 1998). We did not find a significant association between HLA-C mismatch and the development of aGVHD, but this association needs to be further investigated in a larger prospective study because only 75 donor–recipient pairs were available for analysis in this study.

We found that the use of ATG was associated with a reduction in both acute and chronic GVHD without an increased risk of graft failure, as previously shown in studies using alemtuzumab or ATG (Kottaridis *et al*, 2000; Khouri *et al*, 2001; Mohty *et al*, 2003; Nakai *et al*, 2003; Faulkner *et al*, 2004). Initial clinical trials of matched unrelated RICT or haploidentical RICT appear to be encouraging with the use of T-cell depletion (Sykes *et al*, 1999; Kottaridis *et al*, 2000; Giralt *et al*, 2001; Nagler *et al*, 2001; Chakraverty *et al*, 2002; Maris *et al*, 2003; Niederwieser *et al*, 2003; Wong *et al*, 2003; Goggins & Rizzieri, 2004). Mohty *et al* (2003) reported that a high $CD34^+$ cell dose was associated with an increased incidence of chronic, but not acute, GVHD following RICT with G-CSF-mobilised PBSC. We did not find an association between acute or chronic GVHD and the number of $CD34^+$ cells infused. Interestingly, in contrast to data from a myeloablative setting (Ringden & Nilsson, 1985; Gale *et al*, 1987; Anasetti *et al*, 1990; Weisdorf *et al*, 1991; Nash *et al*, 1992), there was no

Table V. Uni- and multivariate analyses for possible risk factors for overall survival.

	Evaluable (n = 286)					
	Univariate			Multivariate		
	HR	95% CI	P-value	HR	95% CI	P-value
HLA						
Identical	1·00			1·00		
1-MM	1·01	0·67–1·53	0·966	1·03	0·64–1·66	0·899
2 or more MM	3·21	2·14–4·82	<0·001	3·41	2·03–5·73	<0·001
		Trend	<0·001		Trend	<0·001
HCT type						
PBSCT	1·00			1·00		
BMT	1·19	0·63–2·25	0·598	1·53	0·79–2·94	0·205
Previous history of HCT						
No	1·00			1·00		
Yes	1·64	1·18–2·28	0·003	1·42	1·00–2·02	0·052
Recipient age (years)						
<60	1·00			1·00		
≥60	1·05	0·73–1·51	0·808	1·09	0·75–1·60	0·65
Recipient sex						
Female	1·00			1·00		
Male	1·29	0·96–1·74	0·095	1·19	0·88–1·61	0·264
TBI						
No	1·00			1·00		
Yes	1·25	0·86–1·82	0·239	1·28	0·84–1·94	0·25
ATG						
No	1·00			1·00		
Yes	1·15	0·77–1·72	0·482	0·71	0·44–1·16	0·177
GVHD prophylaxis						
CSP alone	1·00			1·00		
CSP + MTX	0·92	0·66–1·27	0·614	0·89	0·64–1·24	0·488
FK + MTX	1·17	0·70–1·98	0·546	0·87	0·48–1·57	0·64
Other	0·89	0·52–1·51	0·66	0·69	0·38–1·25	0·223
Risk						
Standard	1·00			1·00		
High	2·21	1·42–3·43	<0·001	2·06	1·31–3·25	0·002

HLA, human leucocyte antigen; MM, mis-match; HCT, haematopoietic cell transplantation; PBSC, peripheral blood stem cells; BM, bone marrow; TBI, total body irradiation; ATG, anti-thymocyte globulin; CSP, ciclosporin; MTX, methotrexate; FK, tacrolimus.
Multivariable adjusted for all variables listed.

increase in the incidence of aGVHD in elderly patients, as previously reported (Mohty *et al*, 2002; Wong *et al*, 2003). It has been shown in experimental models that donor T-cell responses are enhanced under stimulation with antigen-presenting cells from older mice in the context of proinflammatory milieu (Ordemann *et al*, 2002). The absence of excessive inflammation in RICT may be associated with a similar incidence of GVHD in aged recipients following RICT.

The incidence of cGVHD was similar to that following myeloablative HSCT in Japan (Kanda *et al*, 2003). A greater incidence of cGVHD was observed with increased HLA disparity, although the difference was marginal. Chronic GVHD was influenced by the use of ATG, disease status and the stem cell source, with PBSC grafts having a fourfold risk of cGVHD. It has been shown that the use of PBSC grafts instead of marrow increases the frequency of cGVHD following myeloablative HSCT (Blaise *et al*, 2000; Bensinger *et al*, 2001; Cutler *et al*, 2001). Our study suggest that the use of PBSC grafts is also a risk factor for cGVHD following RICT, although there was a large difference between the number of recipients of a PBSC graft and those who received a bone marrow (BM) graft.

In a conventional allogeneic transplant setting, the incidence of graft failure has been shown to be correlated with the degree of HLA mismatch between donors and recipients (Beatty *et al*, 1985; Anasetti *et al*, 1989; Petersdorf *et al*, 1998, 2001; Petersdorf *et al*, 1997). We found that HLA mismatch was also a risk factor for graft failure following RICT. The incidence of graft failure after HLA-matched RICT was comparable with that after conventional SCT in Japan

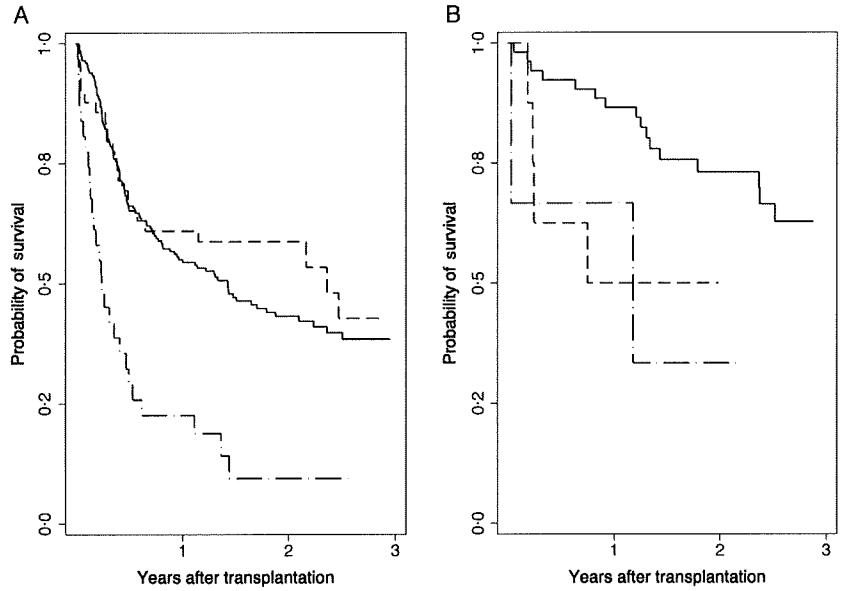

Fig 4. Overall survival (OS) according to the extent of human leucocyte antigen (HLA) mismatch and disease status. The curves represent OS as a function of time after transplantation in relation to the extent of HLA mismatch in (A) patients with a high-risk disease (total, $n = 277$; identical, $n = 198$; one-locus mismatch, $n = 48$ and two to three-loci mismatch, $n = 31$); (B) those with standard-risk disease (total, $n = 63$; identical, $n = 52$; one-locus mismatch, $n = 3$ and two- to three-loci mismatch, $n = 8$).

(Morishima *et al*, 2002; Kanda *et al*, 2003). Thus, the RIC regimens used in this study appear to have been sufficient for achieving donor cell engraftment in these patients. However, we found that the risk of rejection was extremely high (10·8%) in patients who received a graft from a two- to three-loci-mismatched donor in the HVG vector and myeloablative conditioning should be considered in this setting. Previous reports have demonstrated that the incorporation of low-dose TBI in the conditioning or the use of PBSC could reduce the incidence of graft failure (Deeg *et al*, 2001; Maris *et al*, 2003). These associations were not observed in our study probably because rate of graft failure was too low to detect a significant decrease.

The most important factor that affected OS after RICT was multiple HLA mismatch in patients with haematological malignancies. RICT from a two- to three-loci-mismatched donor resulted in a poor outcome, as has been shown in conventional SCT (Beatty *et al*, 1985; Hows *et al*, 1993; Szydlo *et al*, 1997). However, the 2-year OS after one-locus-mismatched RICT was comparable with that after HLA-matched RICT. When stratified according to disease status, one-locus mismatch was a risk factor for survival in patients with standard-risk disease, but not in those with high-risk disease. These results suggest that RICT from a one-locus-mismatched related donor may be warranted in patients with high-risk haematological malignancies when an HLA-matched sibling donor is not available. In previous studies, a younger age of patients (Faulkner *et al*, 2004) and the use of PBSC (Maris *et al*, 2003) were associated with a superior outcome after RICT, but these factors did not influence OS in the present study. However, the present study has several limitations. First, there was a large difference between the number of recipients with an HLA-matched donor and those with an HLA-mismatched donor. Secondly, since the follow-up period was short following RICT, it is too early to determine long-term outcome to these treatment regimens. Thirdly, HLA-C typing and high resolution DNA typing was not routinely performed in HSCT from a related donor.

Nonetheless, the large cohort of patients in the current study allowed us to make several important observations. First, HLA mismatch between the donor and recipient is an important risk factor for graft failure, aGVHD, cGVHD and OS after RICT. Secondly, RICT from a one-locus-mismatched related donor may represent an alternative approach in patients with high-risk haematological malignancies who lacked an HLA-matched sibling donor. These findings should be carefully confirmed in a prospective study.

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