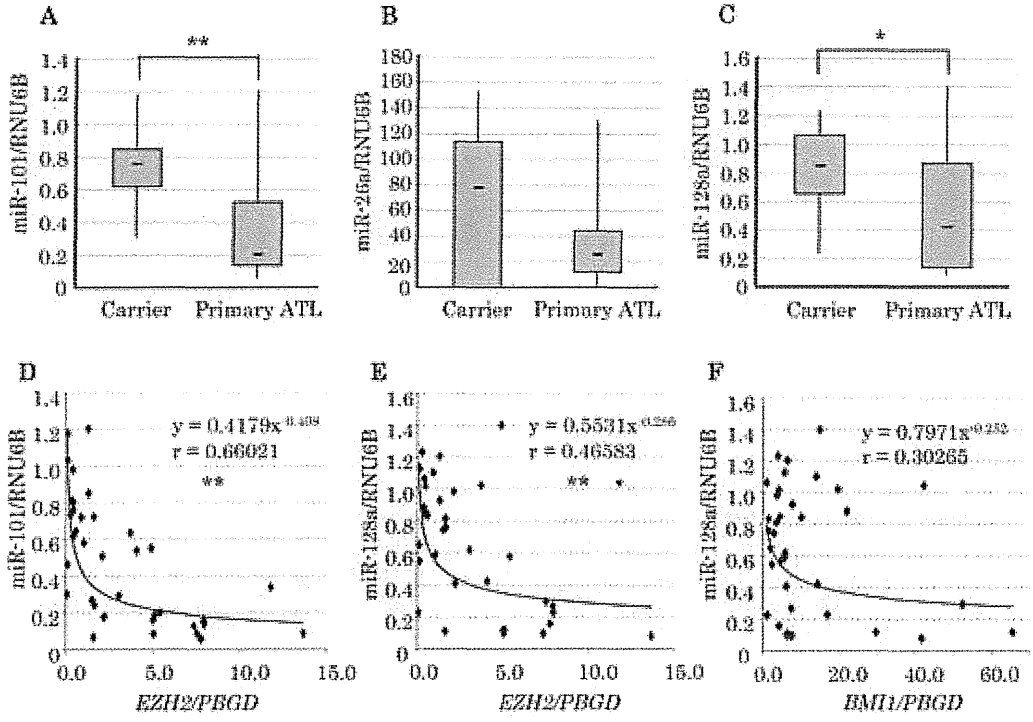
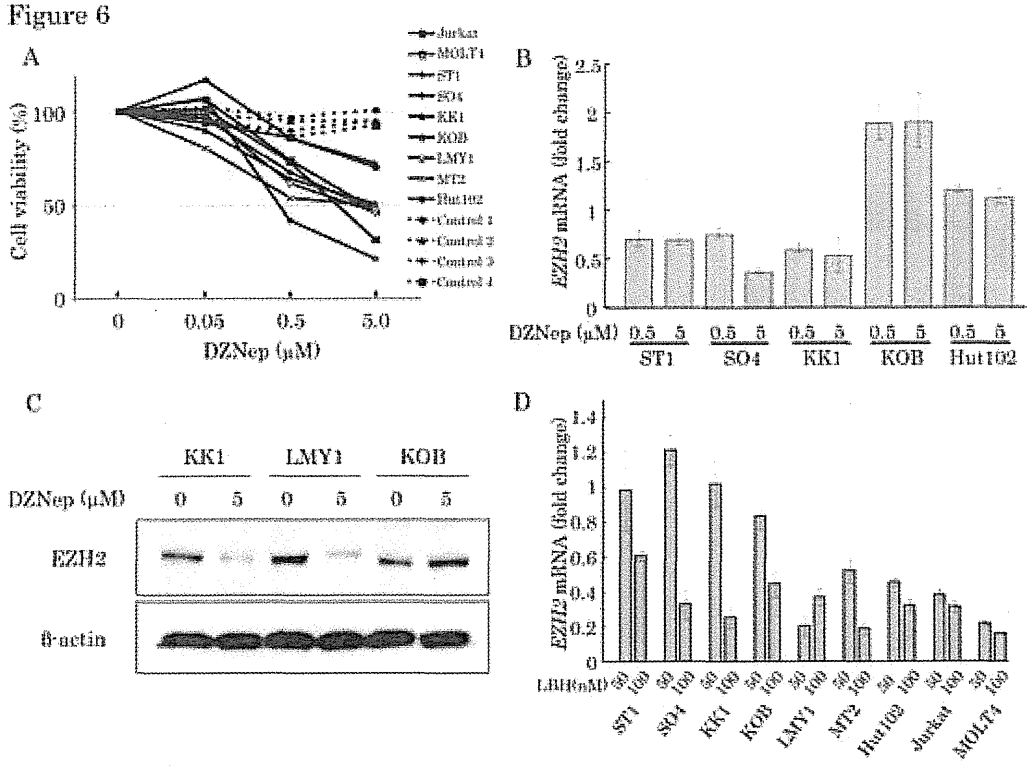
DOI: 10.3324/haematol.2010.028605

Figure 5



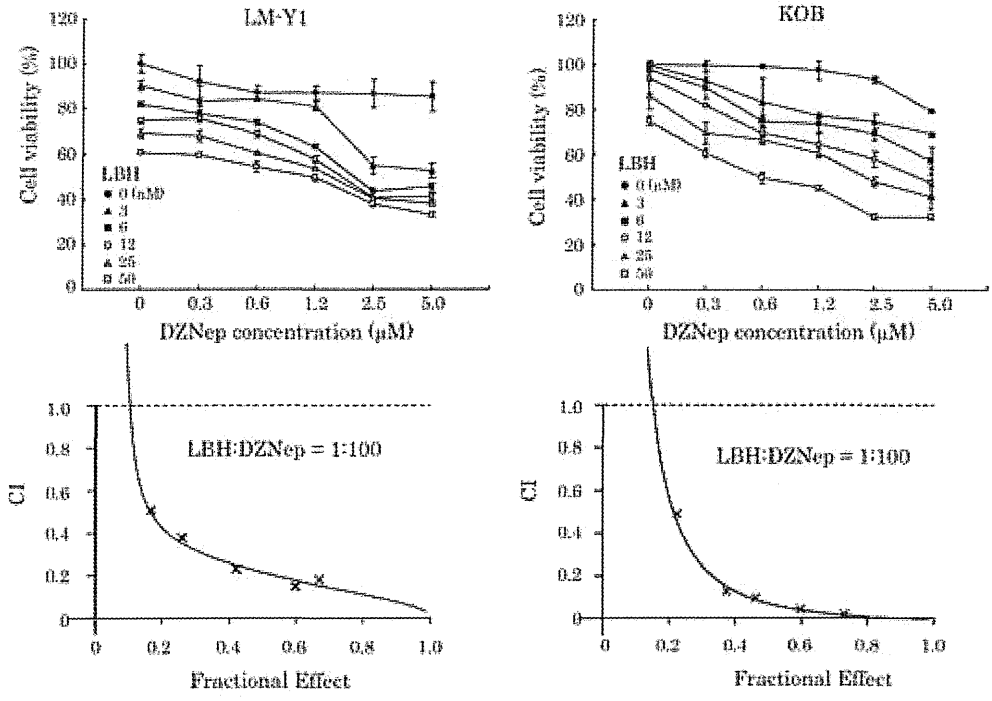
DOI: 10.3324/haematol.2010.028605

Figure 6



DOI: 10.3324/haematol.2010.028605

Figure 6 F



DOI: 10.3324/haematol.2010.028605

Supplementary Appendix

Figure legends for Supplementary Figures

Supplementary Figure 1. RYBP protein expression. Western blot analysis for RYBP protein was performed on primary ATL cells and cells from healthy adults. Most primary ATL samples showed a clear band for RYBP. In contrast, cells from healthy adults lacked the band.

Supplementary Figure 2. Quantitative genomic PCR for miR-101. PCR was performed in two loci, miR-101-1 (chromosome 1p31) and miR-101-2 (chromosome 9p24), in 10 primary ATL samples and cells from 10 HTLV-1 carriers as a control. Both loci were preserved in ATL cells, refuting the possibility that downregulation of miR-101 is caused by genomic loss of the gene.

Supplementary Figure 3. Analysis of 3'-UTR sequence of EZH2 to predict potential target sites for miRNA. In addition to the target sites for miR-101 and miR-26a, there is also a potential target site for miR-128a in the 3'-UTR of EZH2 near one of the miR-101 target sites.

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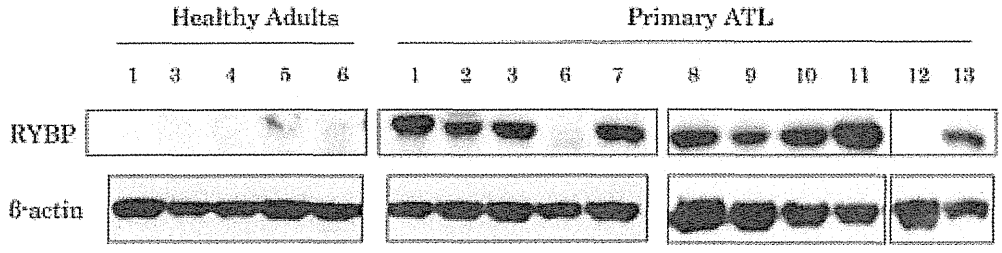
Supplementary Figure 4. Sequence analysis of EZH2. Pyrosequence analysis of EZH2 Try641 was performed in 10 ATL patients and 10 HTLV-1 carriers. Pyrograms of 6 ATL patients are shown. There were no mutations in the examined samples.

Supplementary Figure 1.



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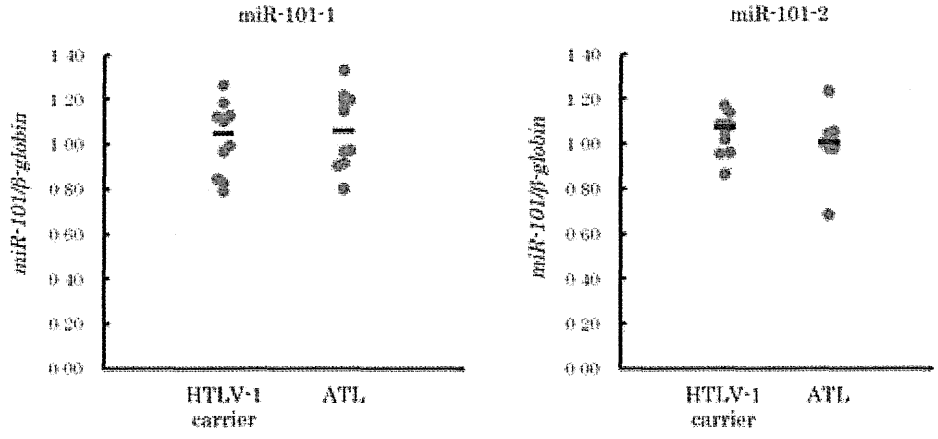
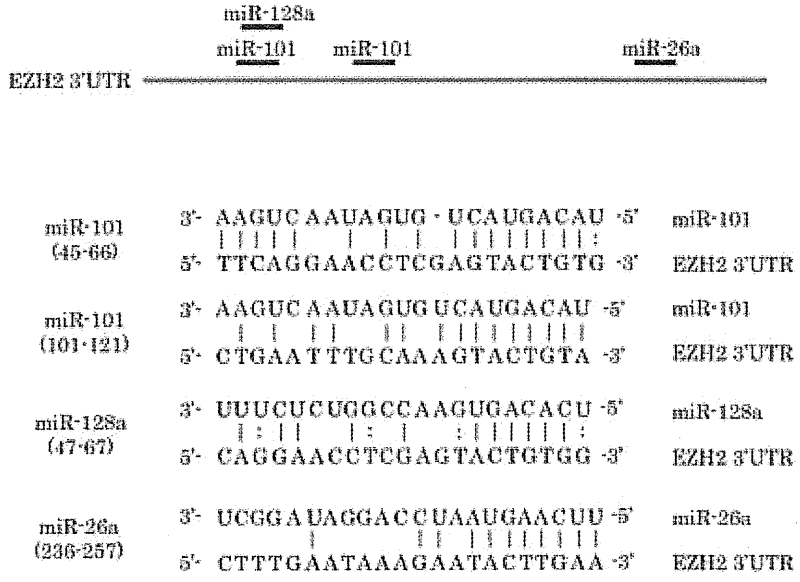
Supplementary Figure 2.



DOI: 10.3324/haematol.2010.028605

Supplementary Figure 3

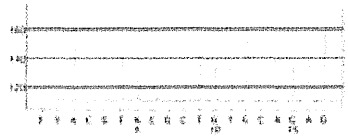


DOI: 10.3324/haematol.2010.028605

Supplementary Figure 4

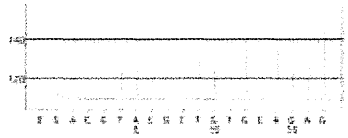
Wild-type sequence: AATACTGTGGAGAGG

ATL patient 1: wild type



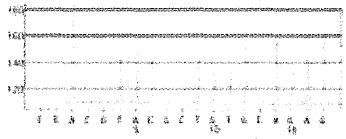
Sequence : AATAC TGTGGAGAGG

ATL patient 2: wild type



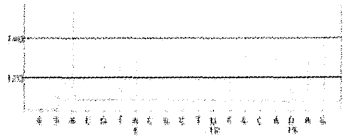
Sequence : AATAC TGTGGAGAGG

ATL patient 3: wild type



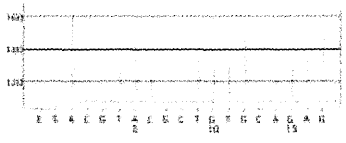
Sequence : AATAC TGTGGAGAGG

ATL patient 4: wild type



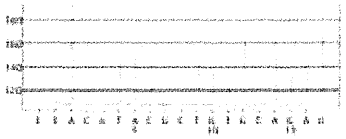
Sequence : AATAC TGTGGAGAGG

ATL patient 5: wild type



Sequence : AATAC TGTGGAGAGG

ATL patient 6: wild type



Sequence : AATAC TGTGGAGAGG

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Differences in the distribution of subtypes according to the WHO classification 2008 between Japanese and German patients with refractory anemia according to the FAB classification in myelodysplastic syndromes

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ABSTRACT

We reported the different clinical features between Japanese and German refractory anemia (RA) patients in FAB classification. We re-analyzed the clinical features by WHO classification revised in 2008. The frequencies of refractory cytopenia with unilineage dysplasia (RCUD) and myelodysplastic syndrome-unclassified (MDS-U) with pancytopenia in Japanese patients were higher than in German patients ($p < 0.001$). Refractory cytopenia with multilineage dysplasia patients showed the most unfavorable prognosis in both countries. The higher frequencies of MDS-U with pancytopenia and RCUD in Japanese patients may influence the different clinical characteristics between Japanese and German FAB-RA patients.

1. Introduction

Myelodysplastic syndromes (MDS) are acquired clonal stem cell disorders characterized by ineffective hematopoiesis with myelodysplasia [1] and are associated with a high risk of progression to acute leukemias [2]. MDS are very heterogeneous in terms of their morphology, clinical features, and survival [3]. There are several reports indicating possible differences in clinical features between Western MDS types and Eastern MDS types [4–9]. The median age of MDS patients in Korea and Thailand were reported to be 57 [8] and 56 [7], respectively. On the other hand, large MDS studies from Western countries showed a median or mean age of 68–73 years [10–13]. We have reported that the clinical features of refractory anemia with excess of blasts (RAEB) or RAEB in transformation (RAEB-t) according to the French–American–British (FAB) classification [14] seemed to be similar between Japanese and Western patients [15]. However, previous reports [5,15] indicated that Japanese MDS patients have a lower frequency of refractory anemia with ringed sideroblasts (RARS) according to the FAB classification and a higher frequency of refractory anemia according to the FAB classification (FAB-RA) than the Western International Prognostic Scoring System (IPSS) study [10], and we reported that the clinical and laboratory features of Japanese FAB-RA patients apparently differ from those of German patients after a precise morphologic consensus (FAB classification: concordance rate, 98.4%; κ, 0.94; $p < 0.001$; prior World Health Organization (WHO) classification (WHO classification 2001) [16]: concordance rate, 83.8%; κ, 0.73; $p < 0.001$) [17]. That was the first comparison report between Western and Eastern FAB-RA patients after confirming morphological consensus. Japanese FAB-RA patients were younger, showed more severe cytopenia(s), a lower frequency of abnormal karyotypes, a lower frequency of MDS with isolated del(5q) (5q- syndrome), and a more favorable prognosis in terms of the overall survival (OS) and leukemia free survival (LFS) in our previous study.

MDS subtypes in the WHO classification 2001 [16] was revised in 2008 (WHO classification 2008) [18]. Refractory anemia (RA), refractory neutropenia (RN), and refractory thrombocytopenia (RT) were combined into refractory cytopenia with unilineage dysplasia

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(RCUD) in the WHO classification 2008. The diagnosis of MDS-unclassified (MDS-U) according to the WHO classification 2008 can be made in the following instances:

1. Patients with the findings of RCUD or refractory cytopenia with multilineage dysplasia (RCMD) but with 1% blasts in the peripheral blood (PB) (PB blasts type).
2. Cases of RCUD which are associated with pancytopenia (RCUD/pancytopenia type).
3. Patients with cytopenia(s) with 1% or fewer blasts in the PB and fewer than 5% in the bone marrow (BM), unequivocal dysplasia in <10% of the cells in one or more myeloid lineages, and who have cytogenetic abnormalities (cytogenetic abnormalities type).

MDS-U (PB blasts type) is classified as RAEB according to the FAB classification because of 1% blasts in the PB. MDS-U (cytogenetic abnormalities type) is not diagnosed as MDS according to the FAB classification because of unequivocal dysplasia. Thus, FAB-RA patients are classified as RCUD, RCMD, MDS with isolated del(5q) (5q- syndrome) or MDS-U (RCUD/pancytopenia type) according to the WHO classification 2008. In the present study, we re-analyzed in detail the clinical features of Japanese and German FAB-RA patients by using revised MDS subtypes in the WHO classification 2008.

2. Patients and methods

The dataset of consecutive patients with primary FAB-RA of our previous study [17] (total 728 consecutive patients: Japan, 131 cases; Germany, 597 cases) were used for the present retrospective analysis. Japanese patients of this dataset were diagnosed at the Saitama Medical University Hospital, Nagasaki University Hospital or affiliated hospitals between April 1976 and January 1997. German patients were diagnosed at the Department of Hematology, Oncology and Clinical Immunology of the Heinrich-Heine University between January 1973 and December 2002. Patients who had previously been treated with anti-neoplastic drugs or ionizing radiation were excluded from the study. Patients without the available necessary data for the WHO classification 2008 were excluded from the present study. Cytogenetic analyses were performed with a trypsin-Giemsa banding technique on BM cells from aspirates. Ordinarily 20–30 metaphases were examined. Cytogenetic aberrations were grouped according to the IPSS publication [10]. Thresholds for cytopenia(s) were defined as those of the IPSS (hemoglobin (Hb) <10.0 g/dL, absolute neutrophil count (ANC) $<1.8 \times 10^9$/L, and platelet $<100 \times 10^9$/L). Criteria for dysplasia were defined as those of a previous German report [19]. Hypoplastic BM was defined as <30% cellular in patients <60 years old, or <20% cellular in patients ≥60 years old [20]. If hypoplastic BM and certain dysplasia more than 10% in one or more of major myeloid cell lines were present, a diagnosis of hypoplastic MDS was made. Patients were reclassified according to the definition of WHO classification 2008 for MDS subtyping by using PB and BM findings, morphologic findings, and cytogenetic findings of the previous dataset [17]. Comparisons of the clinical features at the time of diagnosis and OS and LFS were analyzed by using the dataset of our previous study [17]. OS was measured from the date of diagnosis until death due to any cause, the date of stem cell transplantation, or until the last patient contact. LFS was measured from the date of diagnosis until the date of diagnosis of acute leukemia. This study was approved by the Institutional Review Board of Saitama International Medical Center, Saitama Medical University, Saitama, Japan.

2.1. Statistical methods

The chi-square test and the nonparametric Mann–Whitney test were used to compare the proportions of patients and continuous data, respectively. The Kaplan–Meier method was used to generate the estimate of cumulative probabilities of OS and LFS. The difference in the cumulative probabilities within subcategories of patients was compared using a two-sided log-rank test. A two-sided p value of <0.05 was considered to be statistically significant. All statistical analyses were performed with the use of StatView (version 5.0, SAS Institute, Cary, NC).

3. Results

3.1. Comparison of frequencies of subtypes according to the WHO classification 2008 between Japanese and German FAB-RA patients

A total of 295 patients (Japan, 102 cases; Germany, 193 cases) could be classified according to the WHO classification 2008. A total of 433 patients (Japan, 29 cases; Germany, 404 cases) could not be classified according to the WHO classification 2008 due to a deficit of either cytogenetic data or adequate peripheral blood data, and 427 patients presented without available cytogenetic findings (Japan, 29 cases; Germany, 398 cases). There were 6 patients (Germany, 6 cases) without any data of peripheral blood.

MDS-U (PB blasts type) is classified as RAEB according to the FAB classification. MDS-U (cytogenetic abnormalities type) is not diagnosed as MDS according to the FAB classification due to unequivocal dysplasia. Therefore, patients with MDS-U (PB blasts type) or with MDS-U (cytogenetic abnormalities type) were not included in the previous dataset. Because the previous dataset used in the present study was that of FAB-RA patients, dysplasia existed in at least one lineage and the frequency of blasts in PB was <1% in all patients. Therefore, all MDS-U patients in the present study were diagnosed as RCUD/pancytopenia type. Most Japanese FAB-RA patients were classified as RCUD, RCMD, or MDS-U (RCUD/pancytopenia type) according to the WHO classification 2008 (Table 1A). Most German FAB-RA patients were classified as RCUD, RCMD, or 5q- syndrome (Table 1B). The frequency of RCUD in Japanese FAB-RA patients (45%) was significantly higher than that in German FAB-RA patients (19%) ($p < 0.001$). The frequency of patients with bicytopenia in Japanese RCUD patients was 59%, but that in the German RCUD patients was only 19%. Among 46 Japanese RCUD patients, number of patients with single cytopenia was 17 cases (37%) including 2 RA, 4 RN and 11 RT cases. Among 37 German RCUD patients, number of patients with single cytopenia was 22 cases (59%) including 7 RA, 11 RN and 4 RT cases. Frequency of RT was 2% of German FAB-RA patients. The frequency of RT of Japanese FAB-RA patients (11%) was higher than that of German FAB-RA patients. The frequency of MDS-U in Japanese FAB-RA patients (29%) was significantly higher than that in German FAB-RA patients (3%) ($p < 0.001$). The frequency of RCMD in Japanese FAB-RA patients (25%) was significantly lower than in German FAB-RA patients (58%) ($p < 0.001$). The frequency of 5q- syndrome in Japanese FAB-RA patients (3%) was significantly lower than in German FAB-RA patients (20%) ($p < 0.001$) (Table 1C).

3.2. Comparison of clinical and laboratory features at the time of diagnosis between Japanese and German patients could be classified according to the WHO classification 2008

The age of patients in RCUD, MDS-U and RCMD subtypes did not differ between the two countries. The MDS-U (RCUD/pancytopenia type) subtype was younger than other subgroups in Japanese patients. The gender ratios in the RCUD

Table 1
Laboratory features at the time of diagnosis in FAB-RA patients who could be classified according to the WHO classification 2008.

	RCUD	MDS-U	RCMD	5q- synd
(A) Japanese patients, $n = 102$				
Patients = n (%)	46(45)	28(29)	25(25)	3(3)
Gender (male/female)	28/18	12/16	11/14	2/1
Age (years)	57(16–86)	51(15–82)	63(16–88)	60(59–74)
Neutrophils ($\times 10^9$/L)	1.89 (0.44–4.69)	1.10 (0.26–1.77)	1.28 (0.05–10.24)	0.73 (0.50–2.54)
Hemoglobin (g/dL)	10.2 (3.0–14.3)	6.9 (4.2–9.1)	8.2 (2.9–14.0)	6.3 (4.6–10.8)
Platelets ($\times 10^9$/L)	41 (4–246)	29(7–98)	50(13–390)	207(134–212)
Abnormal karyotype = n (%)	12(26)	6(21)	9(36)	3(100)
Hypoplastic bone marrow = n (%)	3(7)	3(11)	0(0)	0(0)
(B) German patients, $n = 193$				
Patients = n (%)	37(19)	6(3)	111(58)	39(20)
Gender (male/female)	20/17	1/5	80/31	14/25
Age (years)	62(20–80)	56(19–59)	63(15–86)	62(32–78)
Neutrophils ($\times 10^9$/L)	1.92 (0.36–8.72)	1.41 (0.48–1.50)	1.60 (0.21–19.40)	1.95 (0.61–6.78)
Hemoglobin (g/dL)	11.0 (5.2–15.4)	9.4 (5.5–9.8)	9.2 (5.1–16.9)	8.7 (3.0–12.2)
Platelets ($\times 10^9$/L)	128 (2–840)	33(10–90)	102(9–999)	250(28–1540)
Abnormal karyotype = n (%)	12(32)	3(50)	47(42)	39(100)
Hypoplastic bone marrow = n (%)	3(8)	2(33)	13(12)	5(13)
	Japan vs Germany			
(C) Comparison between Japanese and German patients				
(1) RCUD patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.532$			
Age (years)	$p = 0.150$			
Neutrophils ($\times 10^9$/L)	$p = 0.466$			
Hemoglobin (g/dL)	$p = 0.087$			
Platelets ($\times 10^9$/L)	$p < 0.001$			
Abnormal karyotype (%)	$p = 0.526$			
Hypoplastic bone marrow (%)	$p = 0.782$			
(2) MDS-U patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.239$			
Age (years)	$p = 0.557$			
Neutrophils ($\times 10^9$/L)	$p = 0.821$			
Hemoglobin (g/dL)	$p = 0.036$			
Platelets ($\times 10^9$/L)	$p = 0.752$			
Abnormal karyotype (%)	$p = 0.150$			
Hypoplastic bone marrow (%)	$p = 0.156$			
(3) RCMD patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.007$			
Age (years)	$p = 0.401$			
Neutrophils ($\times 10^9$/L)	$p = 0.494$			
Hemoglobin (g/dL)	$p = 0.016$			
Platelets ($\times 10^9$/L)	$p = 0.030$			
Abnormal karyotype (%)	$p = 0.561$			
Hypoplastic bone marrow (%)	$p = 0.072$			
(4) 5q- synd patients				
Frequency	$p < 0.001$			
Gender (male/female)	$p = 0.290$			
Age (years)	$p = 0.920$			
Neutrophils ($\times 10^9$/L)	$p = 0.144$			
Hemoglobin (g/dL)	$p = 0.370$			
Platelets ($\times 10^9$/L)	$p = 0.188$			
Abnormal karyotype (%)	N/A			
Hypoplastic bone marrow (%)	$p = 0.509$			

Values for presentation characteristics are given as median and range where applicable. N/A, not applicable; RCUD, refractory cytopenia with unilineage dysplasia; MDS-U, MDS-unclassified; RCMD, refractory cytopenia with multilineage dysplasia; 5q- synd, MDS with isolated del(5q).

and MDS-U subtypes were not significantly different between the two countries. The frequency of male patients in Japanese RCMD subgroup was significantly lower than that in German RCMD subtype. Japanese patients had significantly lower platelet counts than German patients in both the RCUD and RCMD subtypes. Japanese MDS-U (RCUD/pancytopenia type) and RCMD patients showed significantly lower Hb concentrations than German MDS-U (RCUD/pancytopenia type) and RCMD patients. Japanese RCUD patients showed a tendency towards lower Hb concentrations than German RCUD patients. The ANC did not differ significantly between the two countries in RCUD, MDS-U (RCUD/pancytopenia type), and RCMD patients (Table 1). The frequency of cytogenetic abnormalities in the Japanese FAB-RA patients was significantly lower than in German patients ($p < 0.001$) (Tables 1 and 2). The frequencies of cytogenetic abnormalities in the RCUD, MDS-U (RCUD/pancytopenia type), and RCMD subtypes were not significantly different between the two countries (RCUD, $p = 0.526$; RCMD, $p = 0.561$; MDS-U (RCUD/pancytopenia type), $p = 0.150$). The frequency of isolated del(5q) in Japanese FAB-RA patients was significantly lower than in German patients

Table 2
Cytogenetic findings at the time of diagnosis in FAB-RA patients who could be classified according to the WHO classification 2008.

	RCUD	MDS-U	RCMD	5q- synd	Total
(A) Japanese patients, $n=102$					
Patients = n	46	28	25	3	102
Good	37(80.4%)	23(82.1%)	16(64.0%)	3(100%)	79(77.5%)
Normal	34(73.9%)	22(78.6%)	16(64.0%)	0(0%)	70(68.6%)
–Y	0	1	0	0	1
del(5q)	0	0	0	3	3
del(20q)	3	0	0	0	3
Intermediate	8(17.4%)	3(10.7%)	4(16.0%)	0	15(14.7%)
Poor	1(0.2%)	2(7.2%)	5(20.0%)	0	8(7.8%)
Complex (≥3 abnormalities)	0	1	4	0	5
Chromosome 7 anomalies	1	1	1	0	3
(B) German patients, $n=193$					
Patients = n	37	6	111	39	193
Good	27(73.0%)	3(50.0%)	72(64.9%)	39(100%)	141(73.1%)
Normal	25(67.6%)	3(50.0%)	64(57.7%)	0(0%)	92(47.7%)
–Y	2	0	2	0	4
del(5q)	0	0	0	39	39
del(20q)	0	0	6	0	6
Intermediate	4(10.8%)	2(33.3%)	23(20.7%)	0	29(15.0%)
Poor	6(16.2%)	1(16.7%)	16(14.4%)	0	23(11.9%)
Complex (≥3 abnormalities)	5	0	9	0	14
Chromosome 7 anomalies	1	1	7	0	9

Good indicates normal, –Y, del(5q), del(20q); poor, complex (≥3 abnormalities) or chromosome 7 anomalies; intermediate, other abnormalities not listed in good and poor subgroups.

($p<0.001$) (Table 2). The most frequent cytogenetic aberration in the intermediate cytogenetic risk according to the IPSS publication was trisomy 8 (4 German RCMD cases, 3 Japanese RCUD cases, 1 Japanese MDS-U case). The frequencies of hypoplastic BM were not significantly different between the two countries in the RCUD and MDS-U (RCUD/pancytopenia type) subtypes. In the RCMD subtype, there were no Japanese patients presenting with findings concordant with hypoplastic BM. However, the frequency of German RCMD patients with hypoplastic BM was 12% (Table 1).

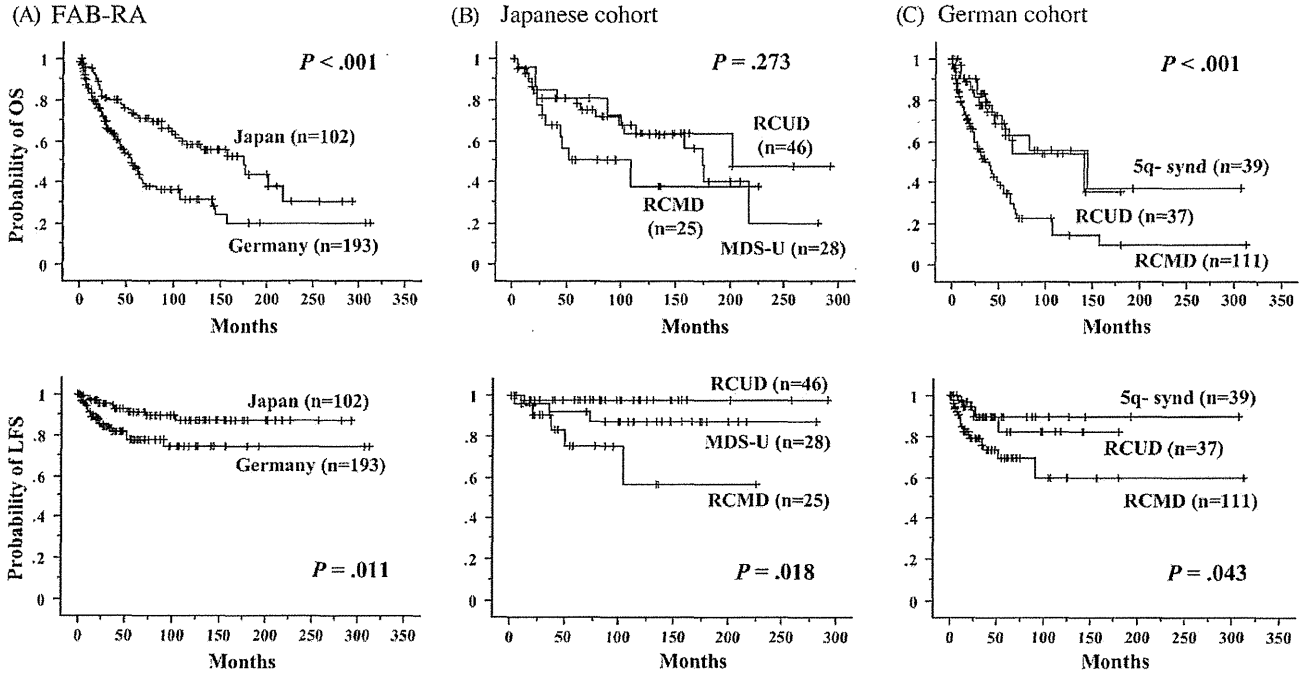

Fig. 1. Cumulative overall survival and leukemia free survival of FAB-RA patients who could be classified according to the WHO classification 2008. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) In FAB-RA patients who could be classified according to the WHO classification 2008, Japanese patients had a more favorable OS than German patients ($p<0.001$). Japanese patients had a more favorable LFS than German patients ($p=0.011$). (B) In Japanese FAB-RA patients who could be classified according to the WHO classification 2008, RCMD patients showed the least favorable OS and LFS compared with the other subtypes excluding a rare subtype (5q- syndrome subtype). RCUD patients showed more favorable OS and LFS than RCMD patients (OS, $p=0.128$; LFS, $p=0.004$). MDS-U (RCUD/pancytopenia type) patients tended to show more favorable OS and LFS than RCMD patients (OS, $p=0.218$; LFS, $p=0.137$). (C) In German FAB-RA patients who could be classified according to the WHO classification 2008, RCMD patients showed the least favorable OS and LFS compared with the other subtypes excluding a rare subtype (MDS-U (RCUD/pancytopenia type) subtype). RCUD patients showed more favorable OS and LFS than RCMD patients (OS, $p=0.003$; LFS, $p=0.075$). 5q- syndrome patients showed more favorable OS and LFS than RCMD patients (OS, $p=0.002$; LFS, $p=0.043$).

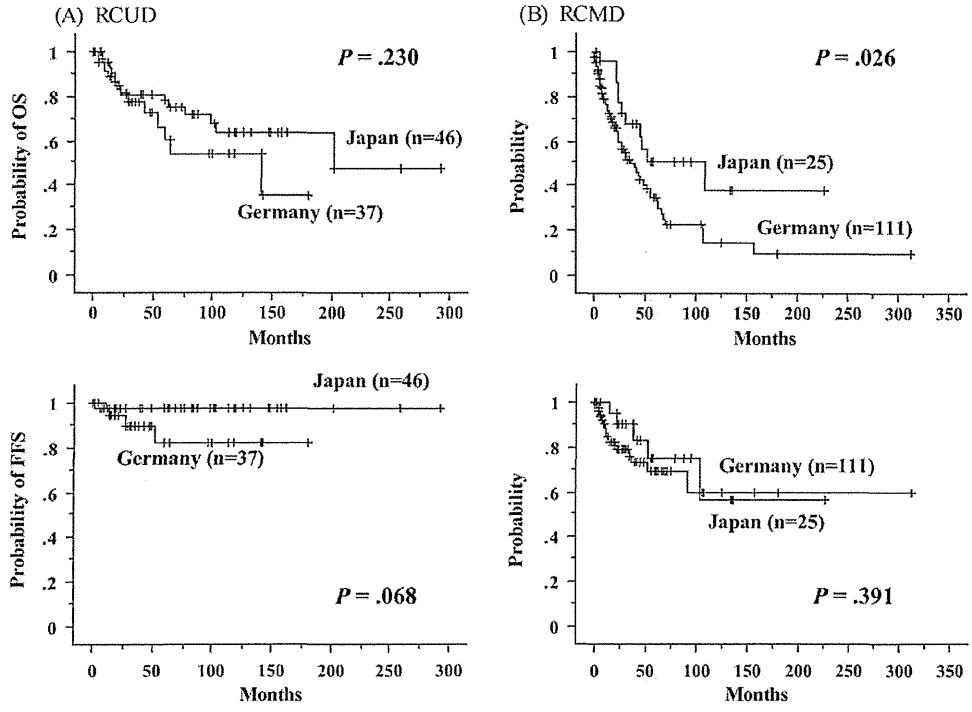

Fig. 2. Comparison of cumulative overall survival and leukemia free survival of RCUD and RCMD between Japanese and German patients. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) The OS of RCUD patients was not significantly different between the two countries ($p = 0.230$). Japanese RCUD patients tended to show a more favorable LFS than German RCUD patients ($p = 0.068$). (B) Japanese RCMD patients showed a more favorable OS than German RCMD patients ($p = 0.026$). The LFS of RCMD patients was not significantly different between the two countries ($p = 0.391$).

3.3. Prognosis

Follow-up periods ranged from 1 to 292 months (median, 78 months) in Japanese FAB-RA patients who could be classified according to the WHO classification 2008. Follow-up periods in German patients ranged from 0 to 313 months (median, 23 months). During the follow-up period, 9 Japanese patients and 27 German patients progressed to acute myeloid leukemia (AML). Forty Japanese patients (9 AML, 15 infection, 7 bleeding, 1 heart failure, 2 others (non-hematological causes), 6 unknown) and 81 German patients (24 AML, 16 infection, 7 bleeding, 2 heart failure, 5 others (non-hematological cause), 27 unknown) died.

For the OS, Japanese FAB-RA patients who could be classified according to the WHO classification 2008 had a more favorable prognosis than German FAB-RA patients (OS median survival: Japan, 117 months; Germany, 55 months; $p < 0.001$). In LFS, Japanese FAB-RA patients who could be classified according to the WHO classification 2008 had a more favorable prognosis than German FAB-RA patients (10% LFS: Japan, 74 months; Germany, 14 months; $p = 0.011$) (Fig. 1A). RCMD patients showed the least favorable OS and LFS compared with the other subtypes excluding rare subtypes (Japan, 5q -syndrome subgroup; Germany, MDS-U (RCUD/pancytopenia type) subgroup) in both countries (Fig. 1B and C). The OS of RCUD patients was not significantly different between the two countries (OS median survival: Japan, 202 months; Germany, 141 months; $p = 0.230$). Japanese RCUD patients tended to show a more favorable LFS than German RCUD patients (LFS median survival: Japan, more than 292 months; Germany, 27 months; $p = 0.068$) (Fig. 2A). Japanese RCMD patients showed a more favorable OS than German RCMD patients (OS median survival: Japan, 109 months; Germany, 36 months; $p = 0.026$). The LFS of RCMD patients was not significantly different between the two countries (10% LFS: Japan, 38 months; Germany, 10 months; $p = 0.391$) (Fig. 2B). Follow-up periods ranged from 1 to 282 months (median, 114 months) in Japanese MDS-U (pancytopenia type) patients. In contrast, follow-up periods ranged from 15 to 46 months (median, 31 months) in German MDS-U (RCUD/pancytopenia type) patients. In addition, there were only 6 German MDS-U (RCUD/pancytopenia type) patients. Because of the short follow-up periods and the small number of German patients, the comparison of OS and LFS between the two countries was not adequate in the MDS-U (RCUD/pancytopenia type) subgroup. For the same reasons as for the MDS-U (RCUD/pancytopenia type) subtype, the comparison of OS and LFS between the two countries was not adequate in the 5q-syndrome subtype.

4. Discussion

There was no centralized pathology review in this study. However, we previously reported that morphologic diagnosis between the German and Japanese hematologists was in line [17]. Morphologic diagnosis of this study was performed by the same Japanese and German hematologists. Therefore, we believe that there may be extremely little differences between the interpretations of pathologists in Germany versus Japan.

Concerning the frequencies of subtypes of the WHO classification 2008, Japanese FAB-RA patients differed from German patients. The frequency of RCUD in Japanese FAB-RA patients was higher than in German patients. The frequency of RCMD in Japanese FAB-RA patients was lower than in German patients. The frequency of RT of Japanese FAB-RA patients was higher than that of German patients. The frequency of 5q- syndrome in Japanese FAB-RA patients was lower than in German patients. Morel et al. [21] and Greenberg et al. [10] reported that the frequencies of isolated del(5q) in patients with all MDS subtypes were 4.7% and 5.9%, respectively. Several reports have already indicated that MDS with isolated del(5q) is rare in Japanese patients. Toyama et al. [5] and Matsushima et al. [6] (Toyama

et al., 2.0%; Matsushima et al., 1.5%) reported that Japanese MDS patients had a lower frequency of isolated del(5q) than patients in Western reports. Most interestingly, the frequency of MDS-U (RCUD/pancytopenia type) in Japanese FAB-RA patients was significantly higher than in German FAB-RA patients. It is suggested here that the frequencies of each MDS subtype cannot be solely judged by the results of the present study. However, in the previous consecutive dataset [17] of the present study including the patients classified according to the WHO classification 2008, the frequency of Japanese FAB-RA patients with pancytopenia (35.1%) was significantly higher than in German patients (13.1%) ($p < 0.001$). Therefore, it is very likely that the frequency of the MDS-U (RCUD/pancytopenia type) subtype in Japanese patients is higher than that in German patients. We believe that the different frequencies of RCUD and MDS-U (RCUD/pancytopenia type) between two countries are noticeable and important for discussing the differences in clinical features between these two countries.

Japanese FAB-RA patients were younger than German FAB-RA patients in our previous study [17]. In contrast, the age of Japanese patients was not significantly different from that of German patients in the RCUD, MDS-U and RCMD subgroups in the present study. However, the comparison of age in the present study is problematic. Cytogenetic findings are necessary for a diagnosis according to the WHO classification 2008. Therefore, patients in the previous data set without available cytogenetic data were excluded from the present study. In German patients with advanced age, the frequency of patients where cytogenetic examinations were performed was low. In German patients, the age of patients without available cytogenetic data (median, 74 years) was significantly higher than in patients with available cytogenetic data (median, 63 years) ($p < 0.001$). In contrast, the age of Japanese patients without available cytogenetic data (median, 60 years) was not significantly different from Japanese patients with available cytogenetic data (median, 56 years) ($p = 0.542$). The age of German patients without available cytogenetic data (median, 74 years) was significantly higher than that of Japanese patients without available cytogenetic data (median, 60 years) ($p < 0.001$). Therefore, it was considered that the age of German patients in the present study was not representative. MDS-U (RCUD/pancytopenia type) patients (median, 51 years) tended to be younger than FAB-RA patients excluding the MDS-U (RCUD/pancytopenia type) subtype (median, 58 years) in Japanese patients. The German MDS-U (RCUD/pancytopenia type) patients also tended to be younger than other subtypes.

We previously reported that Japanese FAB-RA patients showed more severe cytopenia(s) [17]. The MDS-U (RCUD/pancytopenia type) subtype showed more severe cytopenia(s) in the present study. The frequency of MDS-U (RCUD/pancytopenia type) in Japanese patients was higher than that in German patients. The high frequency of the MDS-U (RCUD/pancytopenia type) subtype in Japanese patients may largely influence the unique characteristics (younger age and more severe cytopenia(s)) of the Japanese FAB-RA patients that were clarified by our previous report [17].

We reported that the frequency of cytogenetic abnormalities in Japanese FAB-RA patients were lower than in German patients in previous study [17]. The cause of this finding was the low frequency of 5q- syndrome in Japanese FAB-RA patients.

We reported that Japanese FAB-RA patients presented with a favorable overall OS and LFS in previous study [17]. The OS and LFS of Japanese and German FAB-RA patients who could be classified according to the WHO classification 2008 in the present study were similar to our previous report. Several guidelines [22–24] have been published in Western countries. To adapt these Western guidelines to Asian patients, some modifications may be required, taking into account ethnic differences. Nevertheless, no difference was found in LFS between Japanese and German RCMD patients, Japanese RCMD patients showed a more favorable OS than German RCMD patients. It was reported that transfusion dependency was an adverse prognostic factor in MDS patients [3]. Most Japanese patients with Hb concentrations lower than 7.0 g/dL had received red cell transfusion. In contrast, most German patients with Hb concentrations lower than 9.0 g/dL had received red cell transfusion. This difference in threshold for the induction of transfusion between the two countries may influence the different OS between the two countries. The frequency of German patients with Hb concentrations lower than 9.0 g/dL (41%) was higher than that of Japanese RCMD patients with Hb concentrations lower than 7.0 g/dL (28%). In fact, RCMD patients with Hb concentrations lower than 9.0 g/dL tended to show a more unfavorable OS than RCMD patients with Hb concentrations of 9.0 g/dL or more in German patients (OS median survival: Hb lower than 9.0 g/dL, 30 months; Hb at least 9.0 g/dL, 48 months; $p = 0.054$).

Reports of several Eastern countries showed consistently unique characteristics of Eastern MDS, like young age, and a low frequency of RARS and 5q- syndrome [5,8,9,15] and the absence of a prognostic impact of cytopenia [7,8,17], although environmental factors differ between the countries. Therefore, we consider that there are genetic differences between East and West, rather than environmental factors.

In conclusion, the frequency of RCUD and MDS-U (RCUD/pancytopenia type) in Japanese patients was higher than in German patients. In particular, MDS-U (RCUD/pancytopenia type) patients occupied approximately 30% among Japanese FAB-RA patients, but MDS-U was rare (3%) in German patients. Concerning the age at the time of diagnosis, the MDS-U (RCUD/pancytopenia type) subtype was apparently younger than other subgroups in Japanese patients. The cytopenia(s) of the MDS-U (RCUD/pancytopenia type) subtype were more severe than in the RCUD and RCMD subtypes in Japanese patients. RCMD patients showed the less favorable OS and LFS than the other subtypes in both countries. The frequency of RCMD in Japanese patients was lower than that in German patients. We believe that the different frequencies of MDS subtypes according to the WHO classification 2008 between Japanese and German FAB-RA patients underlie the different clinical characteristics of FAB-RA patients between the two countries.

Conflict of interest statement

The authors reported no potential conflict of interest.

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Contributors. A.M. designed the research, performed morphological analyses, collected data, analyzed data and wrote the manuscript. U.G. and I.J. designed the research, performed morphological analyses, collected data and analyzed data. M.T. designed the research, performed morphological analyses and analyzed data. M.I. collected data, performed morphological analyses and analyzed data. M.B. designed the research and analyzed data. A.K., C.S. and N.G. performed morphological analyses and collected data. K.A., Y.M. and T.H. collected data.

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ORIGINAL ARTICLE

Comparative analysis of remission induction therapy for high-risk MDS and AML progressed from MDS in the MDS200 study of Japan Adult Leukemia Study Group

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Abstract A total of 120 patients with high-risk myelodysplastic syndrome (MDS) and AML progressed from MDS (MDS–AML) were registered in a randomized controlled study of the Japan Adult Leukemia Study Group (JALSG). Untreated adult patients with high-risk MDS and MDS–AML were randomly assigned to receive either idarubicin and cytosine arabinoside (IDR/Ara-C) (Group A) or low-dose cytosine arabinoside and aclarubicin (CA) (Group B). The remission rates were 64.7% for Group A (33 of 51 evaluable cases) and 43.9% for Group B (29 out of 66 evaluable cases). The 2-year overall survival rates and disease-free survival rates were 28.1 and 26.0% for Group A, and 32.1 and 24.8% for Group B, respectively. The duration of CR was 320.6 days for Group A and 378.7 days for Group B. There were 15 patients who lived longer than 1,000 days after diagnosis: 6 and 9 patients in Groups A and B, respectively. However, among patients enrolled in this trial, intensive chemotherapy did not produce better survival than low-dose chemotherapy. In conclusion, it is necessary to introduce the first line therapy excluding the chemotherapy that can prolong survival in patients with high-risk MDS and MDS–AML.

For the Japan Adult Leukemia Study Group.

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Keywords MDS · MDS–AML · JALSG MDS200 · Induction therapy · HSCT

1 Introduction

Myelodysplastic syndrome (MDS) is a group of disorders in which abnormalities occur at the level of hematopoietic stem cells [1], leading to disturbance in the production of blood cells characterized by ineffective hematopoiesis [2], decrease in the number of peripheral blood cells and morphological/functional abnormalities in blood cells [3]. Allogeneic hematopoietic cell transplantation (allo-HCT) is the most effective curative therapy for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) [4]. However, for patients with high-risk MDS (those with refractory anemia with excess of blasts in transformation (RAEB)-t and some patients with RAEB) and patients with acute myeloid leukemia progressed from MDS (MDS–AML), chemotherapy aimed at remission is being used. The reasons for this are that MDS often affects elderly people [5], suitable donors are not always available at the time of disease onset, the necessity of pretransplant conditioning chemotherapy is controversial [6, 7] with a lack of sufficient evidence, and the optimal timing for transplantation varies widely depending on disease type [8].

On the other hand, reduced-intensity conditioning has extended the use of allo-HSCT to patients otherwise not eligible for this treatment due to older age or frailty [9]. However, allo-HSCT using traditional myeloablative preparative regimens is not easily tolerated by the elderly or frailer patient, and may lead to prohibitive treatment-related mortality rates. Most patients treated in the past were younger and devoid of comorbid clinical conditions. Novel reduced-intensity regimens have recently made allogeneic transplants applicable to the elderly, providing the benefit of the graft-versus-leukemia effect to a larger number of patients in need [10].

Low-dose chemotherapy, which has been used in clinical practice for 20 years, reduces the number of myeloblasts, improves pancytopenia and induces remission not only in MDS patients but also in some MDS–AML patients [11]. Common antineoplastic agents used in low-dose chemotherapy include cytosine arabinoside (Ara-C), aclarubicin (ACR), melphalan and etoposide. Nevertheless, despite improved Ara-C and regimens, the prognosis of AML in patients beyond 60 years of age remains dismal [4]. Low-dose antineoplastic drug therapy is still being used in some patients with MDS, which is common in elderly people, especially when the patient is at risk due to poor general condition or organ disorder [12].

The Japan Adult Leukemia Study Group (JALSG) previously conducted a pilot study for the treatment of high-risk MDS and MDS–AML to compare low-dose monotherapy with low-dose Ara-C plus granulocyte colony-stimulating factor (G-CSF) and multiple drug therapy with Ara-C plus Mitoxantrone plus VP-16. Later, JALSG conducted studies using a single protocol (JALSG MDS96) in 1996, in which remission induction and post-remission therapies using Ara-C and IDR in patients with high-risk MDS (RAEB-t) and in those with MDS–AML were performed, after which the efficacy and safety of these therapies were evaluated [13]. Furthermore, a randomized controlled study (JALSG MDS200) of intensive chemotherapy (IDR/Ara-C) or low-dose chemotherapy (CA) for high-risk MDS was also performed by JALSG.

Here, we present and analyze the results of the JALSG MDS200 study to assess and evaluate the validity of the MDS200 protocol for MDS treatment.

2 Patients and methods

2.1 Patient eligibility

A total of 120 patients were initially registered into the JALSG MDS200 study between June 2000 and March 2005. They were assigned into two groups, namely, Groups A and B (Table 1). Patients aged 15 years or more and diagnosed as having high-risk RAEB with high International Prognostic Scoring System score [14], RAEB-t or MDS-AML were eligible for this study. MDS–AML denotes secondary AML transformed from MDS.

Other eligibility criteria were as follows: patients with a performance status (PS) of 0–2 (ECOG); patients whose key organs other than the bone marrow retain intact function; patients who have not undergone any chemotherapy, except for pretreatment that does not affect the outcome of the main therapy; and patients who have given informed consent. Informed consent was obtained after carefully explaining the protocol and before registration.

2.2 Study protocol

The MDS200 protocol (Fig. 1) was designed based on the results of MDS96, and involved a dose-attenuation plan and allowed a wider range of chemotherapy. Patients were randomly assigned to either Group A or B.

In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age ($\geq$60 years), (2) hypoplastic bone marrow and (3) PS $\geq$ 2. Patients with no risk factor received the standard dose, those with 1 risk factor received 80% of the dose and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS96). In therapy B, the use of

Table 1 Characteristics of patients

Group	A ($n = 53$)	B ($n = 67$)	P value (A vs. B)
Age (range)	63 (23–77)	61 (32–81)	0.505
Gender			
Male	37	52	0.332
Female	16	15	
Disease type			
HR-RAEB	4	11	0.269
RAEB-T	22	29	
MDS–AML	27	27	
Infection			
Presence	10	11	0.726
None	43	56	
Karyotype[a]			
Good	23 (44.2%) $n = 52$	21 (33.9%) $n = 62$	0.524
Int	11 (21.2%)	15 (24.2%)	
Poor	18 (34.6%)	26 (41.9%)	
PB (range)			
WBC (/μL)	2,500 (700–64,240)	2,720 (600–43,700)	0.665
Hb (g/dL)	8 (4.7–12.6)	7.9 (4.4–12.7) $n = 66$	0.562
Plt (/μL)	5.8 (0.2–31.4)	5.9 (0.5–36.7)	0.363
BM (range)			
Blast (%)	30 (4–95) $n = 51$	24.2 (1.9–96) $n = 66$	0.171
Biochemical data (range)			
LDH (IU/L)	296 (132–882)	303.5 (111–906) $n = 66$	0.998
CRP (mg/dL)	0.5 (0–20.2)	0.35 (0–11.7) $n = 66$	0.292

Patients who met all of the inclusion criteria and did not meet any of the stated exclusion criteria were included the study. The disease types were classified by FAB classification

Statistical analysis between Group A and Group B was done using χ^2 test or Mann–Whitney U-test

MDS myelodysplastic syndrome, *HR-RAEB* high risk-refractory anemia excess of blasts with high International Prognostic Scoring System Score, *RAEB-T* refractory anemia excess of blasts in transformation, *MDS–AML* MDS overt leukemia, *WBC* white blood cell, *Hb* hemoglobin, *Plt* platelet, *LDH* lactate dehydrogenase, *CRP* C-reactive protein, *PB* peripheral blood, *BM* bone marrow

[a] Shows IPSS risk

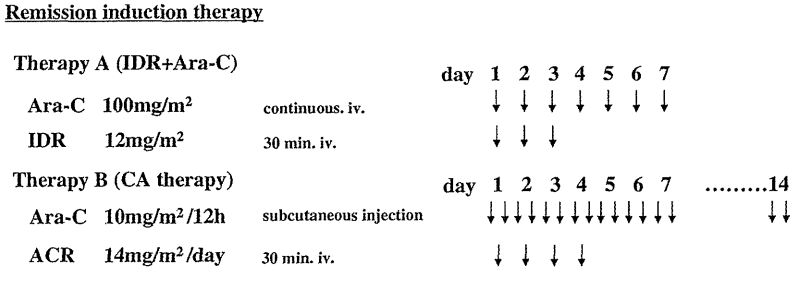

Fig. 1 Japan Adult Leukemia Study Group—myelodysplastic syndrome (JALSG MDS200 Protocol). In therapy A, the dose was adjusted according to a dose attenuation plan based on the presence of risk factors. The following 3 factors were regarded as risk factors: (1) Age (≥60 years), (2) hypoplastic bone marrow and (3) PS ≥ 2. Patients with no risk factor received the standard dose, those with 1 risk factor received 80% of the dose, and those with 2 or more risk factors received 60% of the dose (equivalent to the dose of MDS-96). In therapy B, the use of CAG therapy involving co-administration of G-CSF was allowed. *IDR* idarubicin, *Ara-C* cytosine arabinoside, *ACR* aclarubicin, *G-CSF* granulocyte colony-stimulating factor, *iv* intravenous injection, *min* minutes

CAG therapy involving the co-administration of granulocyte colony-stimulating factor (G-CSF) was allowed.

Untreated adult patients (≥15 years) with MDS (RAEB, RAEB-t or MDS–AML) were randomly assigned to receive either IDR/Ara-C (Group A) or CA (Group B) [15]. Complete remission (CR) rate, CR duration, overall survival (OS) rate and disease-/relapse-free survival (DFS/RFS) rate were compared between the two groups.

Consolidation therapy and maintenance therapy were performed in accordance with JALSG MDS96 [13].

2.3 Evaluation of response

Response to treatment was evaluated in accordance with JALSG criteria [13]. CR was considered achieved when the following conditions remained for at least 4 weeks. For the bone marrow: blasts accounting for ≤5% of all cells; absence of blasts with Auer body; and presence of normal erythroblasts, granulocytes and megakaryocytes. For peripheral blood: absence of blasts; neutrophils ≥ 1,000/ml; platelets ≥ 100,000/μL; and no evidence of extramedullary leukemia. CR duration was defined as the duration from the day when CR is achieved to the day of relapse or death, OS or DFS as the duration from the day of initiation of treatment to the day of death and DFS as the duration in which CR patients survived without relapse. Patients who were treated with HCST were not censored at the date of transplantation. All toxicity was graded using the World Health Organization criteria [16].

2.4 Statistical analysis

The primary endpoint of this study is DFS. Assuming a 1-year DFS rate of 60% in the Group A and 40% in the Group B, this design required the randomization of 200 patients. Eligible patients were randomized according to age, sex and disease type. Differences in background factors (e.g., age, gender and disease type) between Groups A and B were statistically analyzed using the χ^2 test or Mann–Whitney *U*-test. Probability of OS and DFS were estimated according to the method of Kaplan and Meier.

3 Results

3.1 Recruitment of patients and suspension of the study

The initially registered 120 patients were assigned into two groups, namely, Groups A and B. The clinical characteristics of the registered patients are shown in Table 1. The present protocol was originally planned to recruit 200 patients for Groups A and B within 3 years. However, the recruitment pace was slower than expected and thus the study period was extended from 3 years to 4.5 years. At the end of 2004, that is, after 4.5 years from the start of the study, the number of registered patients was only 113 in Groups A and B, which was 56.5% of the target number. At that point, the committee members discussed the progress of the MDS200 study and decided to suspend it at the end of March 2005. Since the final total number of patients did not reach the target number, we did not statistically compare DFS between Groups A and B, which was the primary endpoint of this study.

3.2 Characteristics of patients

There were no clear differences in the clinical characteristics of the patients between Groups A and B, such as FAB subtype, initial blood cell count, presence of infection, distribution in the karyotype group and biochemical data, as well as sex distribution (male/female ratio, 37/16 = 2.315 in Group A, and 52/15 = 3.467 in Group B).

3.3 Treatment outcome

The remission rates were 64.7% in Group A (33 out of 51 evaluable cases) and 43.9% in Group B (29 out of 66 evaluable cases). The 2-year overall survival (OS) rates were 28.1% in Group A and 32.1% in Group B, and the 2-year DFS rates were 26.0% in Group A and 24.8% in Group B. The mean duration of CR was 320.6 days (median: 213 days) in Group A and 378.7 days (median: 273 days) in Group B (Table 2). Reflecting the intensity of the remission induction chemotherapy, the period of WBC (<1,000/μL) after the therapy was longer in Group A than in Group B (19 days and 4 days, respectively). There were more grade 3 or 4 adverse events during the remission induction therapy in Group A (19 out of 53 evaluable patients) than in Group B (13 out of 67 evaluable patients). This difference was mostly attributable to infectious episodes (17 patients in Group A and 4 patients in Group B). In terms of bleeding episodes, 1 patient in Group A and 2 in Group B had grade 3/4 adverse events. The numbers of

Table 2 Treatment outcome (Group A vs. B)

	Group A (n = 53)	Group B (n = 67)
Remission rate (%)	64.7	43.9
Mean duration of remission (days)	320.6 (median: 213)	378.7 (median: 273)
2-Year survival rate (%)	28.1	32.1
2-Year disease-free survival rate (%)	26.0	24.8

The remission rates, 2-year overall survival (OS) rates and 2-year disease-free survival (DFS) rates are shown as percentages

early death in remission induction chemotherapy (death within 30 days) were 1 patient in Group A and 3 patients in Group B (Table 3). The cause of death in each group was infection or tumor progression. The completion rate of consolidation therapies were 37.3% in Group A (12 out of 33 evaluable cases), 37.9% in Group B (11 out of 29 evaluable cases). On the other hand, the maintenance therapies were completed 21.2% in Group A (7 out of 33 evaluable cases), and 15.2% in Group B (5 out of 33 evaluable cases). The numbers of dose attenuation in Group A were 30 patients of 100% dose, 21 patients of 80% or 60% dose and 2 patients of unknown.

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) was performed in 11 out of 50 patients (22%) in Group A and 19 out of 66 patients (28.8%) in Group B. Among those who received allo-HSCT, the transplantation was performed during the first remission in 40%, 21% of patients in Groups A, B, respectively.

There were 15 patients who lived longer than 1,000 days after diagnosis: 6, 9 patients in Groups A, B, respectively. Regarding the transplantation among long-term survivors, 3 out of 6 patients were transplanted in Group A, 6 out of 9 in Group B. Comparing the achievement of CR among these patients in Groups A and B, all 6 patients in Group A achieved CR, but only 4 out of 9 patients in Group B achieved CR.

Table 3 Toxicity of the induction therapy

	A (n = 53) (range)	B (n = 67) (range)	P value (A vs. B)
Period of WBC <1,000 (day)	19 (0–44) n = 49	4 (0–50) n = 63	<0.0001
Toxicity (grade 3/4)			
Presence	19	13	0.427
Bleeding	2	1	ND
Infection	17	11	0.04
Others	2	2	ND
Early death (<30 days)	1	3	ND

Statistical analysis between Groups A and B was performed using the χ^2 test or Mann–Whitney U-test

ND not done

4 Discussion

In this MDS200 study, patients with high-risk MDS and AML transformed from MDS (MDS–AML) were treated with either intensive or low-dose remission induction therapy, followed by intensive post-remission therapy that was the same as in the JALSG MDS96 study [13].

Although we did not perform statistical comparison of DFS or OS between these two treatment groups due to the insufficient number of patients enrolled, the results suggest that there was no significant difference, that is, survival curves were superimposable (Figs. 2, 3). Intensive chemotherapy similar to that for AML can produce a CR rate of 64.7% for high-risk MDS and MDS–AML patients, whereas low-dose induction therapy can result in a CR rate of 43.9%. However, among the patients enrolled in this trial, the difference in CR rate did not lead to better survival as described above. In terms of adverse events, patients who received intensive treatment had more grade 3 or 4 adverse events, particularly infectious events with a longer period of leukopenia. There was no increase in the number of patients succumbing to early death (death within 30 days after the

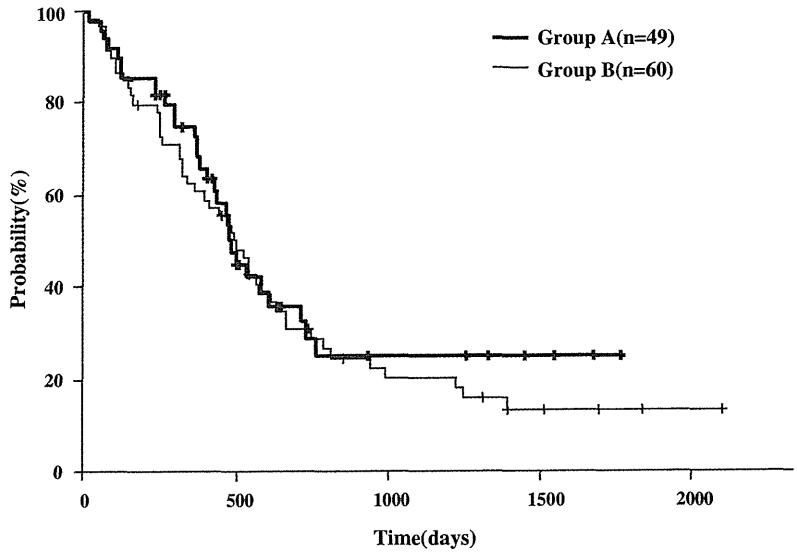

Fig. 2 Overall survival. Survival was calculated from the date of the start of treatment to the date of death due to any cause or to the date of the most recent follow-up. These data were not censored at the time of HSCT. All randomized patients were not included this data in each group. Due to this reason, some patients were not known to be CR or not, but known to be alive or not