Table 2. Prognostic factors affecting overall survival of total entry series

Variables	Unfavorable factors	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Comparison with risk factors					
EBV	Positive	3.5 (2.3-5.5)	<0.0001	2.5 (1.5-4.1)	0.001
B symptom	Present	3.2 (2.0-5.1)	<0.0001	2.0 (1.2-3.5)	0.008
LDH	>normal	2.6 (1.6-4.1)	<0.0001	2.0 (1.2-3.4)	0.011
PS	2-4	2.4 (1.6-3.8)	<0.0001	—	—
Age	>60 y	2.0 (1.2-3.1)	0.006	—	—
Stage	III/IV	1.8 (1.1-2.8)	0.010	—	—
Extranodal disease	>1 site	1.5 (0.9-2.3)	0.083	—	—
Comparison with IPI category					
IPI	HI/H	2.1 (1.4-3.3)	0.001	2.0 (1.3-3.1)	0.003
EBV	Positive			3.3 (2.1-5.3)	<0.0001

Abbreviations: CI, confidential interval; LDH, lactate dehydrogenase; PS, performance status; IPI, International Prognostic Index.

years (*P* = 0.0008), the presence of B symptoms (*P* = 0.0058), and LDH level equal to or more than normal value (*P* = 0.040). Clinical stage, PS, and extranodal involvement of more than one site were nonsignificant factors. In multivariate analysis, the factors that turned out to correlate significantly with survival were B symptom (*P* = 0.0026) and age (*P* = 0.0045). Because the relative risk associated with each of the two factors was comparable, we constructed a prognostic model by combining these prognostic variables in the following way: patients with a score of 0 (*n* = 18), no adverse factors; patients with a score of 1 (*n* = 39), one factor; and patients with a score of 2, two factors (*n* = 21). This prognostic model for age-related EBV+ B-cell LPDs was able to efficiently identify three groups of patients with different outcomes (Fig. 3*B*; *P* < 0.0001). For the patients with scores of 0, 1, and 2, the median overall survival times were 56.3, 25.2, and 8.5 months, respectively.

Discussion

We recently have documented 22 cases named as senile EBV-associated B-cell LPDs arising in elderly patients aged ≥60 years without predisposing immunodeficiencies, suggesting that this disease has a relationship with an immunologic deterioration derived from the aging process (6). Among 1,792 large B-cell

LPD cases examined by EBERs *in situ* hybridization, 156 cases harbored EBV without underlying immunodeficiency-related diseases. This larger series revealed that 149 (96%) of these patients are more than 40 years of age, the increasing positive percentages of which were observed in parallel with the elder patient populations (≥40 years) for all cases examined and reached the highest peak at ages ≥90 years. These data provided additional evidence that EBV-positive B-cell LPDs without predisposing immunodeficiency mainly occur in elderly patients, although seven patients were found to be <40 years of age. Considering these rare cases, the term of "age related" may be more appropriate than that of senile for further understanding the overall age distribution of EBV-positive B-cell LPDs without predisposing immunodeficiency.

This study was predominantly a comparison of clinical features in age-related EBV+ B-cell LPDs and EBV-negative DLBCLs. An analysis of 96 patients with age-related EBV-positive B-cell LPDs, in which the clinical data were available, highlighted the clinical features of this disease—high age at onset, frequent association with poor prognostic components of IPI, and aggressive clinical course. These features were significantly different from those of EBV-negative DLBCL besides more frequent involvement of the skin, supporting the concept that age-related EBV-associated B-cell LPDs constitute a distinct disease with a broad spectrum. However, it could not be definitively concluded whether this disease

Table 3. Prognostic factors affecting overall survival of age-related EBV-positive B-cell LPDs

Variables	Unfavorable factors	Univariate analysis		Multivariate analysis	
		Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
B symptoms	Present	2.3 (1.3-4.3)	0.0058	2.6 (1.4-4.8)	0.0026
Age	>70 y	2.4 (1.4-4.3)	0.0008	2.5 (1.3-4.8)	0.0045
LDH	>normal	1.9 (1.0-3.4)	0.040	—	—
Stage	III/IV	1.8 (1.0-3.2)	0.062	—	—
PS	2-4	1.2 (0.7-2.1)	0.57	—	—
Extranodal disease	>1 site	1.3 (0.7-2.3)	0.38	—	—
IPI category	HI/H	1.8 (1.0-3.2)	0.064	—	—

Abbreviations: LPDs, lymphoproliferative disorders; CI, confidence interval; EBV, Epstein-Barr virus; LDH, lactate dehydrogenase; PS, performance status; IPI, International Prognostic Index.

represented a heterogeneous group of disorders including several lymphoma subtypes.

The morphologic spectrum of age-related EBV+ B-cell LPDs seems to be broader than has been previously realized (data not shown). This disease comprised a spectrum ranging from polymorphic proliferation, sometimes suggestive of a reactive process, to large-cell lymphomas mostly consisting of transformed cells and, therefore, was subdivided into two subtypes, i.e., polymorphic and large-cell lymphomas, based on morphology and conventional immunophenotyping in our previous report (6). However, in the present study, we failed to show any statistical difference in the clinical profiles between these two subgroups. Indeed, several cases had areas that seem more monomorphic in the same or other tissues, thus indicating a continuous spectrum between polymorphic and large-cell lymphoma subtypes. The results that we found in the histologic subgrouping of age-related EBV+ B-cell LPDs seemed to parallel those of the post-transplant LPDs, in which current classification schemes are not fully predictive of prognosis (15, 21). Further investigation should be done to refine the distinction of age-related EBV+ B-cell LPDs into more homogeneous categories with prognostic relevance.

The prognosis of age-related EBV+ B-cell LPDs was significantly poorer than that of EBV-negative tumors. One possible explanation is that the EBV association as a biological marker seemed to be closely associated with the higher IPI index because 35% of patients with this disease were categorized in the high-risk IPI group, which is higher than 15% of the present series of EBV-negative DLBCL or 19% of DLBCL reported by the Non-Hodgkin's Lymphoma Classification project (22, 23). The other is the age distribution and performance status of the patients (Table 1). Due to higher age or poorer PS, many patients with age-related EBV-positive B-cell LPDs might not maintain the intensity of chemotherapy. However, subgroup analyses by age or the IPI also showed that age-related EBV-positive B-LPDs had lower CR rate and inferior overall survival compared with EBV-negative DLBCLs. Multivariate analysis in all cases further identified EBV association and IPI category as an independent prognostic factor. These findings emphasized that age-related EBV-positive B-cell LPDs merits separate consideration because of the diagnostic and therapeutic problems it poses.

Indeed, in multivariate analysis, two host-related factors, i.e., age older than 70 years and the presence of B symptoms, were prognostically significant. In the present series of age-related EBV+ B-cell LPDs, the IPI scoring system did not seem to work with the same efficacy as in DLBCLs for identifying subsets of patients with different prognoses. However, the extension of the disease (clinical stage and extranodal involvement of more than one site) and the biology or cell turnover of the tumor (LDH level) were no longer significant. These findings further supported our assertion that this disease is distinct from DLBCLs and significantly influenced by the host immune status in outcome of patients. Our prognostic model based on the two simple clinical variables of age older than 70 years and the presence of B symptoms also seemed to better define the clinical outcome of age-related EBV+ B-cell LPDs categorized as a single group with an overall superior predictive capacity as compared with IPI (log-rank, 0.0002 versus 0.1). Of course, an external validation study should be done on the larger series of cases in the future.

It is presumed that the pathogenesis of age-related EBV-positive B-cell LPDs has a close relation with an immunologic deterioration or senescence in immunity derived from the aging process because this disease seemed analogous in many respects to that immunodeficiency-associated LPDs, such as EBV association, waxing and waning of disease, and polymorphic proliferation of large bizarre B cells (16). Aging in humans is known to be associated with impaired immune status such as increased infections, the more global phenomenon termed "immune senescence" (24). Indeed, in the present series, 28% of the age-related EBV+ B-cell LPD cases examined were immunohistochemically positive for EBNA2, indicating the reduced immunity to EBV, i.e., type III latency which is believed to occur only in the setting of profound immunodeficiency (25). EBV DNA in peripheral blood mononuclear cells was more frequently detected in healthy individuals older than 70 years of age (8 of 9, 89%) than in ones <70 years (1 of 11, 9%) using real-time PCR (26). Yanagi et al. also showed that EBNA-2 IgG antibodies evoked in young children by asymptomatic primary EBV infections remain elevated throughout life using sera, suggesting the intervention of reactivation of latent and/or exogenous EBV superinfection (27). These data provided additional support on the speculation that age-related decline in immunity may be contributing to the pathogenesis of age-related EBV+ B-cell LPDs.

Biological interfaces may be assumed between age-related EBV+ B-cell LPDs and other EBV-associated B-cell neoplasms such as lymphomatoid granulomatosis and plasmablastic lymphoma, the distinction of which is currently based on the constellation of clinical, morphologic, and immunophenotypic features (28, 29). In our series, nine cases showed pulmonary involvement and four ones had gingival lesions at presentation, posing the differential diagnostic problems from lymphomatoid granulomatosis and plasmablastic lymphoma, respectively, although they were not prototypic in morphology as the latter two. Classic Hodgkin lymphoma (CHL) is also well known to have EBV harboring in 30% to 50% of the cases with achieving a general consensus of the B-cell derivation of the H-RS cells in most (30, 31). Interestingly, three population-based studies of Clarke et al. (32), Stark et al. (33), and more recently, Jarrett et al. (34), without selection bias documented that a marked survival disadvantage in older EBV-positive CHL patients as compared with EBV-negative CHL cases, which was contrasted with no effect of EBV status on the clinical outcome of HL patients selectively enrolled in clinical trails, with a tendency of their relatively younger age distribution (35, 36). As the interpretation for this age-related influence of EBV on clinical outcome of CHL patients, Gandhi et al. (37) and Jarrett et al. (34) clearly indicated that a decline in cellular immunity to EBV with age may contribute to the pathogenesis of EBV+ CHL in older patients. This standpoint is tempting to speculate that EBV+ CHL and age-related EBV+ B-cell LPDs may constitute a continuous spectrum. Our study may also raise an even more fundamental question: whether biological properties, such as an interaction or balance between latent EBV infection and host immunity, precede the morphologic and immunophenotypic evaluation for further understanding the overall clinicopathologic profiles of EBV-associated B-cell LPDs and/or lymphomas. Much still needs to be learned about the detailed clinicopathologic

features, the immunology, and the molecular biology of these diseases in a further study.

Innovative therapeutic strategies such as immunotherapy against EBV should be explored for age-related EBV+ B-cell LPD patients (38, 39), because conventional combination chemotherapy had only a limited effect in an analysis of this larger series. For poor risk patients with aggressive lymphomas such as DLBCL, the superiority of high dose chemotherapy with stem cell support over conventional method is now under confirmation (40 - 42). This therapeutic approach may not, however, be suitable for age-related EBV+ B-cell LPDs because the older age distribution of the patients, many (70%) of which were more than 65 years old, made the application of high-dose chemotherapy difficult enough. Rituximab is a non-cytotoxic drug that showed efficacy when adding to cyclophosphamide-Adriamycin-vincristine-prednisone (CHOP) on elderly patients with DLBCL (43). In our present series, only one case was documented to have received chemotherapy combined with rituximab for an initial treatment, preliminarily providing a good efficacy of this agent on age-related EBV+ B-cell LPD. Now, we are conducting prospective clinical trials to test the efficacy of chemotherapy with rituximab as a multi-institutional study on age-related EBV+ B-cell LPD patients.

In conclusion, the current study elucidates that age-related EBV-associated B-cell LPDs constitute a distinct clinicopathologic group in contrast with EBV-negative DLBCLs, in which conventional chemotherapy has a limited efficacy for this disease. A study to test the efficacy of rituximab with chemotherapy for age-related EBV+ is now ongoing. In the future, less toxic treatment strategy such as a cell therapy for EBV-specific viral antigens will be needed and should be evaluated in clinical trials.

Acknowledgments

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Chronic graft-*versus*-host disease after allogeneic bone marrow transplantation from an unrelated donor: incidence, risk factors and association with relapse. A report from the Japan Marrow Donor Program

Shinichi Ozawa,[1][*] Chiaki Nakaseko,[1][*]
Miki Nishimura,[1] Atsuo Maruta,[2] Ryuko
Cho,[1] Chikako Ohwada,[1] Hisashi
Sakamaki,[3] Hiroshi Sao,[4] Shin-ichiro
Mori,[5] Shinichiro Okamoto,[6] Kouichi
Miyamura,[7] Shunichi Kato,[8] Takakazu
Kawase,[9] Yasuo Morishima[9] and
Yoshihisa Kodera[7] for the Japan Marrow
Donor Program[†]

[1]*Division of Haematology, Department of Clinical
Cell Biology, Chiba University Graduate School of
Medicine, Chiba,* [2]*Department of Haematology
and Chemotherapy, Kanagawa Cancer Centre,
Kanagawa,* [3]*Department of Haematology, Tokyo
Metropolitan Komagome Hospital, Tokyo,*
[4]*Department of Haematology, Meitetsu Hospital,
Aichi,* [5]*Haematology and Haematopoietic Stem
Cell Transplantation Division, National Cancer
Centre Hospital, Tokyo,* [6]*Division of
Haematology, Department of Medicine, Keio
University School of Medicine, Tokyo,*
[7]*Department of Internal Medicine, Japanese Red
Cross Nagoya First Hospital, Aichi,* [8]*Department
of Paediatrics, Tokai University School of
Medicine, Kanagawa, and* [9]*Department of
Haematology and Cell Therapy, Aichi Cancer
Centre, Aichi, Japan*

Summary

Chronic graft-*versus*-host disease (GVHD) remains the major cause of late morbidity and mortality after allogeneic stem cell transplantation. We retrospectively analysed 2937 patients who underwent bone marrow transplantation from an unrelated donor (UR-BMT) facilitated by the Japan Marrow Donor Program (JMDP) and survived beyond day 100 after transplantation. The cumulative incidence of chronic GVHD (limited + extensive) or extensive chronic GVHD at 5 years post-transplant was 45·8% and 28·2%, respectively. On multivariate analysis, seven variables predicting chronic GVHD were identified: recipient age over 20 years, donor age over 30 years, primary diagnosis of chronic myeloid leukaemia, human leucocyte antigen (HLA)-A or -B mismatch, total body irradiation-containing regimen, platelet count not having reached $50 \times 10^9/l$ by day 100, and prior acute GVHD. Among 2609 patients with haematological malignancy, overall survival was significantly higher in patients with limited chronic GVHD but lower in patients with extensive chronic GVHD compared with those without chronic GVHD. The cumulative incidence of relapse among patients with limited or extensive chronic GVHD was significantly lower than that among patients without chronic GVHD. Our results suggest that limited chronic GVHD provides a survival benefit to patients with haematological malignancies by reducing the risk of relapse without increasing the risk of death from chronic GVHD.

Keywords: chronic graft-*versus*-host disease, unrelated bone marrow transplantation, Japan Marrow Donor Program, relapse, graft-*versus*-leukaemia effect.

Haematopoietic stem cell transplantation (HSCT) has become established as one of the curative therapies for haematological malignancies and other haematological or immunologic disorders (Armitage, 1994). However, various late complications of HSCT rather than relapse decrease the quality of life of HSCT recipients (Socie *et al*, 1999; Kiss *et al*, 2002). Among late complications that may occur beyond 100 d post-transplant, chronic graft-*versus*-host disease (GVHD) affects approximately 30–70% of long-term survivors depending on the degree of human leucocyte antigen (HLA)-mismatch with the donor and the source of the stem cells, and remains a major cause of late morbidity and mortality post-transplantation (Atkinson *et al*, 1990; Sullivan *et al*, 1991; Vogelsang, 2001; Lee *et al*, 2002; Farag, 2004). Despite improvements in other areas of supportive care, little significant progress has been made in the management of chronic GVHD (Vogelsang, 2001). Patients with chronic GVHD have decreased performance status, impaired quality of life, and increased risk of mortality (Duell *et al*, 1997; Socie *et al*, 1999). In spite of its adverse effects, chronic GVHD is associated with a lower incidence of leukaemia relapse by a graft-*versus*-leukaemia (GVL) effect that is comparable or greater than that ascribed to acute GVHD (Weiden *et al*, 1981; Sullivan *et al*, 1989; Kataoka *et al*, 2004).

Bone marrow transplantation (BMT) from an unrelated volunteer donor (UR-BMT) has become established as an accepted treatment for patients in need of HSCT who do not have a HLA-matched sibling donor (Kernan *et al*, 1993; Hansen *et al*, 1998; Kodera *et al*, 1999; Davies *et al*, 2000). The incidence of chronic GVHD is assumed to be higher after UR-BMT than after transplants from an HLA-matched sibling donor. Previous studies have identified the incidence and risk factors for chronic GVHD after sibling transplant (Storb *et al*, 1983; Ringden *et al*, 1985; Atkinson *et al*, 1990; Remberger *et al*, 2002); however, there are no definite data available on the incidence and risk factors for chronic GVHD among patients who have undergone UR-BMT. The Japan Marrow Donor Program (JMDP) was established in December 1991. We previously analysed the data of 1298 patients who underwent UR-BMT facilitated by the JMDP between 1993 and 1998 to identify the effect of HLA matching on acute GVHD, chronic GVHD, engraftment, survival and relapse (Morishima *et al*, 2002). In that study, HLA-A and/or HLA-B allele mismatch and patient age were found to be significant risk factors for the occurrence of chronic GVHD. The current study extended the analysis to include the data of 2937 patients who underwent UR-BMT facilitated by the JMDP between January 1993 and June 2004 and survived for at least 100 d post-transplant to clarify the incidence and risk factors for chronic GVHD, and the effect of chronic GVHD on survival and relapse in UR-BMT recipients.

Patients and methods

Patients and transplant procedure

Between January 1993 and June 2004, 2937 Japanese patients who underwent UR-BMT through the JMDP, engrafted and survived for at least 100 d after UR-BMT were included in this analysis. We excluded patients who survived <100 d after UR-BMT to exclude the effect of early mortality. Because peripheral blood stem cell harvest has not been performed through the JMDP, all transplants were BMTs. Baseline characteristics and follow-up data were obtained using standard report forms designed by the JMDP. Follow-up reports were submitted at 100 d, 1 year, and annually thereafter post-transplantation. A final clinical survey of these patients was performed on 1 November 2004. The median follow-up time was 822 d (range, 100–4129 d). Informed consent was obtained from the patients and donors according to the Declaration of Helsinki.

The characteristics of the patients and donors are summarised in Table I. The median age of the patients was 27 years and the median age of the donors was 33 years. As much as 59·7% of the patients and 59·5% of the donors were male. The number of patients with a haematological malignancy was 2667 (90·8%). Transplantation was performed according to the protocol of each centre, and therefore the conditioning regimen and GVHD prophylaxis varied among patients. A conditioning regimen containing anti-thymocyte globulin (ATG) was used in 203 patients (6·9%), and a conditioning regimen containing total body irradiation (TBI) was used in 2329 patients (79·3%). Only 14 patients (0·5%) received T cell-depleted marrow.

HLA matching and typing

According to the donor selection criteria of the JMDP, patients received marrow transplants from serologically HLA-A, -B and -DR antigen completely matched or serologically 1 antigen mismatched donors. Genomic typing of HLA-A, -B and -DR antigens was also performed. 68·5% of the donors were fully HLA-matched by both serological and genomic typing.

Statistical analysis

The incidence of chronic GVHD was the primary endpoint of our study. Diagnosis of chronic GVHD and its clinical grading were performed according to the standard criteria at each institution (Atkinson, 1990). Chronic GVHD was graded as limited (localised skin or single organ involvement) or extensive (generalised skin or multiple organ involvement). The cumulative incidence of chronic GVHD was calculated from the time of transplantation. To evaluate potential risk factors for developing chronic GVHD, the time-dependent

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Table I. Characteristics of the patients who underwent UR-BMT and donors.

Number of patients	2937
Median age of patients, years (range)	27 (0–67)
Patient sex (male/female), *n*	1753/1184
Diagnosis, *n*	
Haematological malignancy	
AML	793
ALL	768
CML	604
MDS	285
NHL	168
Others	49
Non-malignant disease	
AA	191
Hereditary disorders	68
Conditioning, *n*	
ATG	203
TBI	2329
GVHD prophylaxis, *n*	
CsA + MTX	1545
FK506 + MTX	1118
Others	274
Median age of donors, years (range)	33 (20–52)
Donor sex (male/female), *n*	1748/1189
Sex (recipient/donor), *n*	
Male/male	1151
Male/female	602
Female/female	587
Female/male	597
HLA disparity, *n*	
Full match	2012
Class I one locus or one allele mismatch	286
Class II one locus or one allele mismatch	473
Others	166
Blood-type disparity, *n*	
Match	1535
Major mismatch	677
Minor mismatch	616
Major–minor mismatch	72
Bone marrow treatment, *n*	
No	1529
Yes	
Removal of red blood cells	764
Removal of plasma	750
T cell depletion	14
Time from diagnosis to BMT, months	
<13	1180
13–24	865
≥25	865
Median time from BMT to WBC = $1 \cdot 0 \times 10^9/l$, d (range)	17 (1–99)
Platelet count = $50 \times 10^9/l$ by day 100 from BMT, *n*	
Yes	2714
No	223
Prior acute GVHD, *n*	
No	884
Grade I	915
Grade II	793

Table I. *Continued*

Grade III	281
Grade IV	64

AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; AA aplastic anaemia; ATG, anti-thymocyte globulin; TBI, total body irradiation; GVHD, graft-*versus*-host disease; CsA, ciclosporin A; MTX, methotrexate; FK506, tacrolimus; HLA, human leucocyte antigen; BMT, bone marrow transplantation; WBC, white blood cell count.

Cox proportional hazard regression model was used for univariate and multivariate analyses (Cox, 1972). Factors with a *P*-value of 0·2 or less in the univariate analysis were included in the multivariable analysis. Factors that remained significant were retained in the final model.

Patients were also analysed for overall survival (OS) and relapse. To illustrate the effect of chronic GVHD on relapse and survival, semi-landmark plots were constructed (Baron *et al*, 2005). In patients who developed chronic GVHD, the post-transplant day of development of chronic GVHD was defined as the landmark day; in patients who did not develop chronic GVHD, post-transplant day 112, which was the median day of occurrence of chronic GVHD, was defined as the landmark day. OS was calculated from the landmark day to death from any cause or date of last contact. Relapse was defined on the basis of evidence of the respective malignancy and its cumulative incidence was plotted as a function of time since the landmark day.

Survival analyses were performed by the Kaplan–Meier method (Kaplan & Meier, 1958) and the log-rank test was used for univariate comparisons. The cumulative incidences of chronic GVHD and relapse were calculated using the Gray method, considering death without chronic GVHD or death without relapse, respectively, as the competing risk (Gray, 1988). For most of the statistical analyses, the Statistical Package for the Social Sciences (SPSS) software version 11 (SPSS Inc., Chicago, IL, USA) was used. Analyses of cumulative incidences were carried out with package 'cmprsk' of the R statistical software 2.1.0 (the R Foundation for Statistical Computing, Vienna, Austria; available at http://www.r-project.org). All *P*-values were two-sided and differences were considered to be statistically significant when *P* < 0·05. Differences with *P*-values > 0·10 are reported as not significant (NS), whereas differences with *P*-values between 0·05 and 0·1 are reported in detail.

Results

Incidence and severity of chronic GVHD

Among the 2937 patients, 1267 (43·1%) developed chronic GVHD, of whom 268 patients (21·2%) had *de novo* onset of

chronic GVHD. The median time to onset of chronic GVHD was 112 d following transplant. The 5-year cumulative incidence of chronic GVHD was 45·8%, and that of extensive chronic GVHD was 28·2% (Fig 1A). Fig 1B shows the cumulative incidences of chronic GVHD according to the primary diagnosis.

Risk factors for developing chronic GVHD

Multivariate analysis for risk factors for the development of chronic GVHD included the 2909 patients in whom data on the variables with $P \leq 0.2$ in the univariate analysis were available (Table II). Recipient age ≥20 years, donor age ≥30 years, primary diagnosis of chronic myeloid leukaemia (CML),

HLA-A or -B mismatch by serological or genomic typing, total body irradiation (TBI)-containing regimen, platelet count <50 × 10^9/l by day 100, and prior acute GVHD remained in the optimal model on multivariate analysis and increased the risk of chronic GVHD significantly. Aplastic anaemia (AA) and hereditary disorders were significantly associated with a low incidence of chronic GVHD.

When the patients were divided by age decade, the incidence of chronic GVHD was significantly lower in recipient groups aged <10 years and 10–19 years; however, among recipients aged ≥20 years, there were no differences in the incidence of chronic GVHD (Fig 2). When the donors were divided by age decade, the cumulative incidence of chronic GVHD was significantly lower among patients transplanted from donors aged 20–29 years than among patients transplanted from donors aged ≥30 years ($P = 0.005$, method of Gray). No differences in the incidence of chronic GVHD were found among patients transplanted from donors aged ≥30 years.

Prior acute GVHD was the strongest risk factor for chronic GVHD (Table II and Fig 1C). Among patients with no history of acute GVHD ($n = 870$), risk factors for chronic GVHD on multivariate analysis were recipient age ≥20 years {hazard ratio (HR) = 1·45 [95%confidence interval (95% CI), 1·06–1·98], $P = 0.019$}, donor age ≥30 years [HR = 1·54 (95%CI, 1·19–2·00), $P = 0.001$] and one locus mismatch or one allele mismatch at HLA-A/-B loci [versus full match, HR = 1·50 (95% CI, 1·02–2·20) $P = 0.039$]. Among patients with a history of grade II–IV acute GVHD ($n = 1107$), platelet count <50 × 10^9/l by day 100 [HR = 1·30 (95%CI, 1·00–1·67), $P = 0.048$] was the only risk factor on multivariate analysis.
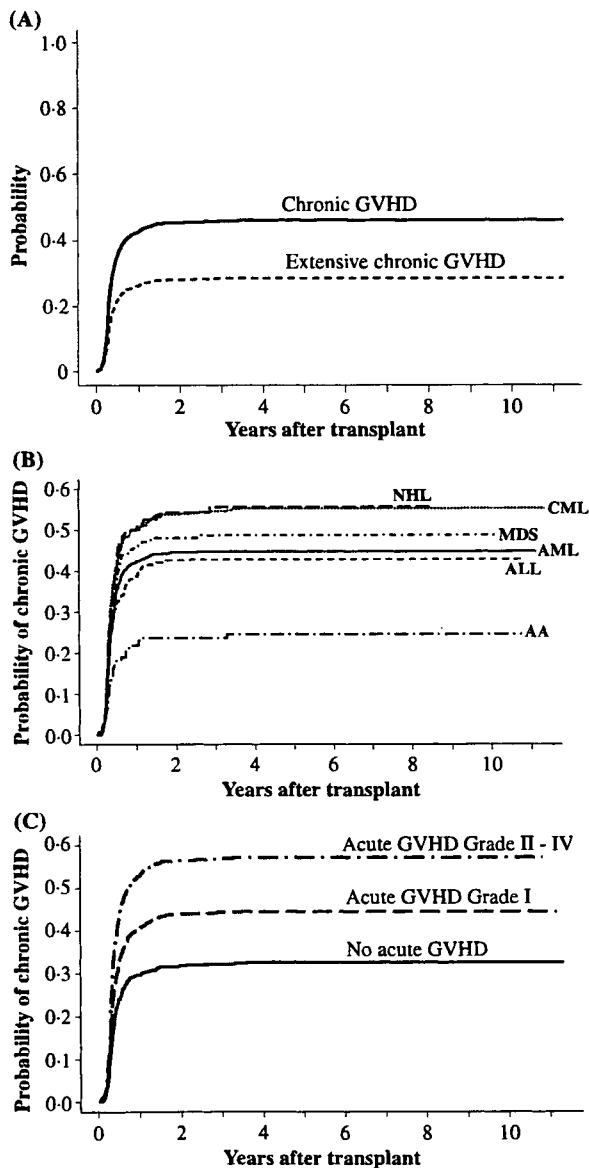

(A)

(B)

(C)

Fig 1. Cumulative incidence of chronic GVHD after UR-BMT. (A) Cumulative incidences of chronic GVHD (limited + extensive) and extensive chronic GVHD. The 5-year cumulative incidence of chronic GVHD was 45·8% (95% CI, 43·9–47·7%) and that of extensive chronic GVHD was 28·2% (95% CI, 26·5–29·9). Competing risks were death without chronic GVHD and death without chronic extensive GVHD (19·3% and 24·4%, respectively). (B) Cumulative incidences of chronic GVHD according to the primary diagnosis. The 5-year cumulative incidence and competing risk were 44·7% and 25·4% among patients with acute myeloid leukaemia (AML, solid line), 42·9% and 25·7% among patients with acute lymphoblastic leukaemia (ALL, dashed line), 49·0% and 16·1% among patients with myelodysplastic syndrome (MDS, dot-dash line), 55·3% and 12·8% among patients with chronic myeloid leukaemia (CML, dotted line), 55·7% and 11·5% among patients with non-Hodgkin lymphoma (NHL, long-dash line), and 24·4% and 6·1% among patients with aplastic anaemia (AA, dot-long dash line), respectively. (C) Cumulative incidences of chronic GVHD according to the severity of prior acute GVHD. The 5-year cumulative incidence was 32·4% among patients without a history of acute GVHD (solid line), 44·4% among patients with a history of grade I acute GVHD (dashed line), and 57·3% among patients with a history of grades II–IV acute GVHD (dot-dashed line). Competing risks were 20·0% without prior acute GVHD, 20·2% for grade I, and 17·9% for grades II–IV.

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Table II. Univariate and multivariate analyses of risk factors for the development of chronic GVHD.

Factor	Univariate analysis			Multivariate analysis ($n = 2909$)		
	n	HR (95% CI)	P-value	n	HR (95% CI)	P-value
Recipient age						
0–19 years	972	1·0		961	1·0	
≥20 years	1965	1·41 (1·24–1·59)	<0·0001	1948	1·19 (1·04–1·36)	0·013
Recipient sex						
Female	1184	1·0				
Male	1753	1·11 (0·99–1·25)	0·07			NS
Donor age						
20–29 years	1007	1·0		994	1·0	
≥30	1930	1·28 (1·14–1·45)	<0·0001	1915	1·20 (1·07–1·36)	0·003
Sex matching						
Match	1738	1·0		1721	1·0	
Female to male	602	1·01 (0·87–1·16)	0·94	595	1·05 (0·91–1·22)	NS
Male to female	597	0·89 (0·77–1·03)	0·11	593	0·85 (0·74–0·99)	0·03
Diagnosis						
AML	793	1·0		787	1·0	
ALL	768	0·92 (0·79–1·08)	0·31	764	0·89 (0·76–1·04)	NS
MDS	285	1·13 (0·92–1·38)	0·25	283	1·11 (0·90–1·36)	NS
CML	604	1·27 (1·09–1·48)	0·002	602	1·19 (1·02–1·39)	0·03
NHL	168	1·32 (1·04–1·67)	0·02	166	1·18 (0·93–1·50)	NS
AA	191	0·43 (0·32–0·60)	<0·0001	190	0·51 (0·37–0·71)	0·0001
Other haematological malignancies	49	1·05 (0·67–1·65)	0·83	49	0·94 (0·60–1·48)	NS
Hereditary disorders	68	0·47 (0·29–0·77)	0·003	68	0·56 (0·34–0·93)	0·02
Time from diagnosis to BMT						
<13 months	1180	1·0				
13–24 months	865	1·05 (0·92–1·20)	0·45			
≥25 months	865	0·98 (0·85–1·12)	0·71			
Blood type disparity						
Match	1535	1·0				
Major mismatch	677	1·03 (0·89–1·18)	0·73			
Minor mismatch	616	1·08 (0·94–1·24)	0·30			
Major minor mismatch	72	1·12 (0·79–1·58)	0·54			
HLA disparity						
Full match	2012	1·0		1991	1·0	
Class I one mismatch	286	1·26 (1·05–1·51)	0·01	285	1·26 (1·05–1·52)	0·01
Class II one mismatch	473	1·03 (0·88–1·20)	0·73	468	0·90 (0·77–1·05)	NS
≥2 mismatches	166	1·31 (1·05–1·64)	0·02	165	1·14 (0·91–1·43)	NS
Preparative regimen TBI for conditioning						
Non-TBI regimen	608	1·0		601	1·0	
TBI-based regimen	2329	1·23 (1·06–1·42)	0·005	2308	1·16 (1·00–1·35)	0·04
ATG for conditioning						
No	2718	1·0				
Yes	203	0·58 (0·44–0·75)	0·0001			NS
GVHD prophylaxis						
CsA + MTX	1545	1·0				
FK506 + MTX	1118	1·00 (0·88–1·19)	0·93			
Treatment of bone marrow						
No	1529	1·0				
Yes	1384	1·06 (0·95–1·19)	0·29			
Platelet recovery (50 × 10^9/l or more by 100 d from BMT)						
Yes	2714	1·0		2688	1·0	
No	223	1·33 (1·10–1·61)	0·003	221	1·34 (1·10–1·63)	0·004

Table II. *Continued*

Factor	Univariate analysis			Multivariate analysis (n = 2909)		
	n	HR (95% CI)	P-value	n	HR (95% CI)	P-value
Days from BMT to WBC recovery						•
<Day 18	1622	1·0				
≥Day18	1314	0·90 (0·80–1·00)	0·049			NS
Prior acute GVHD						
No	884	1·0		869	1·0	
Grade I	915	1·54 (1·31–1·80)	<0·0001	911	1·47 (1·25–1·72)	<0·0001
Grade II–IV	1138	2·28 (1·98–2·64)	<0·0001	1129	2·08 (1·80–2·42)	<0·0001

CI, confidence interval; AML, acute myeloid leukaemia; ALL, acute lymphoblastic leukaemia; CML, chronic myeloid leukaemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin lymphoma; AA aplastic anaemia; ATG, antithymocyte globulin; TBI, total body irradiation; GVHD, graft-versus-host disease; CsA, ciclosporin A; MTX, methotrexate; FK506, tacrolimus; HLA, human leucocyte antigen; BMT, bone marrow transplantation; WBC recovery, the first of three consecutive days with a persistent white blood cell count >1·0 × 10^9/l; HR, hazard ratio.
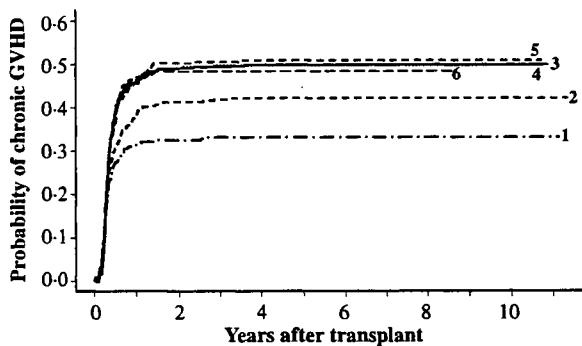

Fig 2. Cumulative incidence of chronic GVHD according to recipient's age decade. The competing risk was death without chronic GVHD. The 5-year cumulative incidence and competing risk were: 32·9% and 14·1% among patients aged 0–9 years (line 1; dot-dash line), 42·1% and 18·8% among those aged 10–19 years (line 2; dash line), 49·1% and 15·1% among those aged 20–29 years (line 3; solid line), 49·4% and 23·1% among those aged 30–39 years (line 4; dotted line), 51·0% and 23·2% among those aged 40–49 years (line 5; dash line), and 48·3% and 25·6% among those aged >50 years (line 6; long-dash line), respectively.

Influence of chronic GVHD on OS and relapse

We analysed how chronic GVHD affects the prognosis after UR-BMT among 2877 patients (Fig 3). Patients with limited chronic GVHD had significantly better prognosis than patients with extensive chronic GVHD (log-rank test, P < 0·0001) or patients without GVHD (P = 0·009), whereas patients with extensive chronic GVHD had significantly poorer prognosis (versus without chronic GVHD, P = 0·003). The same tendencies were observed among 2609 patients with a haematological malignancy. On multivariate analysis using the Cox proportional hazard model with chronic GVHD as a time-dependent covariate, patients with extensive chronic GVHD had significantly increased mortality and patients with limited chronic GVHD had a survival advantage compared with those
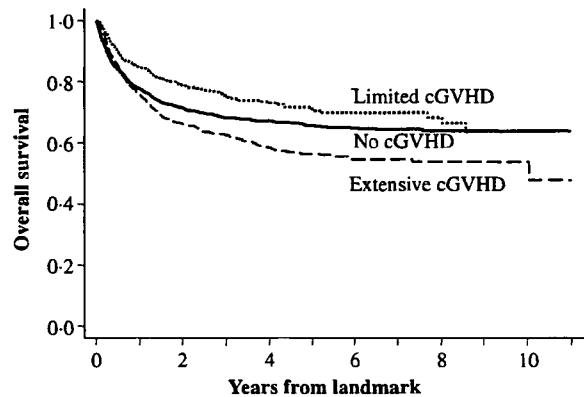

Fig 3. Overall survival according to chronic GVHD grading. The OS of all patients who survived beyond 100 d post-transplant according to chronic GVHD grade (n = 2877), is shown. The 5-year OS rate was 71·1% (95% CI, 66·4–75·8) among those with limited chronic GVHD (n = 489), 56·4% (95% CI, 52·3–60·5) among those with extensive chronic GVHD (n = 771), and 65·9% (95% CI, 63·2–68·5) among patients who did not develop chronic GVHD (n = 1617). The landmark day was the day of onset of chronic GVHD for patients with chronic GVHD, and it was day 112 from transplant, which was the median day of the onset of chronic GVHD, for patients without chronic GVHD. No chronic GVHD, solid line; limited chronic GVHD, dotted line; extensive chronic GVHD, dashed line.

without chronic GVHD (Table III). However, patients with chronic GVHD had a lower cumulative incidence of relapse than patients without chronic GVHD (versus limited chronic GVHD, P = 0·049; versus extensive chronic GVHD, P = 0·009). There was no difference in relapse rate between patients with limited chronic GVHD and those with extensive chronic GVHD. The 5-year probability of relapse was 15·8% (95% CI, 12·1–19·5) among patients with limited chronic GVHD, 15·3% (95% CI, 12·3–18·4) among patients with extensive chronic GVHD, and 21·0% (95% CI, 18·5–23·6) among patients without chronic GVHD.

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Table III. Multivariate analysis of prognostic factors in patients with haematological malignancies.

Factor	HR (95% CI)	P-value
Recipient age		
≥20 years	1·54 (1·30–1·83)	<0·0001
Donor age		
≥40 years	1·18 (1·18–1·38)	0·04
Diagnosis		
CML (*versus* AML)	0·67 (0·55–0·82)	0·0001
HLA disparity		
Class I one mismatch (*versus* full-match)	1·58 (1·27–1·97)	0·0001
≥2 mismatches (*versus* full-match)	1·52 (1·14–2·01)	0·0038
Platelet recovery (≥50 × 10⁹/l by day 100 from BMT)		
No	1·58 (1·24–2·01)	0·0002
Prior acute GVHD		
Grade II–IV (*versus* No prior acute GVHD)	1·60 (1·31–1·95)	<0·0001
Relapse		
Yes	11·62 (10·06–13·41)	<0·0001
Secondary malignancies		
Yes	6·23 (3·28–11·83)	<0·0001
Chronic GVHD		
Limited (*versus* No)	0·67 (0·54–0·83)	0·0003
Extensive (*versus* No)	1·21 (1·03–1·43)	0·02

CI, confidence interval; AML, acute myeloid leukaemia; CML, chronic myeloid leukaemia; GVHD, graft-*versus*-host disease; HLA, human leucocyte antigen; HR, hazard ratio.

Discussion

In the present study, the 5-year cumulative incidence of chronic GVHD was 45·8% and that of extensive chronic GVHD was 28·2%. These cumulative incidences, especially the cumulative incidence of extensive chronic GVHD, are slightly lower than those of the data of the National Marrow Donor Program (NMDP) (Kollman *et al*, 2001) and other previous reports (Sullivan, 1999). Notably, nearly 100% of the recipient and donor pairs in the present study were composed of a single ethnic population of Japanese people. Recently, Oh *et al* (2005) reported that Japanese and Scandinavian people had significantly lower incidences of acute GVHD than American and Irish people in HLA-identical sibling BMT. Because Japanese people have been geographically isolated for a long period of time historically, Japanese people are genetically more similar than people of the USA. or Western countries and it is unclear whether our results apply to other more diverse genetic groups.

Our previous study revealed two significant risk factors for chronic GVHD by multivariate analysis: HLA-A/-B allele mismatch and patient age (Morishima *et al*, 2002). In the current extended analysis, seven risk factors were found to be significant for the development of chronic GVHD on multivariate analysis.

Zecca *et al* (2002) reported that the incidence of chronic GVHD in children after HSCT was 27%, which was assumed to be lower than that in adult recipients. In the current analysis, the incidence of chronic GVHD among patients <20 years of age was significantly lower than that among patients over 20 years of age. However, there was no significant difference in the incidence of chronic GVHD when adult patients over 20 years of age were grouped by age decade, although the OS rate was significantly lower in older adults than in younger adults, probably because of an increased incidence of death from other causes rather than chronic GVHD.

Donor age ≥30 years was a significant risk factor for the development of chronic GVHD and it also tended to decrease the survival rate. Kollman *et al* (2001) also reported that younger donor was a significant predictor of lack of development of chronic GVHD. Although the reason for this is not well understood, our findings suggest that donors of younger age may be preferable when selecting from comparably HLA-matched volunteer donors.

In our previous study (Morishima *et al*, 2002), HLA-C allele mismatch also tended to increase the incidence of chronic GVHD, while HLA-DR/-DQ mismatch showed no effect. Petersdorf *et al* (2004) showed that a single HLA-C mismatch conferred increased risk of mortality compared with matches. Greinix *et al* (2005) also showed that HLA class I mismatch, as detected by high-resolution typing, had a significant impact on the development of chronic GVHD and survival of UR-BMT recipients. The present study returned the same result as that in the previous report, although the effect of HLA-C was not analysed.

Previous analysis of risk factors for chronic GVHD after HLA-identical sibling BMT (Atkinson *et al*, 1990) revealed that the strongest risk factor for chronic GVHD was the existence of prior acute GVHD. In that report, several risk factors including recipient age >20 years predicted a higher risk of chronic GVHD in patients with a history of grade I acute GVHD or without a history of acute GVHD; however, among patients with a history of moderate to severe acute GVHD, no other risk factor predicted the development of chronic GVHD. In our study, recipient age and donor age were important risk factors for *de novo* onset of chronic GVHD, whereas in patients with a history of moderate to severe acute GVHD, patient age and donor age were not risk factors for chronic GVHD. These results are similar to the results of the other report (Atkinson *et al*, 1990). Remberger *et al* (2002) revealed that CML was a risk factor for chronic GVHD. We also identified that the incidence of chronic GVHD among patients with CML was significantly higher than that among patients with acute myeloid leukaemia (AML).

Whether the primary disease was a haematological malignancy or not significantly affected the development of chronic GVHD. In our previous study, among patients with AA who underwent UR-BMT, the incidence of chronic GVHD was

30% (Kojima et al, 2002), and it was 24·4% in the present extended analysis. Moreover, we found that the incidence of chronic GVHD among patients with hereditary disorders was significantly low in multivariate analysis. This finding might be due to the difference in treatment strategies for patients with haematological malignancy and those with AA. Immunosuppressive agents might be stopped or decreased earlier in patients with haematological malignancy than in those with non-malignant disease in order to induce the GVL effect.

Limited chronic GVHD had a significant impact on increasing patient survival, whereas patients with extensive chronic GVHD had a poor prognosis. In patients with a haematological malignancy, we found no significant difference in relapse rates between patients with limited chronic GVHD and those with extensive chronic GVHD, indicating that extensive chronic GVHD does not provide a strong GVL effect compared with limited chronic GVHD.

We used the grading system of limited and extensive chronic GVHD, which was originally proposed in 1980 based on the clinicopathological findings in 20 patients (Shulman et al, 1980). However, this grading system has several limitations. Akpek et al (2001, 2003) proposed a new prognostic model by analysing GVHD-specific survival and suggested that three factors, i.e. skin involvement, platelet count and progressive-type onset, significantly influence the survival of patients who developed chronic GVHD. However, a recent Japanese report showed that Japanese patients could not be accurately classified when these proposed prognostic models were used because the manifestation of chronic GVHD differed between Japanese and Western ethnic populations (Atsuta et al, 2006). We have started to collect more detailed information on Japanese patients with chronic GVHD, such as organ involvement, treatment strategy, and treatment outcome, to establish prognostic models.

In conclusion, this large-scale study demonstrated the incidence of chronic GVHD after UR-BMT in a single Japanese ethnic population and provides strong evidence for seven risk factors for chronic GVHD after UR-BMT. This study also suggests that limited chronic GVHD provides a survival benefit to patients with a haematological malignancy by reducing the risk of relapse without increasing the risk of death from chronic GVHD. Extended intervention and clinical trials are necessary to overcome extensive chronic GVHD.

Acknowledgements

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Appendix 1

The following centres participated in the bone marrow transplantations facilitated by the JMDP: Asahikawa Medical College Hospital, Asahikawa Red Cross Hospital, Sapporo Medical University Hospital, Sapporo Hokuyu Hospital, Hokkaido University Hospital, Asahikawa City Hospital, Hakodate City Hospital, Hirosaki University Hospital, Aomori Prefectural Central Hospital, Akita University Hospital, Iwate Medical University Hospital, Miyagi Cancer Centre, Tohoku University Hospital, Yamagata University Hospital, Fukushima Medical University Hospital, Ibaraki Children's Hospital, Tsukuba University Hospital, Tsuchiura Kyodo General Hospital, Jichi Medical School Hospital, Dokkyo Medical University Hospital, Saiseikai Maebashi Hospital, Gunma University Hospital, Saitama Medical University Hospital, Saitama Cancer Centre Hospital, Saitama Children's Medical Centre, Fukaya Red Cross Hospital, National Defense Medical College Hospital, Kameda General Hospital, Matsudo Municipal Hospital, Chiba Children's Hospital, Chiba Aoba Municipal Hospital, Chiba University Hospital, Jikei University Kashiwa Hospital, Keio University Hospital, Toranomon Hospital, National Cancer Centre Central Hospital, International Medical Centre of Japan, National Centre for Child Health and Development, Juntendo University Hospital, Showa University Hospital, Teikyo University Hospital, Tokyo Medical and Dental University Hospital, Tokyo Medical College Hospital, Jikei University Hospital, Tokyo Women's Medical University Hospital, Research Hospital of the Institute of Medical Science-the University of Tokyo, The University of Tokyo Hospital, Tokyo Metropolitan Komagome Hospital, Tokyo Metropolitan Kiyose Children's Hospital, Tokyo Metropolitan Hospital of Fuchu, Toho University Omori Medical Centre, National Hospital Organisation Tokyo Medical Centre, Nippon Medical School Hospital, Japanese Red Cross Medical Centre, Nihon University Itabashi Hospital, Yokohama City University Medical Centre, Yokohama City University Hospital, Kanagawa Cancer Centre, Kanagawa Children's Medical Centre, St. Marianna University School of Medicine Hospital, Tokai University Hospital, Niigata University Medical & Dental Hospital, Nagaoka Red Cross Hospital, Niigata Cancer Centre Hospital, University of Yamanashi Hospital, Saku Central Hospital, Shinshu University Hospital, Nagano Children's Hospital, Nagano Red Cross Hospital, Toyama Prefectural Central Hospital, Kanazawa Medical University Hospital, Kanazawa University Hospital, Ishikawa Prefectual Central Hospital, University of Fukui Hospital, Hamamatsu Medical Centre, Seirei Hamamatsu General Hospital, Shizuoka Children's Hospital, Shizuoka General Hospital, Shizuoka Red Cross Hospital, Hamamatsu University School of Medicine Hospital, Aichi Medical School Hospital, Aichi Cancer Centre Hospital, Anjo Kousei Hospital, Showa Hospital, National Hospital Organisation Nagoya Medical Centre, Fujita Health University Hospital, Nagoya City University Hospital, Nagoya University Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Nagoya Ekisaikai Hospital, Meitetsu Hospital, Mie University Hospital, Yamada Red Cross Hospital, Suzuka Kaisei Hospital, Suzuka General Hospital, Shiga University of Medical Science Hospital, Kyoto Katsura Hospital, Kyoto City Hospital, Kyoto University Hospital, Kyoto First Red Cross Hospital, Kyoto Prefectural University of Medicine Hospital, Social Insurance Kyoto Hospital, Rinku General Medical Centre, Kansai Medical University Hospital, Kinki University Hospital, Matsushita Memorial Hospital, Osaka Medical College Hospital, Osaka City University Hospital, Osaka Red Cross Hospital, Osaka University Hospital, Osaka Medical Centre for Cancer and Cardiovascular Diseases, Osaka Medical Centre and Research Institute for Maternal and Child Health, Kobe City General Hospital, Kobe University Hospital, Hyogo College of Medicine Hospital, Hyogo Children's Hospital, Hyogo Medical Centre for Adults, Tenri Hospital, Nara Medical University Hospital, Wakayama Medical University Hospital, Tottori Prefectural Central Hospital, Tottori University Hospital, Shimane Prefectural Central Hospital, Okayama University Hospital, National Hospital Organisation Okayama Medical Centre, Hiroshima Red Cross Hospital and Atomic-Bomb Survivors Hospital, Hiroshima University Hospital, National Hospital Organisation Kure Medical Centre, Kurashiki Central Hospital, Yamaguchi University Hospital, Tokushima University Hospital, Kagawa University Hospital, Ehime Prefectural Central Hospital, Ehime University Hospital, Matsuyama Red Cross Hospital, Kochi Medical School Hospital, Kurume University Hospital, Kyushu University Hospital, Harasanshin General Hospital, Hamanomachi General Hospital, National Kyushu Cancer Centre, University of Occupational and Environmental Health Hospital, Kokura Memorial Hospital, St Mary's Hospital, Saga Prefectural Hospital, Nagasaki University Hospital, National Hospital Organisation Kumamoto Medical Centre, Oita Prefectural Hospital, Oita University Hospital, Miyazaki Prefectural Hospital, Imamura Hospital, and Kagoshima University Hospital.

ORIGINAL ARTICLE

High-dose chemotherapy and autologous peripheral blood stem cell transfusion for adult and adolescent patients with small round cell sarcomas

K Yamada[1], M Takahashi[1,3], M Ogura[2,4], Y Kagami[2,5], H Taji[2], Y Kamiya[2], H Sugiura[1] and Y Morishima[2]

[1]Department of Orthopaedics, Aichi Cancer Center Hospital, Nagoya, Japan and [2]Department of Hematology and Cell Therapy, Aichi Cancer Center Hospital, Nagoya, Japan

The treatment of small-round-cell tumors (SRCT) in adult patients remains a challenge to clinicians. In the present study, we analyzed the feasibility and efficacy of high-dose chemotherapy (HDCT) followed by autologous peripheral blood stem-cell rescue as a consolidation therapy exclusively for patients with good disease control through a single regimen of induction chemotherapy and local therapy. Twenty-one patients (12 females, median age 22.0 years) were analyzed, including seven cases with rhabdomyosarcoma (RMS) and 14 cases with Ewing's family tumors (EFT). Overall, survival was 46% and failure-free survival (FFS) was 33% at 3 years. Patients with EFT had better FFS than those with RMS, with an estimated 3-year FFS of 50% ($P < 0.01$). There was a single case of possible treatment-related death and two cases of secondary malignancies. This study cannot conclusively determine the beneficial effects of HDCT for improving treatment outcomes in adult SRCTs due to the small number of subjects. However, study findings suggest that a subgroup of patients with EFT may obtain prolonged survival benefits from this therapy.
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Correspondence: Dr K Yamada, Department of Orthopaedics, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa, Nagoya 464-8681, Japan.
E-mail: kyamada@aichi-cc.jp
[3]Current address: Division of Orthopaedic Oncology, Shizuoka Cancer Center Hospital, Naga-izumi, Shizuoka, Japan.
[4]Current address: Department of Hematology, Nagoya Daini Red Cross Hospital, Nagoya, Japan.
[5]Current address: Department of Hematology, Meitetsu Hospital, Nagoya, Japan.
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Introduction

Rhabdomyosarcoma (RMS) and Ewing's sarcoma family of tumors (EFTs), including Ewing's sarcoma of bone, extra-skeletal Ewing's sarcoma and peripheral primitive neuro-ectodermal tumor, belong to the group of neoplasms commonly referred to as 'small round-cell sarcomas.' These tumors share a common morphologic histology and a similar treatment strategy consisting of multi-drug chemotherapy in combination with surgery and/or radiotherapy.[1,2]

A series of recent studies have reported improvements in outcomes of patients with the use of risk-adapted, intensive multimodal therapy, although the group of patients at high risk for treatment failure still remains.[3–5] Given that both diseases are considered to be chemotherapy-sensitive, dose intensification of chemotherapy is presumed to be a key factor in improving outcomes for patients with high risk factors. In particular, high-dose chemotherapy (HDCT) with stem cell rescue has been used in an attempt to improve clinical outcomes in high-risk patients. However, the impact of HDCT with stem cell rescue on the treatment of high-risk RMS or EFTs has yet to be established, as previous data have demonstrated inconsistent benefits.[6–12]

It is of particular importance to identify subsets of high-risk patients for the purpose of risk stratification. Various studies have identified prognostic factors for patients with RMS or EFTs, including older age, presence of metastatic disease, tumor volume >100 ml or axial site involvement.[13–16] In particular, patient age is one of the strongest predictors of outcome both in EFTs and RMS.[17,18] However, the management of adult patients with EFTs or RMS has been developed mainly from experiences with pediatric patients because of the rarity of the disease in adults.[19,20] Poor outcomes in adult patients[21] may be attributed to the fact that an adult may not respond well to treatments designed for children.

On the basis of these considerations, the purpose of this clinical study was to investigate the effects of HDCT on overall survival (OS) and time to disease progression in adults and adolescent patients with EFTs and RMS. In this report, we present the results from a prospective pilot study of a treatment protocol consisting of induction chemotherapy, local treatment, followed by HDCT as a consolidation therapy.

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Patients and methods

Eligibility criteria

Patients between 15 and 55 years of age with primary and high-risk RMS or EFTs, as previously defined, were considered eligible for the present study. The pathologic diagnosis was confirmed by conventional histopathological and immunohistochemical examinations. Molecular genetic studies were not routinely conducted to investigate specific chromosomal translocations. Routine radiological examinations before study enrollment included computerized tomography (CT) and/or magnetic resonance imaging (MRI) of the primary site, plain radiographs and CT scans of the chest and a 99 technetium-diphosphonate bone scan. A performance status of <2, along with adequate organ function, was required.

Adequate organ function was defined as an absolute neutrophil blood cell count $>1.2 \times 10^9$/l, a platelet count $>100 \times 10^9$/l, a bilirubin level <2.0 mg/dl, a serum creatinine level <2.0 mg/dl, creatinine clearance >60 ml/min and cardiac ejection fraction rate $>50\%$.

Signed informed consent for the treatment was obtained from all patients or their legal guardian in accordance with our institutional review board guidelines.

Treatment protocol

The study treatment protocol included induction chemotherapy, local treatment (surgery and/or radiotherapy), followed by additional induction chemotherapy and, finally, HDCT with autologous peripheral blood stem-cell (APBSC) rescue. Induction chemotherapy consisted of a combination of four chemotherapeutic agents administered every 3 weeks, in modification of the CESS-86 regimen[22] (Table 1).

APBSCs were mobilized with the use of granulocyte colony-stimulating factor (G-CSF) after cycles 3 through 6

of the induction chemotherapy. Before stem cell harvesting, bone marrow aspirates and biopsies were performed to exclude bone marrow involvement of the tumor cells or abnormal myelopoietic functions. Harvesting was performed only when histological or cytological analyses indicated no evidence of tumor cells. A CD34 + cell count > 10 CD34 + cells per μl was used as the harvest criterion. Total cell counts of CD34 + cells $>2.0 \times 10^6$/kg were required to proceed to further HDCT.

Imaging of the primary tumor by MRI and/or CT scanning was conducted after cycles 2 and 4 to aid decisions regarding local therapy, and once again before HDCT. Definitions of radiological antitumor effect were as follows: complete response (CR), no detectable tumors; partial response (PR), $>50\%$ reduction in measurable tumors; stable disease (SD), $<50\%$ reduction in measurable tumors and no new lesions; and progressive disease (PD), tumor growth or new lesions.

Local therapy was scheduled to be given after four courses of the induction chemotherapy, except when the primary tumor had been excised before the first visit to our hospital, or when the tumor was considered unresectable. Local therapy was planned individually, taking into consideration various factors such as tumor site, tumor size, tumor resectability, patients' performance status and expected disabilities after resection. Complete surgical resection was the theoretical goal, whenever feasible. Radiotherapy was prescribed in cases where the tumor was either unresectable or incompletely resectable, or where the surgical margin in the excised specimen proved to be inadequate.

Given that the aim of this study was to investigate the effect of HDCT as a consolidation therapy, patients were scheduled to be re-assessed after the induction chemotherapy with regard to the chemotherapeutic effect. If the patients were in CR or PR after four courses of induction chemotherapy, and had undergone definitive local therapy, they were considered to have minimal tumor burden and eligible for HDCT. If the patients were in SD, tumor excision without macroscopic residue was mandatory. If the patients were in PD, they were excluded from the study.

During the course of this study, two myeloablative regimens were used (Table 2). APBSCs were re-infused on day 0, followed by daily administration of G-CSF for enhancement of myeloid reconstitution until the absolute neutrophil count remained above $1000/\mu$l for 3 consecutive days.

Table 1 Induction regimen (VAIA)

	Day	Daily dosage (mg/m²)	Total dosage (mg/m²)
Vincristine	1	1.0	1.0
Actinomycin-D	1–3	0.4	1.2
Doxorubicin (adriamycin)	1–3	20	60
Ifosfamide	1–5	2000	10 000

Table 2 HDCT conditioning regimen

A: CEC		mg/m²	B: MEC		mg/m²
Day −5 to −3	Cyclophosphamide	2000	Day −4 to −1	Melphalan	130
	Etoposide	400		Etoposide	500
	Carboplatin	300		Carboplatin	500
	Dexamethazon	40			
	APBSC rescue			APBSC rescue	
Day 0	$>2.0 \times 10^6$ CD34 + cells/kg		Day 0	$>2.0 \times 10^6$ CD34 + cells/kg	

Abbreviations: APBSC = autologous peripheral blood stem cell; CEC = cyclophosphamide, etoposide and carboplatin; MEC = melphalan, etoposide and carboplatin.

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Toxicities

Toxicities were scored in accordance with the NCI Common Terminology Criteria for Adverse Events (CTCAE).

Statistical methods

OS rate was defined as the time interval from the date of HDCT to death. Failure-free survival (FFS) rate was defined as the time interval from the date of HDCT to disease progression, relapse, second malignancy or death. Survival analyses were conducted using the Kaplan–Meier method.[23] Comparisons of survival rates were performed using the log rank test.

Results

Patient characteristics

Twenty-five patients were enrolled into this study since 1995. Of the 25 enrolled patients (10 males and 15 females) with a median age of 22.0 years (15–35 years), eight patients (32%) had RMS, nine patients (36%) had EFT of the bone, and eight patients (32%) had EFT of the soft tissue. Twelve patients (48%) had metastatic lesions at the first presentation (Table 3).

Post-induction response

All patients received four courses of induction chemotherapy. Two patients received alternative induction chemotherapy before the prescribed regimen (VAIA) because of their huge tumor size (Case 4) and presence of disseminated intravascular coagulation at the first presentation (Case 24).

Twenty-four patients were assessable after induction chemotherapy, excluding one patient who had undergone radical surgery as an initial treatment. The overall response rate was 75% (18 of 24 patients), with 17% achieving CR (four out of 24), 63% achieving PR (15 out of 24), 13% achieving SD (two out of 24) and 8% resulting PD (two out of 24).

Four out of the 25 enrolled patients (16%) did not receive HDCT because two of them declined after finishing induction chemotherapy and the other two had disease progression during the induction chemotherapy. The remaining 21 patients were considered eligible for the HDCT.

The average CD34+ cell count at PBSCT was 4.21×10^6 CD34+ cells/kg body weight (range:1.7–14.9). The average number of harvesting performed was 1.8 (range 1–3). Stem cell harvesting in patients who had bone and/or bone marrow metastases at the first presentation was performed after cycles 5 or 6 to minimize tumor cell contamination, although there were no patients whose bone marrow aspirates or biopsies indicated tumor cell involvement.

Outcome

The median follow-up was 41.0 months (range 10–105 months) for the 21 patients who received HDCT. The final outcome of these patients included 13 DOD (died of disease), seven CDF (continuous disease free) and one NED (no evidence of disease). There were no AWD (alive with disease) cases at the time of this study.

Table 3 Disease characteristics and outcome of the enrolled patients

Patients' number	Age at diagnosis (years)	Gender	Histology	Primary tumor site	Initial metastatic disease	HDCT regimen	Status
1	25	Female	RMS (alveolar)	Buttock	Lung	CEC	DOD
2	16	Male	EFT (bone)	Ilium	None	CEC	DOD
3	15	Female	EFT (bone)	Femur	None	CEC	CDF
4	23	Female	EFT (soft tissue)	Pelvic cavity	None	CEC	DOD
5	20	Male	EFT (soft tissue)	Retroperitneum	Bone	CEC	DOD
6	15	Female	RMS (embryonal)	Shoulder girdle	Bone, BM	CEC	DOD
7	20	Female	RMS (alveolar)	Forearm	BM	CEC	DOD
8	17	Female	RMS (alveolar)	Perineum	None	MEC	DOD
9	27	Female	EFT (bone)	Pubis	None	MEC	CDF
10	29	Female	EFT (soft tissue)	Iliopsoas muscle	None	N/A (PD)	DOD
11	17	Male	EFT (bone)	Femur	None	MEC	CDF
12	35	Male	EFT (bone)	Lumber vertebra	None	MEC	DOD
13	21	Male	RMS (pleomorphic)	Abdominal wall	Lung	MEC	NED
14	27	Female	EFT (bone)	Femur	None	MEC	CDF
15	15	Male	EFT (soft tissue)	Abdomem	Liver	MEC	CDF
16	27	Female	RMS (pleomorphic)	Lower leg	None	N/A (refused)	CDF
17	20	Male	EFT (soft tissue)	Pleura	Lung	MEC	DOD
18	22	Female	EFT (bone)	Femur	None	MEC	CDF
19	27	Female	EFT (soft tissue)	Retroperitoneum	None	N/A (PD)	DOD
20	22	Male	EFT (soft tissue)	Inguinal	None	N/A (refused)	CDF
21	22	Male	EFT (bone)	Rib	Pleura	MEC	DOD
22	25	Female	EFT (soft tissue)	Uterus	Peritoneum	MEC	DOD
23	32	Female	RMS (embryonal)	Foot sole	Bone, BM	MEC	DOD
24	29	Female	EFT (bone)	Pelvis	Bone, BM	MEC	CDF
25	18	Male	RMS (alveolar)	Perianal	LN, muscle, bone	MEC	DOD

Abbreviations: CDF = continuous disease free; DOD = died of disease; EFT = Ewing's family of tumors; LN = lymph node; N/A = not available; NED = no evidence of disease; PD = progressive disease; RMS = rhabdomyosarcoma.
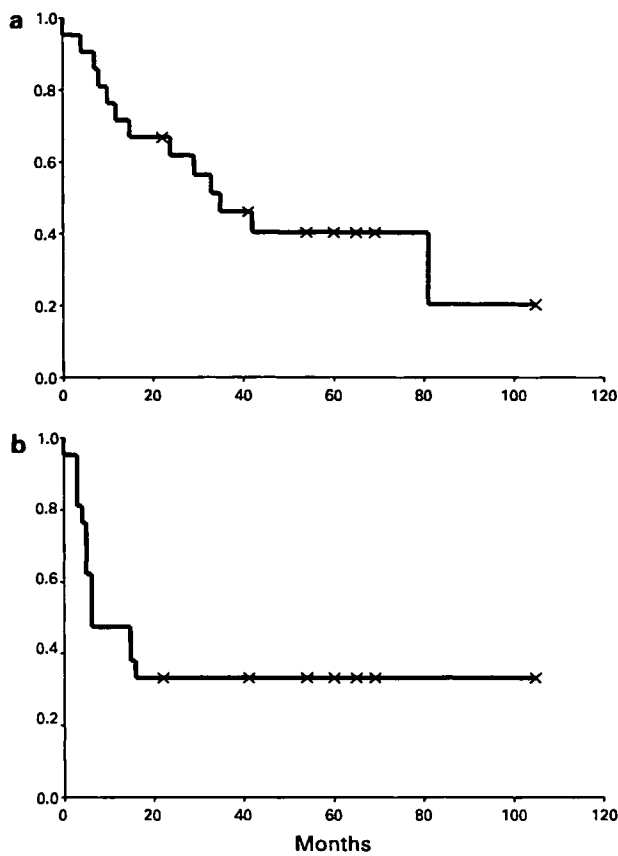
Bone Marrow Transplantation

Figure 1 Kaplan–Meier plot of (a) overall and (b) FFS of 21 patients who underwent HDCT from the time of APBSC reinfusion. Tick marks indicate surviving patients.

Figure 2 Kaplan–Meier plot of (a) overall and (b) FFS of EFT (n = 14) and RMS (n = 7) patients who underwent HDCT from the time of APBSC reinfusion. Tick marks indicate surviving patients.

The median OS and FFS from the date of PBSC reinfusion was 41.2 months (range 0–105) and 6.0 months (range 0–105), respectively. Figure 1 shows the Kaplan–Meier survival curves for OS (Figure 1a) and FFS (Figure 1b). The survival curves indicate that none of the patients relapsed beyond 16 months after HDCT.

Figure 2 shows the Kaplan–Meier survival curves for OS (Figure 2a) and FFS (Figure 2b) in patients with EFT and RMS. FFS for patients with EFT was significantly better compared with those with RMS. However, OS was not significantly different between the two groups of patients. Figure 3a and b show that survival estimates in patients with (M1) and without (M0) metastatic disease at the time of enrollment was significantly different with regard to FFS, but not for OS. Survival estimates comparing HDCT regimens and local treatment modalities were also performed, although there were no significant differences in either OS or FFS.

Toxicity

All patients who received HDCT experienced CTCAE grade 4 leukopenia, neutropenia and thrombocytopenia, which subsequently improved with stem cell re-infusion. One patient (Case 2) presented rapidly progressing left ventricular systolic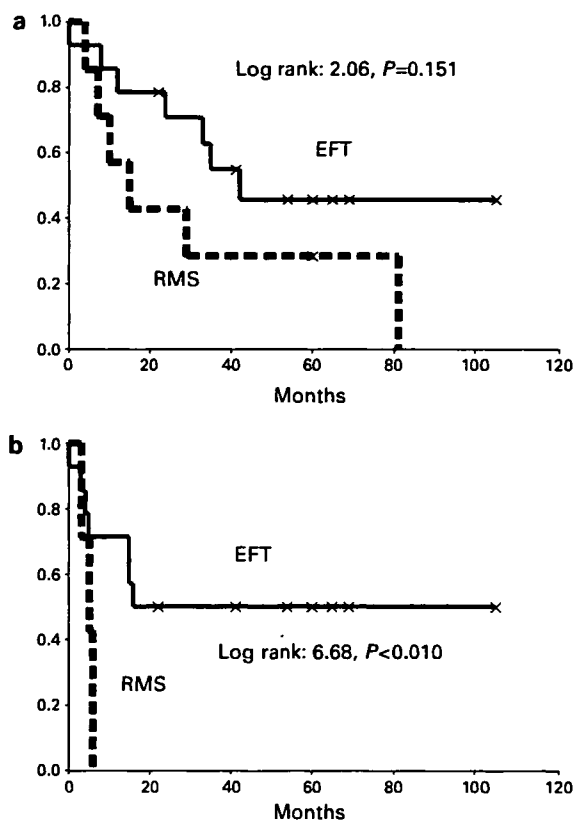 dysfunction and pneumonitis on day 7, which resulted in a fatal cardiac arrest on day 8. As the patient's family did not consent to an autopsy, the definitive cause of the sudden cardiac arrest remains unknown. Nonetheless, it is suspected that cyclophosphamide (CPA)-induced or viral myocarditis was the possible cause.

Treatment-related second malignancy was observed in two patients (Cases 6 and 15). One case (Case 6: myelodysplastic syndrome (MDS)) occurred 34 months after the initial diagnosis of shoulder-girdle RMS with extensive bone/bone marrow metastases. The patient was treated with chemotherapy, followed by allograft bone marrow transplantation, which successfully resulted in complete remission of the MDS. The patient, however, died 51 months after the occurrence of the secondary MDS from graft-versus-host disease-induced pulmonary fibrosis. The second case (Case 15: AML) was diagnosed 23 months after the initial diagnosis of pleural EFT. This patient also received chemotherapy followed by allograft bone marrow transplantation. Given that AML recurred shortly after BM transplantation, the patient died 16 months after the occurrence of the secondary AML.

Discussion

To date, only a small number of studies have examined the clinical significance of HDCT exclusively in adult patients
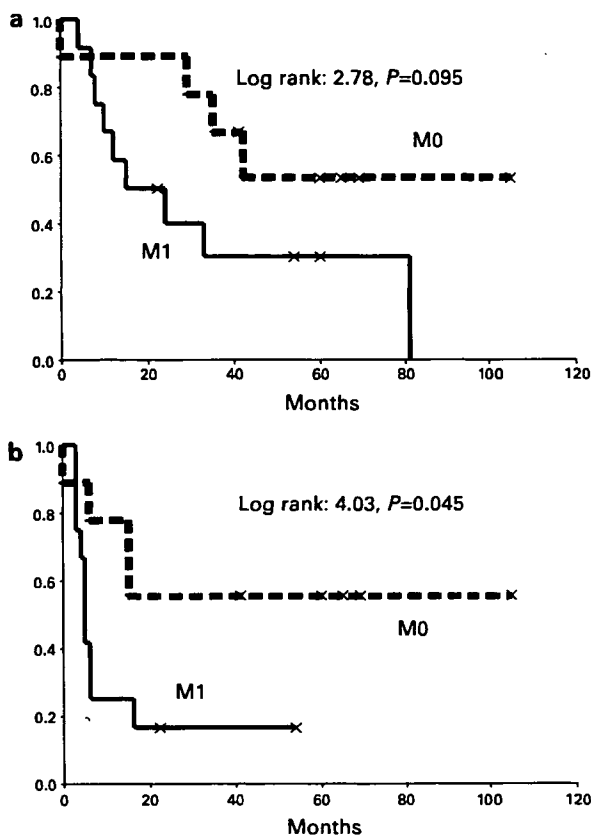
Figure 3 Kaplan–Meier plot of (a) overall and (b) FFS of patients with and without initial metastatic diseases who underwent HDCT from the time of APBSC reinfusion. Tick marks indicate surviving patients. M0: patients without metastatic diseases ($n = 9$). M1: patients with metastatic diseases ($n = 12$).

number of APBSCs were harvested after induction chemotherapy with G-CSF mobilization. Two out of 20 patients received additional mobilization chemotherapy consisting of a single agent. Harvesting APBSCs in the induction phase resulted in fewer chemotherapeutic agents and shorter treatment duration, both of which benefit patients.

Treatment intensification involves considerable risks.[26–28] In the present study, one possible case of treatment-related fatal adverse effect and two cases of secondary malignancy (one MDS and one AML) were observed. Treatment-related deaths in three out of 21 patients (14%) strongly suggest that such intensive treatment must be restricted to patients with a high risk of mortality.

The role of HDCT supported by stem cell rescue as a consolidation therapy for high-risk EFT and RMS patients has yet to be determined conclusively. Numerous reports have described disappointing survival outcomes in patients with EFT and RMS regarding the efficacy of HDCT.[7–13] However, it is difficult to deny completely the role of HDCT in patients with primary high-risk or recurrent diseases and abandon the application of this therapy, especially, as there are only a few new drugs that have been proven effective for refractory conditions. It has also been noted repeatedly that small studies conducted within a single institution cannot provide any definitive conclusions as to whether HDCT should be retained as a component of therapy for patients with high-risk EFT and RMS. A multicenter, prospective, randomized trial must be conducted to answer these difficult questions, although it should be difficult to carry out such a study due to the rare nature of these diseases. Oberlin et al.[29] recently reported on a nation-wide and multicenter scale that consolidation HDCT is feasible and may provide benefits for some EFT patients with refractory conditions.

with RMS or EFT.[24,25] Older age is known to be a significantly negative prognostic factor for both diseases, as previous studies have revealed.[15–18] The present study was conducted at a single institution using a single-arm condition and, as such, we were unable to draw any conclusive results. However, this study demonstrated that a HDCT approach is feasible in subsets of adult patients with RMS or EFT. It should be noted that the median age (22.0 years) of the patients was considerably higher than in previously reported studies.

This study was designed to examine patients with either RMS or EFT, given that treatment strategies for high-risk subsets of both diseases are very similar. The results indicate that RMS patients had very poor outcomes, with no failure-free cases at observation, whereas EFT patients had favorable outcomes, with an FFS rate of 50%. Our findings are consistent with those of Bertuzzi et al.,[12] who also investigated the effects of HDCT in adults. Both study findings suggest that, compared with EFT patients, high-risk RMS patients should not be exposed to intensive treatment using HDCT and PBSC rescue.

Most studies using APBSC as hematopoietic rescue included a mobilization phase with a regimen distinct from the induction phase. In the present study, a sufficient

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