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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).

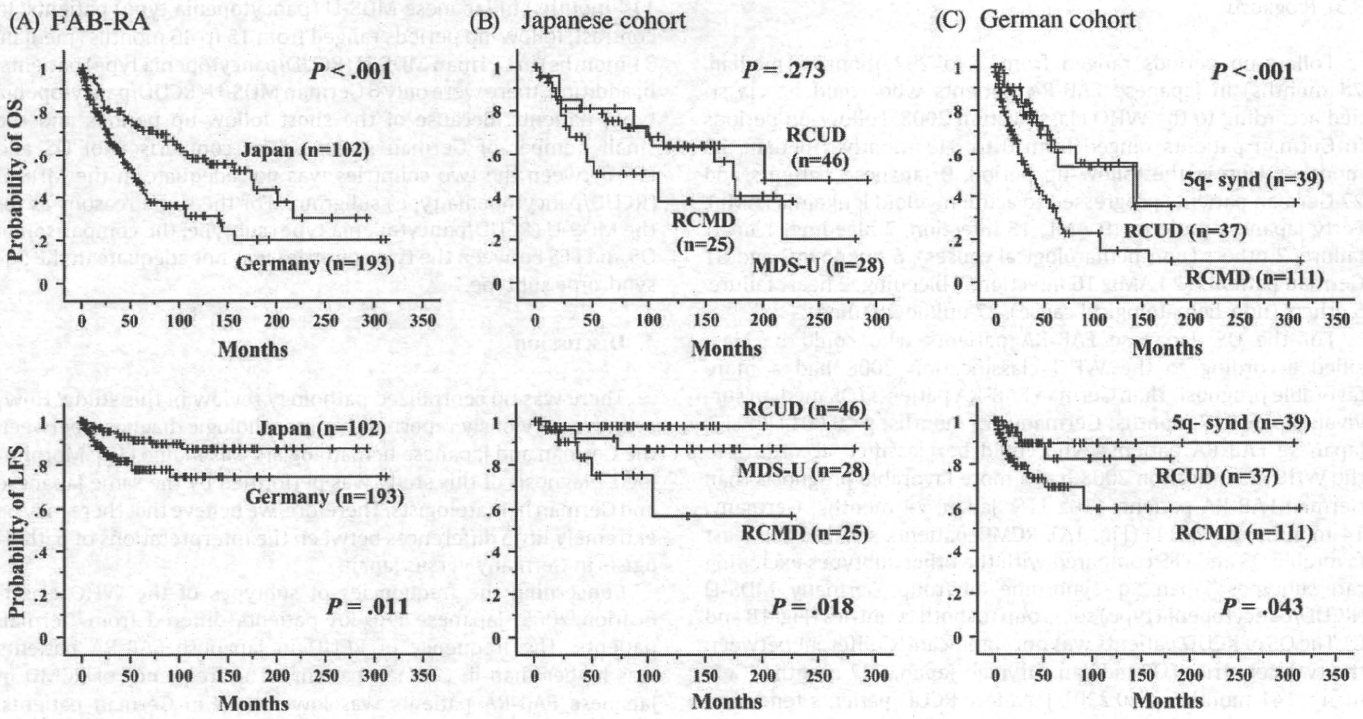

Fig. 1. Cumulative overall survival and leukemia free survival of FAB-RA patients who could be classified according to the WHO classification 2008. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) In FAB-RA patients who could be classified according to the WHO classification 2008, 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, $p=0.043$).

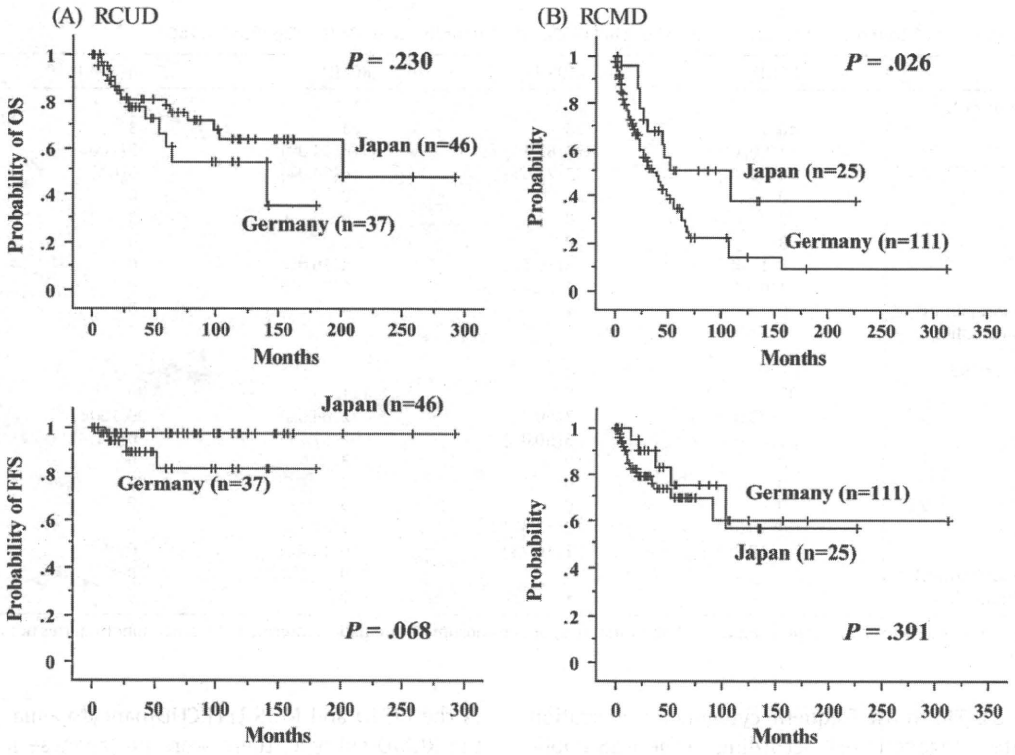

Fig. 2. Comparison of cumulative overall survival and leukemia free survival of RCUD and RCMD between Japanese and German patients. (Top) Overall survival (OS). (Bottom) Leukemia free survival (LFS). (A) The OS of RCUD patients was not significantly different between the two countries ($p = 0.230$). 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

Safety and efficacy of the terminal complement inhibitor eculizumab in Japanese patients with paroxysmal nocturnal hemoglobinuria: the AEGIS Clinical Trial

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Abstract Paroxysmal nocturnal hemoglobinuria (PNH) is a progressive and life-threatening disease characterized by complement-mediated chronic hemolysis, resulting in serious life-threatening complications and early mortality. Eculizumab, a humanized anti-C5 monoclonal antibody that inhibits terminal complement activation, has been shown to reduce hemolysis in PNH patients. The pivotal open-label, 12-week phase II registration study (AEGIS) was designed to evaluate the efficacy and safety of eculizumab in Japanese patients with PNH. This trial achieved its primary endpoint of reducing intravascular hemolysis with high statistical significance. Twenty-seven of the 29 patients responded to eculizumab treatment, resulting in an 87% reduction in hemolysis ($P < 0.0001$) and subsequent improvement in anemia ($P = 0.0003$) despite reduction in transfusion requirements ($P = 0.006$). Fatigue and dyspnea significantly improved within 1–2 weeks of eculizumab treatment and the improvement was independent of changes in hemoglobin. Chronic kidney disease (CKD) was common (66%) and eculizumab treatment improved CKD in 41% of patients at 12 weeks ($P < 0.001$). Elevated thrombotic risk was evident in Japanese PNH patients and eculizumab treatment normalized D-dimer levels in 45% of patients with elevated D-dimers at baseline ($P < 0.001$). The AEGIS results demonstrate that eculizumab is effective, safe and well tolerated in Japanese patients with PNH.

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Keywords Paroxysmal nocturnal hemoglobinuria · Complement-inactivating agents · Hemolysis · Eculizumab · Hematopoietic stem cell

1 Introduction

Paroxysmal nocturnal hemoglobinuria (PNH) is a progressive and life-threatening disease characterized by chronic hemolysis [20, 28, 36]. The 5-year mortality in patients presenting with hemolysis ranges from 15 to 35% and median survival ranges from 10 to 22 years after diagnosis [10, 20, 27, 36]. The disease can present at any age with the median age ranging from early 30s to mid-40s [27]. PNH arises from an acquired genetic mutation in the X-linked phosphatidylinositol glycan-complementation class A (*PIGA*) of hematopoietic progenitor cells leading to clonal deficiency of glycosylphosphatidylinositol (GPI)-linked proteins on the surface membrane of blood cells [38, 41]. This GPI deficiency results in the loss of the complement inhibitor proteins CD55 and CD59 from the surface of hematopoietic cells in PNH patients leading to complement-mediated red cell lysis [3, 28], platelet activation [2], and hemostatic activation with inflammation [40].

Historically, physicians viewed and treated PNH as a disease of anemia. However, the demonstration of the underlying phenotype—deficiency of GPI-linked complement inhibitors CD55 and CD59—indicates that the primary clinical manifestation is the terminal complement activation causing not only lysis of PNH red blood cells but also in parallel platelet, monocyte, and leukocyte activation with consequent inflammation and hemostatic activation [13, 40]. Thus, anemia is only a single consequence of the underlying chronic intravascular hemolysis. Patients suffer severe morbidities and early mortality as a direct result of terminal complement activation with chronic hemolysis including kidney disease, thrombosis, pulmonary hypertension, hemoglobinuria, debilitating fatigue, severe dyspnea, disabling pain, and a poor quality of life (QoL) [15, 18, 27–29, 31, 32]. Thromboembolism (TE) accounts for 40–67% of PNH-related deaths and renal failure accounts for 8–18% of PNH-related deaths [15, 18, 27–29, 31, 32].

Eculizumab is a humanized monoclonal antibody that specifically targets the terminal complement protein C5, thereby inhibiting terminal complement-mediated hemolysis [33]. The efficacy and safety of eculizumab have been evaluated in two multinational phase III studies and a multinational extension trial performed predominantly in the North America, Europe and Australia [4, 21, 22]. These studies demonstrate that eculizumab significantly reduces hemolysis, thrombotic events, renal impairment, pulmonary hypertension, and improves fatigue, QoL, and anemia, while reducing transfusion requirements.

While the clinical course of PNH in both the Western and Asian populations is associated with similar mortality rates and both populations suffer significant hemolysis-mediated symptoms, the clinical manifestation of the disease is perceived to differ between the two populations [27]. The current study was an open-label, single-arm, phase II registration study (AEGIS) designed to evaluate the safety, efficacy, pharmacokinetics and pharmacodynamics of eculizumab in Japanese patients with PNH and to examine the consistency of these results with the previously reported multinational phase III and extension studies of eculizumab.

2 Methods

AEGIS was an open-label, single-arm, multi-center study in Japanese patients who were 12 years of age or older with a diagnosis of PNH for at least 6 months and was conducted at 9 medical centers in Japan. Additional inclusion criteria included: the presence of a population of GPI-deficient red blood cells (PNH Type III RBCs) by flow cytometry $\geq$ 10% at screening; and lactate dehydrogenase (LDH) $\geq$ 1.5 times the upper limit of normal (ULN) within 12 weeks of screening or during the screening period, and platelet count $\geq 30 \times 10^9$/L. Enrolled patients were judged by the physician to require at least one red blood cell transfusion within the past 2 years, although enrollment of patients who had received no red blood cell transfusions was permitted provided that the physician had judged the patient to have required transfusion. All patients were required to give written informed consent. Females of child-bearing potential were required to have a negative pregnancy test (serum HCG) at screening. Sexually active females had to agree to use a reliable and medically approved method of contraception. Exclusion criteria included: platelet count $< 30 \times 10^9$/L at screening; absolute neutrophil count $\leq$ 500/μL at screening; known or suspected hereditary complement deficiency; history of hematopoietic stem cell transplantation; and participation in any other investigational drug trial or exposure to other investigational agent, device, or procedure within 30 days prior to screening. Patients who were pregnant or breast-feeding, or who could become pregnant or intended to conceive during the course of the study (including the post-treatment period and the follow-up visits for early termination) were excluded. Additional exclusion criteria included a history of meningococcal disease and, in the opinion of the investigator, the presence or suspicion of active bacterial infection 2 weeks prior to first dose of eculizumab, or recurrent bacterial infections.

Patients received 600 mg of eculizumab intravenously every 7 ± 2 days for 4 weeks, followed by 900 mg 1 week

later followed by 900 mg every 14 ± 2 days for a total of 12 weeks. All patients were vaccinated with a meningococcal vaccine at least 2 weeks before receiving the first dose of eculizumab.

The primary efficacy endpoint in the AEGIS study was the change in intravascular hemolysis (as measured by change in LDH) at study Week 12 from baseline. Secondary endpoints included change from baseline in the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) [5] scale at study Week 12, change in PNH Type III RBC count at study Week 12, change in transfusion requirements (number of units of packed RBCs transfused), change in plasma-free hemoglobin (free-Hgb) at study Week 12, area under the curve (AUC) for change of LDH, and change in the European Organization for Research and Treatment of Cancer Quality of Life Core 30 (EORTC QLQ-C30) [1] questionnaire score at study Week 12. D-Dimer levels were measured at the central laboratory at baseline, Week 4 and Week 12.

The effect of eculizumab on renal function as measured by an improvement or worsening in chronic kidney disease (CKD) stage during treatment was also evaluated. CKD stages were determined for each patient according to Kidney Disease Outcomes Quality Initiative (KDOQI) CKD published guidelines classification (Stage 5: glomerular filtration rate, GFR < 15 mL/min/1.73 m^2; Stage 4: GFR 15–30 mL/min/1.73 m^2; Stage 3: GFR 30–60 mL/min/1.73 m^2; Stage 2: GFR 60–90 mL/min/1.73 m^2 and evidence of proteinuria; Stage 1: GFR ≥ 90 mL/min/1.73 m^2 and evidence of proteinuria; no CKD: GFR > 60 mL/min/1.73 m^2 and no evidence of proteinuria) [26]. An improvement in renal function was defined as a categorical reduction in CKD stage level or fulfilling the criteria of no CKD. Worsening in renal function was defined as a categorical increase in CKD stage level.

Evaluation of the safety of eculizumab included assessment of adverse events (AEs), assessment of thrombotic events, laboratory measurements, vital signs, ECG, and chest X-ray.

The pharmacokinetics of eculizumab was determined with a validated enzyme-linked immunosorbent assay that detects both free and C5-bound eculizumab [39]. The analytical range of the assay was 10–640 μg/mL. The pharmacodynamics of eculizumab was determined by measuring the capacity of the patient's serum to lyse chicken erythrocytes in a validated standard total human serum-complement hemolytic assay [30].

2.1 Statistical analysis

Changes in LDH, hemoglobin (Hgb), RBC type III, free-Hgb and PRBC transfusion units from baseline to Week 12 were analyzed by Wilcoxon's signed rank test. Transfusion avoidance was evaluated with a McNemar test.

Changes in scores on the FACIT-Fatigue instrument and the EORTC QLQ-C30 instrument from baseline through Week 12 were analyzed with the use of a mixed-effects model, with baseline scores as the covariate, time as the fixed effect, and the patient identifier as the random effect. The proportion of patients returning to normal range of D-dimer was analyzed by exact binomial test.

Changes in the proportion of patients in each CKD stage from baseline were compared using Chi-square analyses and the hypothesis tested the probability of worsening CKD stage was equal to the probability of improving CKD stage.

For each subgroup of patients with a history of bone marrow dysfunction (BMD) that includes aplastic anemia (AA) or myelodysplastic syndrome (MDS), or no history of BMD, the change in LDH, Hgb, FACIT-Fatigue, EORTC from baseline was analyzed by a t test in mixed-effects model and between the two subgroups was analyzed by F test in mixed-effects model with baseline as covariate, subgroup and time as fixed effect, and patient as random effect.

3 Results

3.1 AEGIS patient characteristics

Eculizumab was administered to 29 Japanese patients (14 men and 15 women; median patient age 47 years; range 26–70 years) at 9 institutions (see Table 1). Forty-five percent (45%; 13/29) of patients had a history of AA or MDS. Forty-eight percent (48%; 14/29) of patients were receiving concomitant corticosteroids. The cohort also included 2 patients who were never transfused. These two patients demonstrated clinical signs and symptoms of PNH comparable to patients who had received transfusions. Twenty-seven of 29 patients completed the study.

3.2 Hemolysis

The primary endpoint—reduction of intravascular hemolysis—was achieved with a high level of statistical significance with eculizumab treatment. Eculizumab treatment reduced LDH 87% from a median of 1814 U/L at baseline to 244 U/L at 12 weeks of treatment ($P < 0.0001$; normal range 103–223 U/L). Mean LDH levels decreased from 1845 ± 115 U/L at baseline to 399 ± 99 U/L at 12 weeks (Table 1). A significant reduction in LDH was observed within 1 week of treatment ($P < 0.0001$) and this reduction was sustained throughout the 12-week study (Fig. 1). In two patients, eculizumab serum concentrations were maintained above the effective level of 35 μg/ml but

Table 1 Baseline characteristics, effects of eculizumab on hematologic parameters

Parameter	Baseline
Median age (years) (range)	47 (26–70)
Gender, female	52%
History of AA or MDS	45%
History of thrombosis	17%
Elevated D-dimer	38%
Elevated D-dimer or thrombosis	52%
Chronic kidney disease (CKD)	66%
Concomitant antithrombotic (%)	31%
Concomitant steroids (%)	48%

Parameter	Mean ± SE (median)		*P* value
	Baseline	12 weeks	
LDH (U/L)	1845 ± 115 (1814)	399 ± 100 (244)	<0.0001
RBC type III (%)	43.9 ± 4.5 (39.2)	57.3 ± 4.9 (56.7)	<0.0001
PNH RBC mass ($\times 10^{12}$/μL)	1.2 ± 0.1 (1.2)	1.8 ± 0.2 (1.7)	<0.0001
Transfusion (units/12 weeks)	5.2 ± 1.0 (2.0)	1.5 ± 0.7 (0.0)	0.006
Hemoglobin (g/dL)	7.9 ± 0.3 (7.6)	8.9 ± 0.4 (9.0)	0.0003
Free hemoglobin (mg/dL)	22.6 ± 2.6 (20.0)	2.8 ± 1.0 (1.0)	<0.0001

P value was calculated using signed rank test

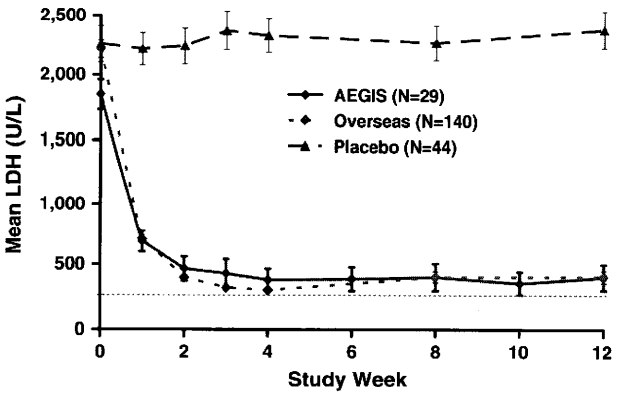

Fig. 1 Comparison of the effects of eculizumab on LDH in AEGIS versus multinational studies (units, U/L). Treatment with eculizumab reduced mean LDH levels from baseline (Week 0) within 1 week of treatment ($P < 0.001$ from baseline) and was sustained for 12 weeks ($P < 0.001$ from baseline) in Japanese-treated patients (AEGIS, *red diamonds*). The 87% reduction in the Japanese patients was similar to the treated patients in multinational overseas study ($P < 0.001$; overseas, *orange diamonds*). Patients not treated with eculizumab did not demonstrate a reduction in LDH (placebo, *blue triangles*). The overseas data consist of the placebo-controlled TRIUMPH and open-label SHEPHERD registration studies [4, 21, 22]. The placebo group is from the TRIUMPH study

without reduction in LDH, although a partial drug response was observed with a reduction in complement activation as measured by in vitro CH50 assays.

3.3 Red blood cell mass and anemia

Eculizumab treatment significantly increased the proportion of PNH type III RBCs from a median of 39.2% at baseline to 56.7% at Week 12 ($P < 0.001$; Table 1). Similarly, the proportion of type II RBCs was also significantly increased from a median of 4.2% at baseline to a median of 5.6% at Week 12 ($P < 0.0001$). Eculizumab treatment significantly increased PNH RBC mass from a mean at baseline 1.2×10^{12}/μL to 1.8×10^{12}/μL at 12 weeks ($P < 0.0001$; Table 1).

Eculizumab treatment significantly reduced the number of PRBC transfusion units from a median 2.0 units (mean of 5.2 ± 1.0 units) per patient in the 12 weeks prior to eculizumab treatment to a median 0.0 units (mean 1.5 ± 0.7) per patient during the 12-week treatment phase ($P = 0.006$; Table 1). Of the 21 patients that had received at least one or more transfusion in the 12-week period prior to treatment, 66% (14/21) did not require transfusion during the 12 weeks of treatment ($P = 0.001$). Despite the decrease in transfusion requirements, Hgb levels increased from a median of 7.6 g/dL at baseline to 9.0 g/dL at Week 12 ($P = 0.0003$; Table 1). However, the statistical improvement in Hgb was first observed 8 weeks after treatment initiation (median 8.7 g/dL at Week 8 vs. 7.6 g/dL at baseline, $P = 0.007$).

Table 2 Effects of eculizumab on fatigue, dyspnea, hemoglobin and free hemoglobin during first 4 weeks of treatment

	Baseline	Change from baseline [mean ± SE, median (*P* value)]			
		Week 1	Week 2	Week 4	Week 12
Fatigue (FACIT Score)	38.5 ± 1.9, 41.0	2.1 ± 1.1, 2.0 (0.04)	4.2 ± 1.0, 4.0 (<0.001)	4.9 ± 1.4, 4.0 (<0.001)	4.1 ± 2.3, 5.0 (<0.001)
Dyspnea (EORTC Score)[a]	37.9 ± 5.2, 33.3	−11.5 ± 4.1, 0.0 (0.006)	−13.8 ± 3.9, 0.0 (<0.001)	−13.8 ± 4.5, 0.0 (<0.001)	−13.8 ± 4.5, 0.0 (<0.001)
Hemoglobin (g/dL)	7.9 ± 0.3, 7.6	N/A	0.2 ± 0.2, 0.1 (NS)	0.4 ± 0.2, 0.25 (NS)	1.0 ± 0.25, 1.0 (<0.001)
Free hemoglobin (mg/dL)	22.6 ± 2.6, 22	−18.3 ± 2.7, −12.0 (<0.001)	−18.6 ± 3.0, −17.0 (<0.001)	−11.9 ± 9.1, −16.0 (0.006)	−19.8 ± 2.7, −17.0 (<0.001)

NS not significant: *P* value based on *t* test

[a] A negative change in EORTC score of fatigue and dyspnea indicates improvement

There was an immediate reduction in median free-Hgb levels at 1 week of eculizumab treatment (from 20.0 mg/dL at baseline to 4.2 mg/dL; $P < 0.001$) compared to baseline levels and this reduction was sustained throughout the study to 1.0 mg/dL at Week 12 ($P < 0.0001$).

3.4 Improvement in FACIT-Fatigue and QoL

Fatigue levels, as measured by the FACIT-Fatigue instrument and confirmed by the EORTC-Fatigue instrument, significantly improved within 2 weeks of eculizumab treatment. Thirty-eight percent of treated patients experienced a clinically meaningful improvement in fatigue (at least a 3-point increase on the FACIT-Fatigue scale) [6, 7] at Week 1 of treatment, 62% at Week 2 and 66% at Week 12. Eculizumab treatment improved fatigue, a mean of 2.1 and 4.2 points on the FACIT-Fatigue scale after 1 and 2 weeks of treatment ($P = 0.04$ and $P < 0.001$ compared to baseline, respectively; Table 2). The improvement in fatigue was sustained, a mean increase of 4.1 points at 12 weeks ($P < 0.001$). The rapid improvement in fatigue measured by FACIT-Fatigue was confirmed by a large mean improvement in EORTC QLQ-C30 Fatigue of 7.1 and 11.1 points by Weeks 1 and 2 ($P < 0.001$ compared to baseline each week).

Treatment with eculizumab demonstrated improvements in QoL ($P = 0.02$) as measured by EORTC QLQ-C30, with statistically significant improvements in global health status ($P = 0.02$), role ($P < 0.001$), physical ($P = 0.02$) and emotional functioning ($P = 0.002$), fatigue ($P < 0.0001$), dyspnea ($P < 0.0001$), and appetite loss symptoms ($P < 0.0001$). Fifty percent of Japanese patients treated with eculizumab improved by at least 10% in global health status at Week 12, a degree of improvement considered clinically meaningful. Eculizumab treatment was associated with a large and rapid improvement in patient-reported dyspnea symptoms with an 11.5 points improvement at Week 1 ($P = 0.02$). Furthermore, 41% of patients reported a major improvement (10% or greater) in dyspnea with eculizumab treatment that was sustained through Week 12.

3.5 Improvement in CKD

Renal dysfunction was common in the study population, with 66% (19 of 29 patients; Table 1; Fig. 3a) of patients demonstrating CKD at baseline. Patients treated with eculizumab for 12 weeks were more likely to improve (41%; 12/29) rather than worsen (3%; 1/29) CKD stage ($P = 0.0002$ improved compared to worsen) and 55% (16/29) of patients had no change in their CKD stage (Fig. 3b). Of the 16 patients with CKD Stage 1–2 at baseline, 11 patients (69%) improved with eculizumab; of the 3 patients with CKD Stage 3–5 at baseline, 1 patient (33%) improved with eculizumab treatment (Fig. 3).

3.6 Thrombotic events and D-dimer levels

There were five patients with a history of TEs (one patient had a cerebrovascular accident and 4 patients had a deep vein thrombosis) prior to study enrollment and there were no reported TEs during eculizumab treatment. At baseline, 11/29 (38%) of patients had D-dimer levels above the ULN. Seven of the 11 patients with elevated D-dimer levels had evidence of CKD. Eculizumab treatment was associated with the normalization of D-dimer levels in 5 of the 11 (45%) of patients with elevated D-dimer ($P < 0.001$).

3.7 AA or MDS subpopulation analysis

Forty-five percent (13/29) of the PNH patients enrolled in the study were also diagnosed with a history of BMD (AA or MDS). Patients with or without BMD showed similar levels of hemolysis, as measured by LDH ($P = 0.74$ between both subpopulations), Hgb ($P = 0.60$), transfusions ($P = 0.82$), FACIT-Fatigue score ($P = 0.66$),

EORTC-Fatigue ($P = 0.62$), and EORTC-Dyspnea ($P = 0.32$) at study entry. Both PNH patient subgroups showed an immediate and sustained reduction in LDH from baseline within the first week of treatment ($P < 0.001$ in each group). Eculizumab treatment improved QoL measures in both patient groups as indicated by significant improvements in FACIT-Fatigue ($P < 0.001$ and $P = 0.008$, respectively), EORTC-Fatigue ($P = 0.001$ and $P = 0.008$, respectively) and EORTC-Dyspnea ($P < 0.001$ and $P = 0.003$, respectively). Hgb was improved with eculizumab treatment at Week 12 from baseline in both groups ($P < 0.05$ and $P = 0.006$, respectively). There was no difference in improvement between the two groups during eculizumab treatment: LDH reduction ($P = 0.51$), increase Hgb ($P = 0.26$), improvement in FACIT-Fatigue ($P = 0.95$), EORTC-Global health ($P = 0.90$), EORTC-Fatigue ($P = 0.94$), and EORTC-Dyspnea ($P = 0.49$).

PNH patients with PNH and BMD experienced significant improvement in renal function. At baseline, 12/13 (92%) of patients with a history of BMD demonstrated CKD. Eculizumab treatment led to 7/13 patients improving CKD, 6/13 patients with no change, and no patients with worsening of CKD ($P = 0.0001$). At baseline, 7/16 (44%) of patients without a history of BMD demonstrated CKD. Eculizumab treatment was associated with a strong trend for improvement ($P = 0.07$) with 5/16 patients improving to a level of no CKD, 10/16 patients with no change, and one patient with worsening of CKD from Stage 0 to Stage 1.

3.8 Never transfused patients

Despite 2 patients never being transfused at baseline, these patients were hemolytic (LDH approximately 7- and 11-fold above normal, respectively), demonstrated significant organ damage with evidence of renal disease (CKD stage 2 and 1, respectively) and thrombosis (1 patient with DVT), and suffered disabling QoL as measured by FACIT-Fatigue and EORTC QLQ-C30 Dyspnea. In both patients, eculizumab treatment resulted in substantial 78–88% reductions in LDH, significant improvements in fatigue (improvements of 5 and 23 points, respectively), improvement in dyspnea in one patient (improvement of 33 points from baseline), and elimination of CKD with no subsequent TE in both patients.

3.9 Pharmacokinetics and pharmacodynamics of eculizumab

Blood samples for PK/PD assessments were collected at all dosing visits in the AEGIS study. Pharmacokinetic analysis showed that eculizumab trough levels reached a median of 85.8 μg/mL (range 20.4–172.5 μg/mL) and peak levels reached a median of 189.9 μg/mL (range 90.6–297.9 μg/mL) at study Week 2. Over the course of the study, both peak and trough levels were maintained above the levels reached at Week 2. No patients at Week 4, and 1 patient each at Weeks 6, 8, and 12 showed serum eculizumab levels below 35 μg/mL, a minimal level required to completely inhibit complement-related hemolysis in serum samples [17]. After the induction period (study Week 8), 93.1% (27/29) of patients showed strong inhibition of hemolysis.

3.10 Safety

Eculizumab was safe and well tolerated in all patients. The majority of AEs (98.3%) were reported as mild or moderate. There were no patient deaths during the study, and no patients withdrew participation due to an AE. There was a single infection-related serious AE (pyrexia) which was not reported as probably or definitely related to drug.

The most frequent treatment-emergent AEs were headache (52%), nasopharyngitis (41%), and nausea (21%). Notably, of 15 patients who reported headache, 14 did so within 1 day of study drug infusion. All headaches were reported as mild or moderate in severity and were effectively treated with over-the-counter medications. The frequency of headaches reduced from 45% during the first 4 weeks to only 14% during the following 8 weeks. Other AEs that were reported with ≥10% incidence were diarrhea (14%), eczema (10%), pyrexia (10%), and vomiting (10%) (Table 3). Most (88%) infection-related AEs were mild and no infection-related events were reported as probably or definitely related to drug. No meningococcal infections were reported during the treatment period. There were no deaths and no pregnancies, major adverse vascular events, or serious hemolysis events during the study.

Table 3 Frequently (≥10%) reported treatment-emergent adverse events

Preferred term	Patients, *n* (%)	
	Eculizumab ($N = 29$)	Placebo[a] ($N = 44$)
Total patient reporting	28 (96.6%)	37 (84.1%)
Serious AE	1 (3.5%)	5 (11.4%)
Withdrawal due to AE	0 (0.0%)	0 (0.0%)
AEs that were mild or moderate	(98.3%)	(97.0%)
Headache	15 (51.7%)	9 (20.5%)
Nasopharyngitis	12 (41.4%)	5 (11.4%)
Nausea	6 (20.7%)	3 (6.8%)
Diarrhea	4 (13.8%)	3 (6.8%)
Eczema	3 (10.3%)	0 (0%)
Pyrexia	3 (10.3%)	2 (4.5%)
Vomiting	3 (10.3%)	3 (6.8%)

[a] First 12 weeks of placebo from the multinational study

4 Discussion

The AEGIS trial results demonstrate that eculizumab is safe, effective and well tolerated in Japanese patients with PNH. The primary endpoint of the study, reduction of hemolysis, was achieved with a high level of statistical significance, demonstrating that treatment with eculizumab significantly suppresses chronic intravascular hemolysis 87% in Japanese patients with PNH. The response to eculizumab was immediate (within 1 week of treatment) and sustained for at least the current 12-week observation period (Fig. 1). These results are consistent with those of the multinational phase III trials of eculizumab which showed a similar 87% reduction in LDH from a median of 2042 U/L at baseline to 265 U/L at 12 weeks ($N = 140$; $P < 0.001$). The reduction in hemolysis in the multinational trials was sustained for at least the 54-month observation period in these studies (2165 U/L at baseline to 274 U/L at 18 months, $P < 0.001$, $n = 171$ and 277 U/L at 54 months, $P = 0.002$, $n = 10$) [37]. Given the globally consistent response to therapy in other multinational studies, it is expected that long-term eculizumab treatment will continue to result in sustained inhibition of hemolysis and reduction in the hemolysis-driven morbidities in Japanese patients.

At baseline, and despite ongoing immunosuppressive therapy in 4/13 patients, levels of hemolysis, fatigue, dyspnea, global health, CKD, anemia, and transfusion requirements were similar in patients with or without a history of AA or MDS. These results demonstrate that intravascular hemolysis significantly contributes to disabling symptoms in patients with PNH, irrespective of whether the patient has or does not have a history of AA or MDS. Further, our data demonstrate that chronic treatment with eculizumab effectively suppressed intravascular hemolysis and significantly improved signs and symptoms of PNH similarly in patients with or without a history of AA or MDS.

The immediate and sustained improvements in fatigue in Japanese PNH patients treated with eculizumab are similar to the improvements observed in the multinational trials. We observed that the significant improvements in fatigue are independent of any improvement in anemia, since the fatigue improvement at Week 1 preceded any observable change in Hgb levels (which did not change until Week 8, Fig. 2) [22]. The burden of fatigue in PNH is frequently underappreciated, and has historically been ascribed to concomitant anemia. The results of the current study as well as the parallel results of the multinational studies demonstrate, however, that in both Japanese and non-Japanese patients with PNH, terminal complement activation leading to intravascular hemolysis causes fatigue, and that fatigue in patients with PNH is independent of the level of

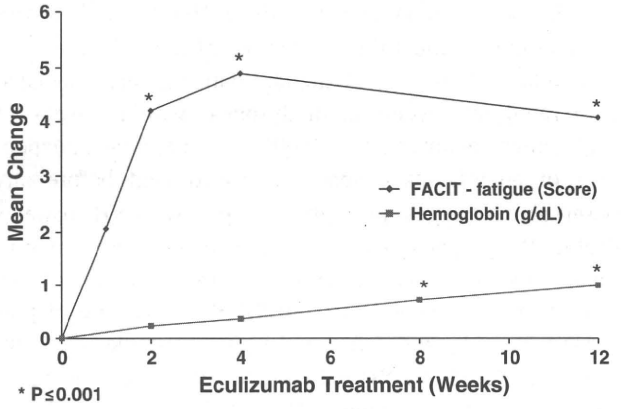

Fig. 2 Effects of eculizumab on FACIT-Fatigue scores. Treatment with eculizumab improved fatigue (as measured by FACIT-Fatigue) within 2 weeks of treatment ($P < 0.001$; *blue diamonds*). A change of 3 points is considered clinically significant. Significant improvement in hemoglobin was not seen in until Week 8 of treatment (*red squares*)

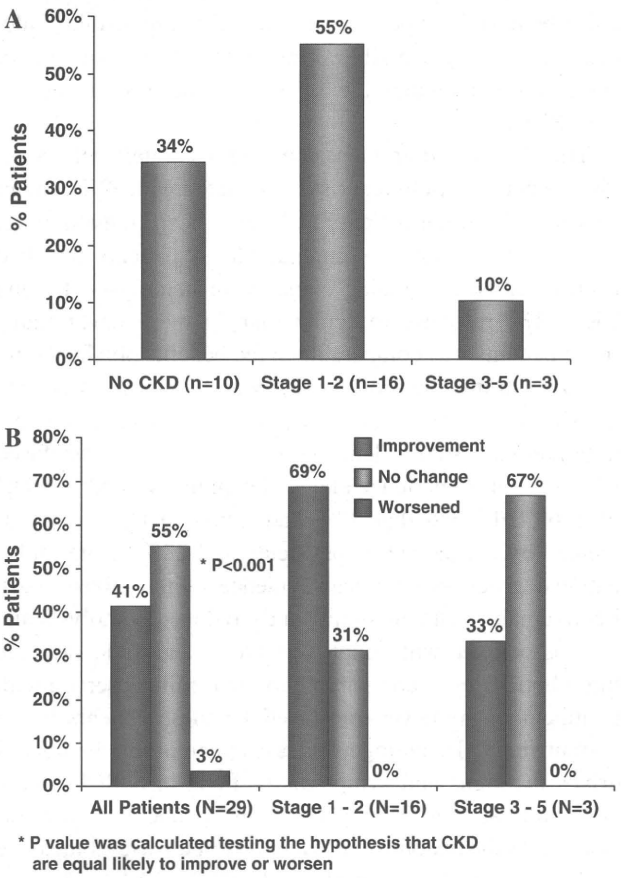

Fig. 3 Baseline and change in chronic kidney disease after first 12 weeks of eculizumab treatment. **a** CKD at baseline. **b** Treatment with eculizumab improved CKD in 41% of all patients ($P < 0.001$). 69% of patients with CKD Stage 1–2 at baseline improved and 33% of patients with CKD Stage 3–5 at baseline improved with eculizumab treatment

anemia. Hence, in patients with PNH, Hgb level alone will not accurately reflect the full burden of the disease.

Similar to fatigue, eculizumab treatment was associated with rapid improvements in dyspnea (within 1 week of eculizumab treatment, $P = 0.006$ from baseline) independent of anemia, as dyspnea improved well before any observable changes in Hgb in Japanese PNH patients (Table 2). Dyspnea is considered a manifestation of pulmonary hypertension and cardiac overload. N-terminal pro-brain natriuretic peptide (NT-proBNP), a measure of pulmonary vascular resistance and right ventricular dysfunction, is elevated in PNH patients, possibly due to elevated hemolysis and subsequent NO depletion [14, 15]. Treatment with eculizumab has been shown to reduce NT-proBNP and the reduction correlated with an increase of NO availability and improvement in dyspnea, both independent of anemia [14, 15]. Taken together, these data further demonstrate that symptoms historically associated with anemia such as fatigue and dyspnea are in fact independent of Hgb levels in patients with PNH. The reduction of terminal complement activation and chronic intravascular hemolysis appears to be directly responsible, independent of any possible improvement in anemia, for significantly reducing the severe morbidities in patients with PNH.

Transfusion requirement does not accurately reflect the disease burden or clinical risks associated with PNH. In our cohort, 2 Japanese patients had never been transfused yet demonstrated hemolysis comparable to patients that had been transfused including evidence of hemolysis, TE and CKD. The response to eculizumab in these never transfused patients was comparable to the benefits obtained with eculizumab treatment of patients who had been previously transfused in our study. Consistent with our data, a separate multinational study demonstrated that never-transfused PNH patients experience elevated hemolysis (median LDH of 1360 U/L) and that 87% had impaired QoL as documented by the patient or physician and 28% of the multinational group had clinical evidence of thrombosis [24]. Eculizumab treatment significantly reduced hemolysis and was associated with improved QoL, and reductions in thrombotic events, comparable to the results observed with treatment of the never-transfused Japanese patients in the current study. Taken together, it is apparent that hemolysis drives the signs and symptoms of PNH in both Japanese and multinational PNH patients independent of transfusions and transfusions do not appear to be a useful measure of the risks or clinical burden suffered by PNH patients.

Renal failure is a consequence of hemolysis and has a significant impact on survival in Japanese patients with PNH, accounting for 18% of deaths [27]. The incidence of renal failure is reported at 10.5% in the Japanese PNH population, similar to that reported in the US PNH population 9.6% [27]. Repetitive exposure to elevated cell-free Hgb causes renal hemosiderin accumulation, tubulointerstitial inflammation, and kidney damage [25]. In addition, NO depletion due to excess free-Hgb leads to alterations in renal blood flow and can have a direct effect on the GFR and renal plasma flow [11, 12, 34, 35]. There is also evidence of microscopic infarction playing a role in chronic renal failure [9, 19]. We found that 37% (7/19) of patients with CKD had elevated D-dimer levels, suggestive that microthrombotic infarctions may also contribute to CKD, but only in some PNH patients. Elevated D-dimer levels are not specific to CKD patients as elevated D-dimer levels were evident in 40% (4/10) of patients with no CDK. We determined that renal dysfunction or damage, as defined by stages of CKD, is common (66%) in Japanese PNH patients enrolled in the study (Fig. 3), similar to the 64% of PNH patients with CKD in the PNH multinational studies and to the 68% of PNH patients with reduced creatinine clearance studied separately [9, 19]. Treatment with eculizumab led to improvement of CKD in 41% of Japanese patients at 12 weeks. While there is no placebo control arm in the current study, the placebo group in multinational PNH did not demonstrate any likelihood of CKD improvement compared to baseline ($P = 0.78$) at 26 weeks [19, 23]. Treatment of Japanese patients with milder CKD Stage 1–2 at baseline was associated with a higher likelihood of improvement in renal function. This result is similar to the PNH multinational trials in which 64% with Stage 1–2 at baseline improved with eculizumab treatment. There were very few patients in the current trial with CKD Stage 3–5 to determine the beneficial impact, although 1 of the 3 patients improved with eculizumab treatment, and no patient worsened. In the PNH multinational clinical trials, 20% of PNH patients with CKD Stage 3–5 showed improvement and 75% remained stable over 18 months. Taken together, these data suggest that eculizumab had a pronounced and beneficial effect on pre-existing CKD within 12 weeks of chronic treatment initiation and initiation of eculizumab treatment earlier in the disease course was more likely to be associated with significant improvement in renal function.

The standard dosing regimen is designed to maintain eculizumab serum levels > 35 μg/mL, which is sufficient to completely and consistently block complement-mediated hemolysis in patients with PNH. In the multinational studies ($N = 195$), 100% of patients showed a strong response to the standard eculizumab dosing regimen as measured by a significant reduction in LDH [4, 21, 22]. In the current trial, two patients treated with eculizumab were not observed to show a rapid and strong reduction in LDH despite eculizumab serum concentration above 35 μg/mL. In these two patients, a partial drug response was observed with a reduction in complement activation as measured by

in vitro CH50 assays. This is an extremely rare event, perhaps unique to Japanese patients. Efforts are underway to examine the molecular linkage between CH50 and LDH in these two patients. Twenty-seven patients who each showed a strong response to treatment were enrolled in an extension trial and continued eculizumab treatment.

The review of safety parameters in this study shows that eculizumab, administered per the specified induction and maintenance dose, appears safe and well tolerated. Most AEs were mild (88%). There were no major adverse vascular events, thrombotic events, infusion reactions or episodes of anaphylaxis reported during the study. No patient discontinued participation in the study due to an AE or died. The safety profile of eculizumab reported in the Japanese trial was also consistent with that reported in the multinational phase III trials of eculizumab. The most frequent treatment-emergent AEs in trial were headache (52%), nasopharyngitis (41%), and nausea (21%), similar to the most common AEs in the SHEPHERD multinational study (headache 52.9%; nasopharyngitis 32%; upper respiratory tract infection 29.9%; nausea 20.6%) [4]. Furthermore, the observation is that most headaches were mild and the kinetics of the reported headaches in the Japanese trial was consistent with the observations in the SHEPHERD multinational study. Specifically, in SHEPHERD, 94% of patients who experienced headache did so within the first 48 h of drug administration and most were restricted to the first 2 weeks of therapy. A rapid increase in levels of nitric oxide has been shown to result in the transient induction of headache through vasodilatation [8], suggesting that headaches observed with eculizumab in the AEGIS and multinational PNH study populations may be related to the initial, rapid therapeutic reductions in intravascular hemolysis, cell-free Hgb, and nitric oxide consumption.

Thrombosis is the leading cause of death due to PNH in the Caucasian population, accounting for 40–67% of deaths [21]. However, past studies have suggested that TE is less prominent in Japanese patients with PNH [27, 28]. In the current study, we note that 5/29 or 17% of patients entering the Japanese PNH trial had a history of TE. This incidence is similar to the 19% observed in the randomized, double blind placebo PNH multinational phase III trial [22]. Previous studies have also demonstrated the ongoing TE risk in PNH patients, despite the absence of clinical evidence of TE [16]. Western patients with PNH have been shown to have subclinical TE (as detected by MRI) elevated levels of prothrombotic (e.g. D-dimer) and pro-inflammatory (e.g. IL-6) markers even without evidence of previous clinical thrombosis [13, 16, 40]. Consistent with these previous studies, we observed that 38% (11/29) of Japanese PNH patients had D-dimer levels above the ULN at baseline. Indeed, 52% (15/29) of Japanese patients were at demonstrably elevated risk for TE as indicated by either an elevated D-dimer measurement and/or history of documented thrombosis.

TE appears to be mediated by terminal complement activation in Japanese PNH patients. The observations that a significant proportion of Japanese PNH patients have elevated D-dimer levels at baseline and that eculizumab treatment normalizes this measure of hemostatic activation further confirm both the elevated risk for TE and the beneficial effect of eculizumab treatment in Japanese PNH patients. Indeed, the substantial reduction of TEs during eculizumab treatment in the multinational PNH study (over 281 patient years) empirically demonstrates that terminal complement activation plays a prominent role in the pathogenesis of thrombosis. There are strong similarities between Western and Japanese PNH patients. It has been clearly demonstrated that PNH evolves from the same somatic genetic mutations in the PIGA gene in both Western and Japanese populations [38, 41]. Additionally, the impact of blocking terminal complement-mediated hemolysis on significant morbidities and potentially life-threatening complications in Japanese patients is similar to that observed in the multinational trials, demonstrating that the physiology of hemolysis does not differ between the Japanese and Western population. It is possible that previously reported minor differences in patient presentation or symptoms may be related to different patterns of diagnosis of PNH or cultural sensitivities to various morbidities, low sample sizes due to rarity of the disease, or unappreciated comorbid factors in the two regions. In the future, participation in a PNH registry may be able to address cultural differences in regard to clinical manifestations in PNH patients.

Chronic eculizumab treatment provided clinically meaningful benefit to PNH patients in this study by reducing the primary manifestation of PNH, chronic intravascular hemolysis, with consequent significant improvements in fatigue, dyspnea, overall QoL, kidney disease, hemostatic activation and measures of thrombotic risk, anemia and transfusion requirements. These results demonstrate that eculizumab treatment provides significant clinical benefit to Japanese patients with PNH and the substantial reductions in morbidities and complications are consistent with the clinical benefits observed in the previous multinational studies.

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