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ORIGINAL ARTICLE

Long-term outcome of immunosuppressive therapy for Japanese patients with lower-risk myelodysplastic syndromes

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Abstract To investigate the long-term usefulness of immunosuppressive therapy (IST) for Japanese patients with lower-risk myelodysplastic syndromes, we retrospectively analyzed 29 MDS patients who were treated with cyclosporine A alone or with anti-thymocyte globulin at a single institute in Japan. A total of 58.6 % of patients showed hematological response to IST. Overall survival of all patients was 74.5 % at 5 years and 48.3 % at 10 years. The major adverse event was the elevation of creatinine level (grade 1 and 2). Eleven patients were still on IST at the time of analysis with, at least, some clinical benefits. Pneumonia was the most frequent cause of death (eight of 12 deaths), followed by bleeding (three of 12); most of the patients who died were non-responders. The presence of paroxysmal nocturnal hemoglobinuria–type cells was significantly associated with both response to IST and long-term survival by univariate analysis. The 10-year overall survival of responders (72.2 %) was significantly superior to that of non-responders (15.6 %, $P < 0.0001$). These results suggest that IST using cyclosporine A provides long-term benefit for Japanese patients with lower-risk MDS.

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Introduction

Myelodysplastic syndromes (MDS) are clonal haematopoietic disorders characterized by cytopenia with ineffective hematopoiesis and transformation to acute leukemia. Although genetic abnormalities in stem cells play important roles, the precise pathophysiology of MDS is unclear. For example, in some cases, ineffective hematopoiesis appears to be caused, at least partly, by an immunological mechanism [1]. Reflecting these complicated disease pathophysiology, the clinical features of MDS including prognosis are quite heterogeneous, which necessitates a system of prognostic evaluation other than classification of MDS. The International Prognostic Scoring System (IPSS) is widely used to classify MDS patients into four risk categories [2], and the IPSS has been recently revised (IPSS-R) [3]. In general, different treatment strategies are taken for those with "lower-risk" and "higher-risk" patients. Since hematopoietic stem cell transplantation (HSCT) is the only curative treatment for MDS, HSCT is the first option for patients with high risk of leukemia transformation. However, MDS patients are tend to be elderly, and most of them be unable to undergo this procedure; therefore, treatments other than HSCT are needed. In terms of "lower-risk" patients most of whom are categorized as low or intermediate-1 risk by IPSS, improvement of ineffective hematopoiesis is the major aim of the treatment.

Immunosuppressive therapies (IST) with anti-thymocyte globulin (ATG) and cyclosporine A (CsA) are used for patients with aplastic anemia and lower-risk MDS [4–8].

Molldrem et al. [9] reported that by ATG administration, 34 % of treated MDS patients achieved transfusion independency. Lim et al. [10] reported the response rate of MDS patients for ATG as 42 % with a 3-year survival rate of 85 % among responders, which was not statistically different from that of non-responders (56 %). IST with CsA alone had a response rate of 50–80 % [11–14]. The combination of ATG and CsA exhibited a higher response rate than ATG alone (45 vs. 21 %). Importantly, the median survival time was significantly longer with IST (8.2 years) than with supportive care alone (5.2 years) [15, 16]. Clinical markers can be used to predict the response to IST for MDS patients. For example, the United States National Institutes of Health has used patient age, history of blood transfusion, and HLA-DR status to predict the efficacy of IST [16].

These reports strongly suggest that IST is beneficial for lower-risk MDS patients; however, reports with long-term observation for Japanese patients are rare. In this article, we evaluated the long-term outcome of IST for MDS patients treated at a single institute in Japan.

Patients and methods

Patients

This is a retrospective study of MDS patients treated with IST at Nagasaki University Hospital from January 1995 to December 2012. This analysis was performed as of March 2013. The diagnosis of MDS was made by clinical features, hematological data of peripheral blood and bone marrow, and cytogenetics, following the French–American–British classification [17] and the World Health Organization (WHO) classification [18]. In our institute, IST was applied to MDS patients with cytopenia with dependency on transfusions of red blood cells (RBCs) or platelets, or neutropenia [absolute neutrophil count (ANC) $< 0.5 \times 10^9$/L], and classified by IPSS as low, intermediate (int) -1 or int-2 (only with less than 5 % blasts in the bone marrow) risk. All patients were ineligible for HSCT because of old age, complications, a lack of suitable donors, or patient's choice.

Marrow cellularity was assessed by core biopsy, bone marrow clot section, and magnetic resonance imaging findings. Hypocellular marrow was defined as bone marrow cellularity of less than 20 % with which MDS patients were also included in this study. Karyotype was assessed by conventional G-band cytogenetics. RBCs and neutrophils that lacked CD55 and CD59 expression were defined as paroxysmal nocturnal hemoglobinuria (PNH)-type cells, which were detected by flow cytometry with monoclonal antibody specific to these antigens [19]. Blood samples of patients obtained after 2008 underwent high-resolution two-color flow cytometry to detect PNH-type cells, as previously reported [20]. PNH-type cells were considered present if they were detected in more than 0.003 % of granulocytes and more than 0.005 % of RBCs in the high-resolution setting. The Ham's test was performed to detect PNH clones in some cases. HLA-DRB1 allele status and the presence of PNH-type cells were assessed before or after the initiation of IST. Informed consent was obtained from all patients.

Transfusion dependence was defined as the requirement for at least 2 units of RBCs per month to maintain hemoglobin values of 90 g/L or less, according to the International Working Group criteria [21].

Treatment

All patients received oral CsA at doses ranging from 2 to 6 mg/kg/day to maintain a trough blood concentration of 150–250 ng/mL. CsA was continued as long as no major toxicities occurred, and the dose was also adjusted by the serum creatinine value. Some patients received CsA and intravenous horse ATG (Lymphoglobuline, Genzyme) at a dose of 10–15 mg/kg/day for 5 days. Patients with neutropenia (less than 0.5×10^9/L) received granulocyte-colony stimulating factor until ANC increased to more than 0.5×10^9/L. For some patients who showed inadequate response to CsA, tacrolimus was administered at a dose needed to maintain a trough concentration of 5–10 ng/mL.

Response criteria

Response criteria were defined according to the International Working Group response criteria [21] with minor modifications. We evaluated the response using the data obtained at the time of best response. Complete remission (CR) was defined as the improvement of all three lineages of peripheral blood parameters with hemoglobin ≥110 g/L, platelet count $\geq 100 \times 10^9$/L, and ANC $\geq 1.0 \times 10^9$/L with no blasts in peripheral blood and blasts in bone marrow less than 5 %. Partial remission (PR) was defined as normalization of peripheral blood counts and at least 50 % reduction of marrow blasts. Hematological improvement (HI) was defined as: (1) HI-erythroid (HI-E), an increase of hemoglobin level more than 15 g/L or reduction of blood transfusion by at least 4 RBC units during 8 weeks; (2) HI-platelet (HI-P), an increase of platelet count more than 3.0×10^9/L for patients starting with $>2.0 \times 10^9$/L, or an increase of more than 2.0×10^9/L and by at least 100 % for patients starting with $<2.0 \times 10^9$/L; and (3) HI-neutrophil (HI-N), an increase of ANC more than 0.5×10^9/L or by at least 100 %. Relapse after CR, PR, or HI was defined as a reduction of ≥50 % from the maximal response level for ANC or platelets, a

reduction in hemoglobin level ≥15 g/L, or the onset of transfusion dependence.

Statistical methods

Continuous variables of the patient subgroups were compared using the Mann–Whitney rank-sum test or Student's *t* tests. Percentages were compared with the Chi-squared test or the Fisher's exact test. Overall survival (OS) was estimated from the start of treatment to the time of death, HSCT, or last follow-up. OS was calculated using the Kaplan–Meier method [22], and the log-rank test or Gehan–Breslow–Wilcoxon test was used to evaluate differences between survival distributions. *P* values less than 0.05 were considered statistically significant. Statistical analyses were performed with GraphPad Prism 6.01 (GraphPad Software, La Jolla, CA, USA).

Results

Patients and treatment

Pre-treatment patient characteristics are shown in Table 1. We recruited 29 patients with a median follow-up of 6.7 years (range, 0.50–18.0 years). There were 14 men and 15 women, and the median age was 60 years (range, 31–83 years). Hemoglobin levels were less than 90 g/L in all patients. Twenty-two patients (76 %) had received RBC transfusions before the initiation of IST. All patients except three had platelet counts less than 100×10^9/L. ANC was less than 1.0×10^9/L in 18 patients (62 %).

Five patients (17.2 %) had hypocellular marrow. Based on the IPSS-R cytogenetics classification system, karyotypes of most patients were in "good" and "intermediate" categories (21 patients, 72.4 %, and 6 patients, 20.7 %, respectively). Although all but two were in Int-1 category by IPSS, IPSS-R, differentiated four patients (13.8 %) as low, 20 patients (69.0 %) as intermediate, and five patients (17.2 %) as high risk. Human leukocyte antigen (HLA)-DRB1 status was tested in 27 patients. Ten patients had at least one allele of HLA-DRB1* 1501, and six patients had DR15 but their DRB1* was 1502, not 1501.

Twenty-three patients were screened for PNH-type cells, and they were found in 12 patients; 7 out of 11 patients tested by high-resolution two-color flow cytometry, 4 out of 11 patients tested by ordinary flow cytometry, and one by Ham test.

Treatment choice of CsA alone or CsA with ATG was determined by the attending physicians who considered age, presence of infection, organ function, and general condition of patients. Although we intended to continue IST for at least 6 months, the duration of IST was 6 months or less in seven patients. Two patients could not continue to receive IST because of complications (elevation of

Table 1 Patient characteristics

	No (%), (range)
No. of patients	29
Median age (years) (range)	60 (31–83)
Sex	
Male	14 (48.5)
Female	15 (51.7)
Median Hb (g/L) (range)	62 (46–90)
Median ANC ($\times 10^9$/L) (range)	0.9 (0.33–2.70)
Median platelet count ($\times 10^9$/L) (range)	19 (4–385)
Bone marrow cellularity	
Hypo	5 (17.2)
Normo/hyper	24 (82.8)
RBC transfusion before IST	22 (75.9)
Cytogenetics category according to IPSS-R	
Very good	1 (3.4)
Good	21 (72.4)
Intermediate	6 (20.7)
Poor	1 (3.4)
FAB	
RA	28 (96.6)
RARS	1 (3.4)
WHO 2008	
RA	12 (41.4)
RCMD	17 (58.6)
IPSS risk category	
Low	1 (3.4)
Int-1	27 (93.2)
Int-2	1 (3.4)
IPSS-R risk category	
Low	4 (13.8)
Int-1	20 (69.0)
High	5 (17.2)
PNH-type cells	
Present	12 (41.4)
Absent	11 (37.9)
Test not performed	6 (20.7)
HLA DRB1	
1501	10 (34.5)
1502 (without 1501)	6 (20.7)
Other	11 (37.9)
Test not performed	2 (6.9)
Treatment	
CsA	24 (82.8)
ATG + CsA	5 (17.2)
Tacrolimus (after CsA)	7 (24.1)

creatinine, and interstitial pneumonia), and five patients discontinued IST because of no effect. At the time of this analysis, 11 responders out of 29 patients were still receiving IST (treatment duration, 0.6–16.1 years).

Response

Eight patients (27.6 %) achieved CR, and no patients had PR. Nine patients (31 %) achieved some hematological improvement, resulting in an overall response rate of 58.6 % (Table 2). HI-E was observed in 16 of 29 patients (55 %) with anemia. Twelve of 26 patients (46.2 %) with thrombocytopenia achieved HI-P, and 9 of 18 patients with neutropenia (50 %) showed HI-N. One patient who did not respond to IST received allogeneic bone marrow transplantation from a suitable unrelated donor 4.8 years after discontinuation of IST. Tacrolimus was administered to 3 patients who lost response to CsA and to 3 patients for

Table 2 Comparison of characteristics of patients between responders (CR and HI) and non-responders

	Responder		Non-responder	*P* (Responder vs. non-responder)
	CR	HI		
No. of patients	8	9	12	
Median age (years)	58.5	64	59	0.870
Range	31–83	56–69	32–71	
WHO				
RA	3	6	5	0.710
RCMD	5	3	7	
IPSS-R				
Low	0	2	2	0.931
Int-1	7	5	8	
High	1	2	2	
RBC transfusion				
Yes	7	4	11	0.095
No	1	5	1	
Median Hb, g/L (range)	59 (46–89)	62 (52–90)	65 (47–77)	0.978
Median ANC ($\times 10^9$/L)	0.87	0.90	1.001	0.989
Range	(0.38–2.48)	(0.6–2.7)	(0.33–2.67)	
Median platelet count ($\times 10^9$/L)	20	14	19	0.834
Range	(0.5–11.8)	(0.5–5.7)	(0.4–38.5)	
Bone marrow cellularity				
Hypo	1	2	2	1.000
Normo/hyper	7	7	10	
HLA DRB1				
Presence of 1501 or 1502	6	4	6	0.317
Other	1	4	6	(15 vs. others)
Test not performed	1	1	0	
Cytogenetics (IPSS-R)				
Very good	0	1	0	0.512
Good	6	6	9	
Intermediate	2	2	2	
Poor	0	0	1	
PNH-type cells				
Present	8	3	1	0.002
Absent	0	3	8	
Test not performed	0	3	3	
Treatment				
CsA	5	8	11	1.000
ATG +CsA	2	1	2	
Tacrolimus	1	0	6	

Table 3 Patient characteristics by the presence of PNH-type cells

	With PNH-type cells	Without PNH-type cells	*P*
No. of patients	12	11	
Median age (years, range)	61 (31–83)	59 (40–71)	0.529
WHO			
RA	5	5	1.000
RCMD	7	6	
IPSS-R			
Low	1	2	0.579
Int-1	9	6	
High	2	3	
RBC transfusion			
Yes	10	8	0.617
No	2	3	
Bone marrow cellularity			
Hypo	1	3	0.231
Normo/hyper	11	8	
HLA DRB			
1501 and/or 1502	10	3	0.008
Other	1	8	(15 vs. others)
ND	2	0	
Cytogenetics (IPSS-R)			
Very good	0	1	0.865
Good	7	6	
Intermediate	3	3	
Poor	2	1	

whom CsA had no effect. One patient who did not respond to CsA achieved CR by tacrolimus. Hematological relapse occurred in 9 of 17 responders. Six of these patients had to discontinue or reduce the dose of CsA before relapse due to the elevation of creatinine level.

There were no statistically significant differences in age, WHO subtype, marrow cellularity, IPSS-R or HLA DRB1*1501 status between responders (CR and HI) and non-responders. There was no specific karyotype that associated with responses to IST, either. However, the presence of PNH-type cells was significantly predictive of the response to IST (Table 2). The presence of PNH-type cells correlated with HLA-DRB1*1501, but not with other factors (Table 3).

Survival

Five and 10-year OS was 74.5, and 48.3 %, respectively (Fig. 1). Median survival time was 8.6 years. In one patient that stopped IST after 5 months without response, leukemia progression occurred 3.8 years after discontinuation of IST. Five- and 10-year OS among responders was 100 and 72.7 %, respectively, which was significantly better than those among non-responders (41.7 and 15.6 %, respectively. $P < 0.0001$, Fig. 2). Factors such as marrow cellularity, IPSS category, IPSS-R category and blood transfusion status were not significant predictors for long-term survival. OS of patients with PNH-type cells was better (but not statistically significantly) than patients without PNH-type cells (Fig. 3). There were 17 patients that survived more than 5 years (long-term survivors). Among long-term survivors, 5 died. One patient received allogeneic HSCT, and one patient was lost for the follow-

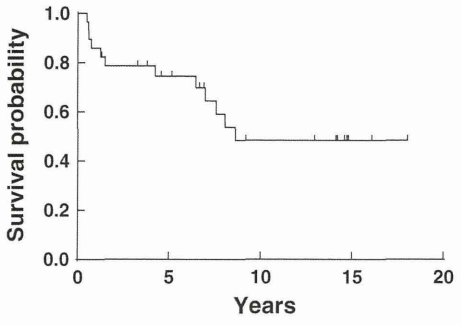

Fig. 1 Overall survival of all patients. Five-year OS was 74.5 %, and 10-year OS was 48.3 % in all patients. Median survival was 8.6 years

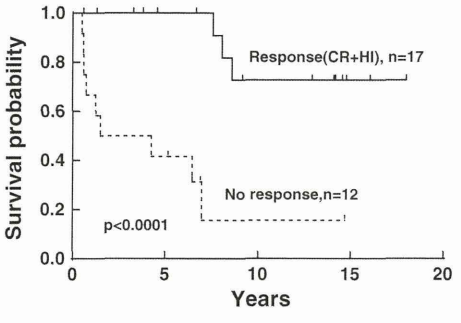

Fig. 2 Overall survival of responders (CR and HI) and non-responders. Five- and 10-year OS among responders was 100 and 72.7 %, and those among non-responders were 41.7 and 15.6 %, respectively

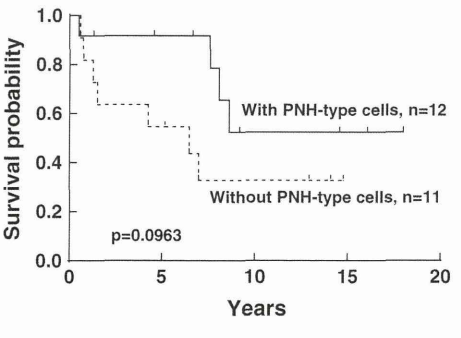

Fig. 3 Overall survival of patients with and without PNH-type cells

up. Except for those died, transplanted, and lost follow-up, eleven out of 15 patients had continued receiving IST. All patients who were on IST showed various levels of clinical benefit of IST continuously, though some of them lost CR or HI by definition.

Adverse events and cause of death

Nine patients experienced elevated creatinine levels and subsequently required reduction of CsA dose, however, there were no grade 3 or 4 acute renal toxicities. Two patients developed interstitial pneumonia. Eight patients died of pneumonia (one of them had also systemic bleeding), 3 patients of bleeding (two cerebral, one gastrointestinal), and one died of an unknown cause.

Discussion

In this study, we reported the long-term results of IST for MDS patients (median observation period, 6.7 years). The overall response rate was 58.6 %, and the median survival time was 8.6 yeas for all patients in this study. Considering that most of the patients (27 of 29 patients, 93.2 %) in this study were in the Int-1 category of IPSS of which median survival time was 3.5 years [2], it is suggested that IST for lower-risk MDS is beneficial even for survival as reported by others. [16] These results confirmed previous reports that IST is a treatment option for MDS, in particular for cytopenia of lower-risk MDS patients, in which survival could be prolonged.

Response rates to IST varied widely by each reports from less than 30 % up to 60 % [5, 7, 23]. This might reflect the difference in each study, such as drugs used for IST, background of patients, and also the observation period. In this study, 24 out of 29 patients were treated with CsA, alone. Though IST using CsA alone seemed to provide inferior efficacy to IST using ATG with or without CsA [16, 24], our results showed certain efficacy of IST with CsA alone for Japanese patients with MDS. It is not clear whether response to IST would be different among ethnic groups, Shimamoto et al. [12] reported relatively high response rate for Japanese by CsA alone. In the present study, which had a very long observation time for most of the patients, 17 of 29 patients (58.6 %) showed a clinical response to IST. Response rates between patients administered CsA alone and those received ATG and CsA did not differ. Six patients who did not respond to CsA or lost their response to CsA received tacrolimus after CsA, and one patient achieved a CR that was maintained for 9 years. Considering diverse reports from many groups, it is necessary to collect data on IST using various immunosuppressive agents for Japanese MDS patients.

PNH-type cells are present in 4–10 % of patients with MDS [10, 19, 25]. Using sensitive flow cytometry [26], PNH-type cells were detected in 12 of 23 patients (52.1 %) in this series. Wang et al. [20] demonstrated that seven of nine MDS-RA patients with PNH-type cells responded to CsA, suggesting PNH-type cells as a response marker. Ishikawa et al. [14] reported that the presence of PNH-type cells was associated with the platelet response to IST. It was also true in our results, showing the presence of PNH-type cells as a statistically significant predictor of hematological response and good prognosis. Since methodological improvements have changed the sensitivity for PNH cells, it would be interesting to re-evaluate its clinical meaning with long-term observation. Although our data came from a retrospective analysis of a limited number of cases, by reflecting the difference in 10-year survival rates of responders and non-responders (72.2 and 15.6 %, respectively. $P < 0.0001$), univariate analysis revealed that the response to IST and presence of PNH-type cells were predictive factors for long-term survival. HLA subtype (DR 15), which was reported previously [27], did not predict response. The recently published IPSS-R divides cases into 3 categories (low-risk: 4 cases; intermediate-risk: 20 cases; high-risk: 5 cases); however, IPSS-R category did not predict response. Bone marrow cellularity was not selected as a predictor of response in this study, which was shown to have predictive value for IST. Considering 3 out of 5 cases with hypoplastic MDS responded to IST in our series, the importance of cellularity would be underestimated because of the small number of cases of this group.

In summary, our study with long-term observation supports previous reports showing the usefulness of IST for Japanese patients with lower-risk MDS.

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Conflict of interest None.

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Role of hematopoietic stem cell transplantation for relapsed acute promyelocytic leukemia: A retrospective analysis of JALSG-APL97

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For patients with relapsed acute promyelocytic leukemia (APL), all-*trans* retinoic acid-based salvage regimens can achieve second complete remission (CR2), but the optimal post-remission strategy for APL patients after CR2 remains unclear. Hematopoietic stem cell transplantation (HSCT) during CR2 might be effective, but data on the role of HSCT for APL patients after CR2 are limited in Japan. We retrospectively analyzed outcomes for 57 relapsed APL patients who achieved CR2 in the JALSG APL97 study. Of those, six received autologous (auto)-HSCT, 21 received allogeneic (allo)-HSCT, and 30 received various regimens other than HSCT. The 5-year event-free survival (EFS) rate, overall survival (OS) rate and cumulative incidence of relapse (CIR) were 50.7%, 77.4% and 51.0% in the non-HSCT group, 41.7%, 83.3% and 58.3% in the auto-HSCT group and 71.1%, 76.2% and 9.8% in the allo-HSCT group, respectively. Both the EFS rate and CIR were significantly better in the allo-HSCT group than in other groups. Allo-HSCT appears effective in APL patients in CR2, with a low relapse rate beyond a relatively early transplantation-related mortality (19%). Among older patients (age ≥40 years), the 5-year OS was significantly better in the non-HSCT group than in the HSCT group (78.0% *vs* 40.5%; $P = 0.04$). Further prospective studies with larger patient numbers are required to confirm the impact of HSCT alone and in combination with arsenic trioxide on outcomes for patients with APL in CR2. (*Cancer Sci* 2013; 104: 1339–1345)

The introduction of all-*trans* retinoic acid (ATRA) has brought about marked progress in the treatment of acute promyelocytic leukemia (APL), but relapse still occurs in approximately 15–25% of patients.[1–3] Most of the relapsed patients were able to achieve second complete remission (CR2) using ATRA-based salvage regimens[4–6] or recent arsenic trioxide (ATO)-based salvage regimens.[7,8] After achieving CR2, most patients need to receive post-remission treatments to reduce minimal residual disease (MRD). A variety of post-remission strategies have been used, including further consolidation chemotherapy,[3] hematopoietic stem cell transplantation (HSCT),[6,9–11] continued treatment with ATO[7,8,12] or a combination of such therapies; however, the optimal post-remission therapy remains controversial. Previous studies have reported that ATO-based post-remission therapy for patients with APL in CR2 resulted in superior survival compared with chemotherapy alone or HSCT alone.[13] Likewise, HSCT strategies for patients with APL in CR2 resulted in better outcomes than chemotherapy alone, despite being associated with high transplantation-related mortality (TRM).[9–11] Moreover, autologous HSCT (auto-HSCT) was much better than allogeneic HSCT (allo-HSCT) for patients in CR2 who achieved molecular remission.[6,9]

Recently, in a phase 2 prospective study, our Japan Adult Leukemia Study Group (JALSG) reported the efficacy of sequential treatment using ATO followed by auto-HSCT for 25 patients with relapsed APL.[14] However, evidence has been lacking in terms of the role of auto-HSCT alone on the cumulative relapse rate or efficacy for patients with APL in CR2 who were ineligible for the phase 2 study regimens. Moreover, in situations where no guidelines regarding the optimal choice of auto- or allo-HSCT in CR2 have been determined, the role of HSCT alone in post-remission therapies for patients with APL in CR2 is yet to be evaluated. Therefore, the present study aimed to evaluate in detail the efficacies of HSCT alone for APL patients in CR2 by comparing outcomes, including cumulative relapse rate, both for APL patients who underwent auto-HSCT or allo-HSCT during CR2 and for those who did not receive HSCT during long-term follow up.

Materials and Methods

Data source. Information on patients with APL in CR2 and the salvage treatment applied were obtained from the JALSG APL97 study.[15] Between May 1997 and June 2002, a total of 302 adult patients with previously untreated de novo APL were registered in this study. The main eligibility criteria included diagnosis of APL with t(15;17) and/or the *PML-RARA* fusion gene and age between 15 and 70 years. For remission induction therapy, patients received ATRA either

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alone or with chemotherapy, followed by three courses of consolidation therapy consisting of cytarabine and anthracyclines. After completing consolidation therapy, patients negative for the *PML-RARA* fusion gene were randomly allocated to undergo either six courses of intensified maintenance chemotherapy or observation alone. More detailed eligibility criteria and the treatment schedule have been described previously.[15] Of the 283 assessable patients with t(15;17) and/or *PML-RARA*, 267 (94.3%) achieved complete remission (CR). Of the 267 patients who achieved CR, 67 (26.1%) experienced a first relapse during the median follow-up duration of 100 months (range, 11–155 months) from first achieving CR.

Salvage treatment in first relapse. All 67 relapses occurred between 1998 and 2005, during which time ATRA was mainly used as the salvage treatment for relapsed patients because ATO was not commercially available in Japan. Among the relapsed patients, two were unable to complete the follow-up survey and 65 received salvage treatment with ATRA alone (n = 17), ATRA plus chemotherapy (n = 33), tamibarotene (Am80) alone (n = 7), chemotherapy alone (n = 6), allo-HSCT alone (n = 1) or unknown (n = 1). Of those patients who received salvage treatments, 58 (89%) achieved CR2.

Of the 58 patients who achieved CR2, 27 had received HSCT (auto-HSCT, n = 6; allo-HSCT, n = 21) during CR2, 30 had not and one was unassessable. Therefore, the present study included 57 patients. We defined 27 patients in CR2 who received HSCT (six auto-HSCT and 21 allo-HSCT) as the HSCT group and 30 patients in CR2 who received regimens other than HSCT as the non-HSCT group. Clinical characteristics of the 57 APL patients in CR2 are summarized in Table 1.

Hematopoietic stem cell transplantation group. Stem cells for auto-HSCT were harvested in CR2 from peripheral blood in all six patients. Peripheral blood stem cell (PBSC) collection was made after mobilization using granulocyte colony-stimulating factor (G-CSF) following chemotherapy. All patients who underwent auto-HSCT achieved molecular CR of *PML-RARA* in bone marrow according to nested reverse transcriptase–polymerase chain reaction (RT-PCR) (n = 3), real-time quantitative PCR (RQ-PCR) (n = 2) or RT-PCR (n = 1) just before PBSC collection. For allo-HSCT, bone marrow cells were used in 15 patients, G-CSF-mobilized PBSC in four patients and cord blood cells in two patients. Donors were unrelated in 13 patients (bone marrow, 11 patients; cord blood, two patients). Seven of 15 patients who were examined for *PML-RARA* in the marrow before allo-HSCT were positive for MRD.

Patients were administered various conditioning regimens for HSCT. All six autografted patients received a myeloablative regimen using total body irradiation (TBI)/cyclophosphamide

Table 1. Clinical characteristics of the 57 APL patients in CR2 according to treatment after CR2

	Auto-HSCT (n = 6) No. (%) or median (range)	Allo-HSCT (n = 21) No. (%) or median (range)	Non-HSCT (n = 30) No. (%) or median (range)	All (n = 57) No. (%) or median (range)
At diagnosis	6	21	30	57
Sex				
Male	3 (50)	16 (76)	18 (60)	37 (64)
Female	3 (50)	5 (24)	12 (40)	20 (36)
Age (years)	40 (24–59)	33 (21–55)	50 (15–70)	45 (15–70)
15–29	2 (33)	8 (38)	5 (17)	15 (26)
30–49	2 (33)	11 (52)	9 (30)	22 (39)
50–70	2 (33)	2 (10)	16 (53)	20 (35)
WBC counts (x10^9/L)	6.5 (2.1–33.7)	3.2 (0.4–46.1)	1.9 (0.1–63.7)	2.7 (0.1–63.7)
<3.0	1 (17)	9 (43)	19 (63)	29 (51)
3.0–10.0	4 (66)	6 (29)	6 (20)	16 (28)
10.0 or higher	1 (17)	6 (29)	5 (17)	12 (21)
At first relapse				
Age (years)	44 (27–60)	36 (22–59)	53 (16–72)	47 (16–72)
First CR duration (months)	22 (10–81)	22 (6–63)	18 (6–90)	21 (6–90)
Salvage treatment				
ATRA alone	1 (17)	3 (14)	12 (40)	16 (28)
ATRA plus chemotherapy	5 (83)	9 (43)	12 (40)	26 (46)
Tamibarotene alone	0	3 (14)	4 (13)	7 (12)
Chemotherapy alone	0	5 (24)	2 (7)	7 (12)
Unknown	0	1 (5)	0	1 (2)
In CR2 achievement				
Age at CR2 (years)	44 (27–60)	36 (22–59)	53 (16–72)	47 (16–72)
Time to HSCT after CR2 (months)	7 (4–20)	5 (1–13)	–	–
Stem-cell source				
Peripheral blood	6	4	–	–
Bone marrow	0	15	–	–
Cord blood	0	2		
Donor	–		–	
HLA-identical sibling	–	8	–	–
Unrelated donor	–	13	–	–

Allo-HSCT, allogeneic HSCT; APL, acute promyelocytic leukemia; ATRA, all-*trans* retinoic acid; Auto-HSCT, autologous HSCT; CR, complete remission; CR2, second complete remission; HLA, human leukocyte antigen; HSCT, hematopoietic stem cell transplantation; Non-HSCT, patients who received regimens other than HSCT; WBC, white blood cell.

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