Long-term outcome of immunosuppressive therapy for Japanese patients with

lower-risk myelodysplastic syndromes

Running title: Immunosuppressive therapy for MDS

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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 analysed 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 five 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.

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 "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 anaemia 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% to 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 neutropaenia [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-colour 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 2-6 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, tacrolims 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 $\geq$110 g/L, platelet count $\geq100\times10^9$/L, and ANC $\geq1.0\times10^9$/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 $>2.0\times10^9$/L, or an increase of more than 2.0×10^9/L and by at least 100% for patients starting with $<2.0\times10^9$/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 $\geq$50% from the maximal response level for ANC or platelets, a reduction in haemoglobin level $\geq$15g/L, or the onset of transfusion dependence.

Statistical methods

Continuous variables of the patient subgroups were compared by 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 by 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).

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 to 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; 7out of 11 patients tested by high-resolution two-colour 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 recieve IST because of complications (elevation of 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 to 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 neutropaenia (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 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-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]. By 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

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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 ponse. 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.

Potential conflict of interest

Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.

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Figure legends

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.

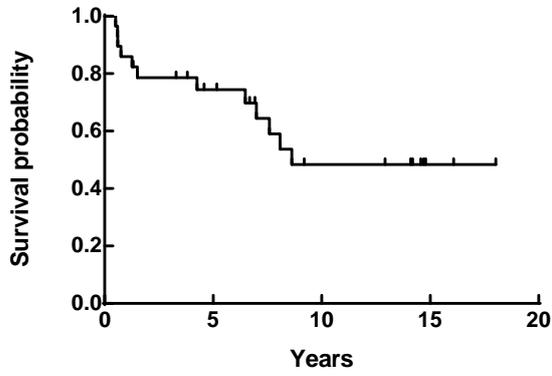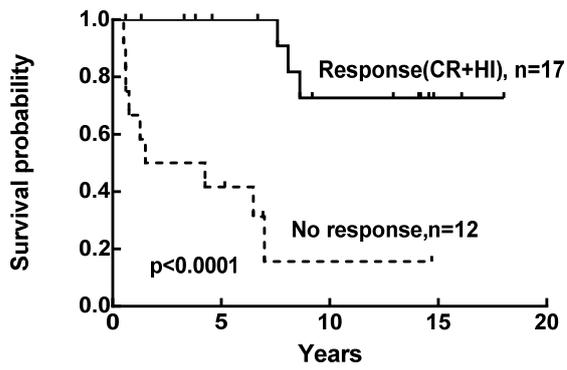Fig 3.   Overall survival of patients with and without PNH-type cells.
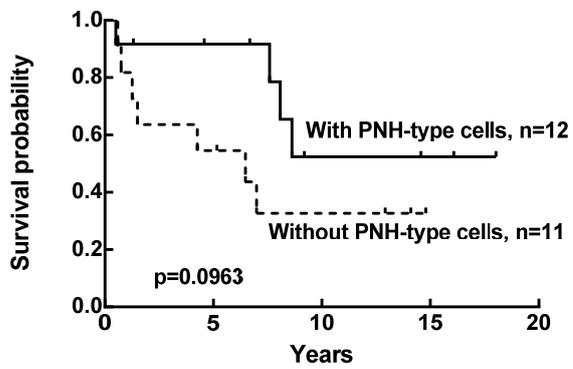
Fig.1

Fig.2

Fig.3

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)

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

non-responders

| | Responder | | Non- | P　(Responder vs. |
	CR	HI	responder	non-responder)
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	59	62	65	0.978
(range)	(46-89)	(52-90)	(47-77)	
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	20	14	19	0.834
count, $\times 10^9$/L				
(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	6	4	6	0.317

1501or 1502				
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 DRB1			
1501and/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	