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Five-day regimen of azacitidine for lower-risk myelodysplastic syndromes (refractory anemia or refractory anemia with ringed sideroblasts): A prospective single-arm phase 2 trial

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Although azacitidine is the first-line drug for higher-risk myelodysplastic syndrome (MDS) patients, its efficacy for lower-risk MDS remains unestablished. Therefore, we conducted a prospective study to examine the efficacy and safety of a 5-day regimen of azacitidine (AZA-5) for lower-risk MDS. The primary endpoint was hematological improvement (HI) after 4 courses of therapy. A total of 51 patients with lower-risk MDS based on the French-American-British (FAB) classification (44 patients with refractory anemia [RA] and 7 patients with refractory anemia with ringed sideroblasts [RARS]) were enrolled from 6 centers in Japan. The median age was 75 years (range: 51-88). These patients received AZA-5 (75 mg/m^2; once daily for 5 sequential days). The median number of AZA-5 courses was 8 (range: 1-57), and 45 patients (88.2%) received more than 4 courses. HI and transfusion independency were seen in 24 patients (47.1%) and 11 patients (39.2%), respectively. A total of 11 patients (21.6%) achieved complete remission or marrow remission. WT1 mRNA levels were not significantly correlated with therapy response. Grade 3 or 4 neutropenia and thrombocytopenia occurred in 26 (51.0%) and 11 (21.5%) patients, respectively. Nonhematological grade 3 or 4 adverse events were observed in 9 patients (17.6%). Together, these results indicate that AZA-5 is feasible and effective for lower-risk MDS patients as well as for higher-risk MDS patients.

KEYWORDS
5-day regimen of azacytidine, lower-risk MDS, multicenter study, prospective trial, Wilms tumor 1

1 | INTRODUCTION

Myelodysplastic syndromes (MDS) are clonal hematopoietic stem cell disorders characterized by cytopenia in peripheral blood and subsequent leukemic transformation in a substantial proportion of the patients.[1] A hypomethylating agent (HMA), azacitidine, was reported to improve overall survival (OS) for higher-risk MDS (including acute myeloid leukemia by WHO classification) with a 7-day administration schedule (AZA-7) compared with conventional therapies.[2] In addition, a phase 1/2 AZA-7 study in Japan demonstrated that AZA was effective, safe and well tolerated in MDS patients. Based on these results, AZA was approved for MDS including all-risk groups in Japan in

2011.[3] However, most previous clinical studies have focused on the efficacy of AZA for higher-risk MDS. The main purpose of the treatment for higher-risk MDS is the control of MDS cells, while that for lower-risk MDS is to improve cytopenia, thereby decreasing the risk of infection and/or bleeding and improving quality of life. Therefore, the optimal administration schedule for lower-risk MDS might be different from that for higher-risk MDS. Most clinical trials of AZA for MDS have adapted the AZA-7 regimen, which would be inconvenient in daily practice due to drug administration on weekends. A previous paper reported that a 5-day regimen of AZA (AZA-5) showed almost equivalent efficiencies and toxicities with AZA-7.[4] In addition, a phase 2 prospective study demonstrated the efficacy and safety of AZA-5 in erythropoietin-unresponsive lower-risk MDS patients.[5,6] However, the efficacy of AZA for lower-risk MDS has not been fully clarified. Therefore, in the present study, we analyzed the efficacy and safety of AZA-5 for untreated Japanese MDS patients with lower-risk MDS, including refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) based on the French-American-British (FAB) classification in the multicenter prospective single-arm phase 2 trial.

2 | PATIENTS AND METHODS

2.1 | Patient eligibility

Untreated MDS patients with lower-risk MDS (refractory anemia [RA] and refractory anemia with ringed sideroblasts [RARS]) based on the FAB classification,[7] who were aged ≧20years old, were eligible for this study. In addition, only the patients who matched at least 1 of the following eligibility criteria were enrolled: a neutrophil count less than 1×10^9/L accompanied by the susceptibility to the bacterial infection without prophylaxis, a transfusion history of red blood cells (RBC) within 3 months before registration, platelet count less than 50×10^9/L or with an apparent bleeding tendency. Other eligibility criteria were as follows: patients with the ECOG performance status (PS) 0-2 and without main organ dysfunction (serum total bilirubin ≤2.0 mg/dL, serum creatinine ≤2.0 mg/dL, and PaO2 ≥ 60 Torr or SaO2 ≥ 90%). Patients with the following conditions were excluded: uncontrolled infection and other active malignancies; and serum positivity for HB antigen, HCV antibody or HIV antibody. This study was approved by the ethics committee of each institute and registered at UMIN-CTR (UMIN000005662), and all of the patients were registered after obtaining written informed consent.

2.2 | Treatment regimen

AZA was administered at 75 mg/m^2 once daily for 5 consecutive days with a 28-day cycle either subcutaneously or intravenously (10-minute infusion). A serotonin (5-HT3) receptor antagonist was routinely administered approximately 30 minutes prior to AZA administration to prevent nausea and vomiting. Dose reduction, delay of initiation, or withdrawal of treatment with azacitidine was carried out as necessary. If grade 3 or 4 nonhematological events according to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 occurred in patients during treatment cycles, it has been stopped to be administered for 21-day. If adverse events are not recovered, treatment was withdrawn. The 28-day interval between AZA treatments allowed most patients to reach nadir values for hemoglobin, platelets and absolute neutrophil count (ANC), and to achieve hematologic recovery prior to their next treatment cycle. AZA-dosing cycles could be delayed and/or modified because of hematologic toxicity by 14 days, as needed, until hematologic recovery. For patients with baseline counts of WBC $\geq 3 \times 10^9$/L, ANC $\geq 1.5 \times 10^9$/L, and platelets $>75 \times 10^9$/L, dose modification or delay could occur if ANC nadir was $\leq 1.5 \times 10^9$/L. For patients with baseline counts of WBC $<3 \times 10^9$/L, ANC $<1.5 \times 10^9$/L or platelets $<75 \times 10^9$/L, dose modification or delay could occur if WBC, ANC or platelet nadir decreased ≥50% from baseline. The dose modification or delay was also contingent on bone marrow cellularity at the time of nadir WBC or platelet counts. If hematological toxicities were not resolved within 21 days, AZA treatment was discontinued and the patients were treated as having dropped off from the study.

The other treatment drugs that would influence the clinical course of MDS, such as cytokines (EPO and G-CSF excepting the use for the accompanied active infection), immunosuppressive therapy, lenalidomide, anti-cancer drugs, anabolic steroids, vitamin D and vitamin K, were prohibited from use during the study.

2.3 | Evaluation of response

The primary endpoint was hematological improvement (HI), based on the IWG criteria 2006, that lasts for more than 8 weeks after 4 cycles of AZA-5 treatment.[8] HI included erythroid response (HI-E), platelet response (HI-P) and neutrophil response (HI-N). The secondary endpoint was the rate of hematologic remission (HR), transfusion independency and HI by the karyotypes, changes in Wilms tumor 1 (WT1) messenger RNA (mRNA) level, treatment continuity and evaluation of adverse events. HR was judged by "Response criteria for altering natural history of MDS" utilized in IWG criteria 2006 and was categorized into complete remission (CR), partial remission (PR) and marrow CR (mCR: defined by ≤5% myeloblasts in the bone marrow) only when it continued more than 4 weeks. Transfusion independency was evaluated in transfusion-dependent patients at baseline. Patients were judged to be transfusion-independent if they did not have red blood cell or platelet transfusion for more than 8 weeks. The expression of WT1 mRNA in peripheral blood (PB) was measured at baseline and after every AZA-5 treatment until 4 cycles at SRL (Tokyo, Japan) using a WT1 mRNA Assay Kit (Otsuka Pharmaceutical, Tokyo, Japan). In this assay, the normal range of WT1 mRNA is <50 copies per 1 μg of RNA.

2.4 | Evaluation of safety

All adverse events (AE) were monitored in patients, who received AZA at least once, from the first administration to day 29 of the last

cycle and evaluated by CTCAE Version 4.0. If the patients dropped off the study before completion of the study protocol, AE were monitored until the next treatment was initiated.

2.5 | Statistical analysis

The primary endpoint of this study was the rate of HI as described above. The expected and threshold rates of HI were estimated to be 40% and 20%, respectively, based on the previous reports using the similar response criteria.[3] With a statistical power of 80% and a 2-sample 1-sided α of .025, the requirement of 44 eligible patients for this study was calculated by means of binomial analysis. Dichotomous variables were compared between different groups using the Wilcoxson test or Fisher's exact test, and results were considered significant if the *P*-value was <.05. All statistical analyses were performed with EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). More precisely, it is a modified version of R commander designed to add statistical functions frequently used in biostatistics.[9]

3 | RESULTS

3.1 | Patient characteristics

A total of 51 patients were enrolled in this study from 6 medical centers between May 2011 and December 2016. Patient characteristics are shown in Table 1; 30 patients were male and 21 patients were female. The median age of the patients was 75 years (range: 51-88). Based on the FAB classification, 44 patients (86.3%) were diagnosed as having RA and 7 patients (14%) as having RARS. When the 2016 revision of the WHO classification was applied, 9 patients (18%) were diagnosed as having MDS with single lineage dysplasia (MDS-SLD), 34 patients (66%) as having MDS with multilineage dysplasia (MDS-MLD) and 5 patients (10%) as having therapy-related myeloid neoplasm, and 3 patients (6%) were classified as "others." [10] Forty-four patients (86%) were classified into low (n = 8, 8%) or intermediate-1 (Int-1) (n = 36, 78%) risk groups, while the remaining patients (n = 7, 14%) were classified into intermediate-2 (Int-2) based on the IPSS risk classification.[11] In addition, 20 patients (39%) were classified as low risk, 21 patients (41%) as intermediate risk and 10 patients (20%) as higher risk, based on the IPSS-R risk classification.[12] According to the MD Anderson Cancer Center (MDACC) lower-risk scoring system[13], in 44 patients (IPSS low and Int-1), 2 patients were classified into category 1/low risk (5%), and 26 patients (59%) and 16 patients (36%) were classified into category 2/intermediate risk and 3/high risk, respectively, suggesting that most patients had intermediate-risk or high-risk disease. Twenty-one patients (41%) were dependent on RBC transfusion, and 7 patients (14%) were dependent on platelet transfusion. Only 2 patients (4%) required both types of transfusion.

TABLE 1 Patient characteristics

Number of patients	51
Median age (range)	75 (51-88)
Gender (M/F)	30/21
FAB classification: Number of patients (%)	
RA	44 (86)
RARS	7 (14)
WHO classification: Number of patients (%)	
MDS-SLD	9 (18)
MDS-MLD	34 (66)
t-MN	5 (10)
Others	3 (6)
Karyotypes (IPSS): Number of patients (%)	
Good	28 (55)
Intermediate	11 (22)
Poor	12 (23)
IPSS: Number of patients (%)	
Low	8 (16)
Int-1	36 (70)
Int-2	7 (14)
IPSS-R: Number of patients (%)	
Low	20 (39)
Intermediate	21 (41)
High, very high	10 (20)
MDACC LR-MDS score: Number of patients (%)	
Category 1/low	2 (5)
Category 2/intermediate	26 (59)
Category 3/high	16 (36)
All transfusion dependency: Number of patients (%)	26 (50.9)
RBC transfusion-dependent	21 (41.1)
PLT transfusion-dependent	7 (13.7)
RBC and PLT transfusion-dependent	2 (3.9)

FAB, French-American-British; Int-1(2), Intermediate-1(2); IPSS, International Prognostic Scoring System; IPSS-R, The Revised International Prognostic Scoring System; MDACC, MD Anderson Cancer Center; MDS-MLD, MDS with multilineage dysplasia; MDS-SLD, MDS with single lineage dysplasia; PLT, platelets; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RBC, red blood cell; t-MN, therapy-related myeloid neoplasms.

3.2 | Treatment outcomes

Among 51 patients enrolled in this study, 45 patients (88.2%) received AZA-5 for more than 4 cycles. The reasons for treatment discontinuation before the 4 cycles were disease progression (n = 2) and adverse events (n = 4). A number of patients required decreasing and delayed azacitidine administration: 6/51 (11.8%) or 26/51 (51.0%), respectively.

As shown in Table 2, HI was observed in 24/51 patients (47.1%), including HI-E (15/40, 37.5%), HI-P (17/33, 51.5%) and HI-N (6/19, 31.5%). CR was achieved in 6 patients (21.6%) and mCR in 5 patients

TABLE 2 Therapeutic response

Hematological improvement	
Any HI	24/51 (47.1%)
HI-E	15/40 (37.5%)
HI-P	17/33 (51.5%)
HI-N	6/19 (31.5%)
Hematological remission	
CR	6/51 (11.8%)
Marrow CR	5/51 (9.8%)
Transfusion independency	
RBC	10/21 (47.6%)
PLT	1/7 (14.3%)

Hematological improvement (HI) was evaluated by International Working Group 2006 response criteria after 4 cycles of AZA-5 treatment.
CR, complete recovery; PC, platelet concentration; RBC, red blood cell concentration.

(9.8%). All patients who achieved CR had shown cytopenia in more than 2 lineages at baseline based on the IPSS criteria.[7] Transfusion independence was seen in 10/21 RBC-dependent patients (47.6%) and 1/7 platelet-dependent patients (14.3%), respectively.

Hematological improvement rates for IPSS and IPSS-R risk groups are shown in Table 3A. Of IPSS risk groups, 5/8 (62.5%) in Low, 18/36 (50%) in Int-1 and 2/7 (28.6%) in Int-2 achieved HI (HI rate: Low + Int-1 vs Int-2, $P = .4247$), indicating that the efficacy of AZA-5 is independent of IPSS risk groups. In addition, among IPSS-R risk groups, 10/20 (50%) in Low, 12/21 (57%) in Intermediate, 1/6 (16.7%) in High, and 1/4 (25%) in Very high groups achieved HI (HI rate: Low + Int vs High + very High, $P = .0805$). Although there was a tendency that AZA-5 was more effective for the Low/Int group than for High/very High groups based on IPSS-R, this difference was not statistically significant. HI rates according to karyotypes based on IPSS or IPSS-R are shown in Table 3B. The HI rates in good, intermediate and poor karyotypes based on IPSS were 16/28 (57.1%), 4/11 (36.4%) and 4/12 (33.3%), respectively (HI rates: Good vs Int + Poor, $P = .1602$).

Similarly, the HI rates in good, intermediate, poor and very poor karyotypes based on IPSS-R were 16/29 (55.2%), 4/11 (36.4%), 2/3 (66.7%) and 2/8 (25%), respectively (HI rates: Good vs Int + Poor + Very poor, $P = .2588$). These results indicate that the efficacy of AZA-5 was observed independently of karyotypes based on IPSS or IPSS-R. Furthermore, the HI rates in categories 1, 2 and 3 based on MDACC LR-MDS score were 1/2 (50%), 13/26 (50%) and 8/16 (50%), respectively.

3.3 | WT1 messenger RNA (mRNA) expression in peripheral blood

Among 21 patients with normal WT1 mRNA expression (≤50 copies/μg of RNA), 10 patients (47.6%) obtained HI, while 1 patient (4.8%) experienced disease progression. In contrast, of 28 patients with WT1 mRNA and more than 50 copies/μg of RNA, 12 patients (42.9%) achieved HI, while 6 patients (21.4%) showed disease progression. There wano significant difference between WT1 mRNA levels before treatment and responses to AZA-5 ($P = .3734$).

We also compared WT1 mRNA levels before and after AZA-5 therapy between responders and nonresponders. Among 22 responders, WT1 mRNA levels increased in 6 patients (22%) regardless of their responses (Figure 1). There was no significant difference in the change of WT1 mRNA levels between responders and nonresponders ($P = .0819$) (Figure 1). These results indicate that WT1 mRNA levels are neither useful to predict nor to evaluate the responses to Aza-5 in MDS patients with RA or RARS.

3.4 | Hematological and nonhematological toxicity

The most common toxicity was hematologic toxicity. As shown in Table 4, neutropenia of grade 3 was observed in 9 patients (17.6%) and of grade 4 in 17 patients (33.3%). Grade 3 and 4 thrombocytopenia occurred in 3 (5.9%) and 8 (15.7%) patients, respectively. Grade 3 anemia occurred in 8 of 51 patients (15.7%). None of the patients dropped out the study due to hematologic toxicities. Nine patients developed grade 3 nonhematological toxicities: febrile neutropenia (FN) in 3 and pneumonia, diverticulitis, renal insufficiency, cerebral infarction, Sweet's syndrome and heart failure in 1 patient. Although FN, pneumonia and diverticulitis were considered to be related with AZA-5, renal insufficiency, cerebral infarction, Sweet's syndrome and heart failure were judged

TABLE 3 Subgroup analysis of hematological responder rate in IPSS and IPSS-R risk group

IPSS				IPSS-R		
Low	**Int-1**	**Int-2**	**Low**	**Int**	**High**	**Very high**
(A) Risk group						
5/8 (62.5%)	18/36 (50%)	2/7 (28.6%)	10/20 (50%)	12/21 (57%)	1/6 (16.7%)	1/4 (25%)
	$P = .4247$			$P = .0805$		
Good	**Int**	**Poor**	**Good**	**Int**	**Poor**	**Very poor**
(B) Karyotypes						
16/28 (57.1%)	4/11 (36.4%)	4/12 (33.3%)	16/29 (55.2%)	4/11 (36.4%)	2/3 (66.7%)	2/8 (25%)
	$P = .1602$			$P = .2588$		

The therapeutic response was evaluated by International Working Group 2006 response criteria. (A) According to risk group and (B) according to karyotype in IPSS and IPSS-R. Dichotomous variables were compared between different groups using the Fisher's exact test. Int, intermediate.

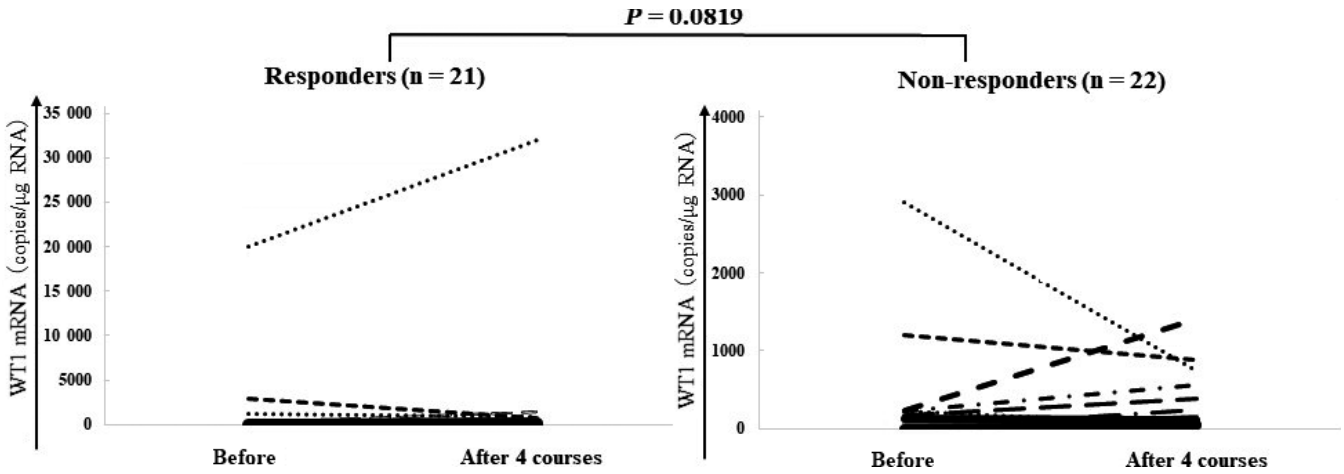

FIGURE 1 Correlation between WT1 mRNA levels and sensitivity to AZA treatment. The differences in change rate of WT1 mRNA levels between AZA responders and nonresponders. Dichotomous variables were compared between 2 groups using the Wilcoxson test. CR, complete remission; HI, hematological improvement; PLT, platelets; RBC, red blood cells

TABLE 4 Reported grade 3 and 4 adverse events

	Grade 1-2	Grade 3	Grade 4
Hematological			
Neutropenia	4	9	17
Hemoglobin decreased	4	8	
Thrombocytopenia	8	3	8
DIC	1		
Nonhematological			
Febrile neutropenia		3	
Pneumonia		1	
Diverticulum		1	
Acute kidney injury	4	1	
Cerebral infarction		1	
Sweet's syndrome		1	
Heart failure		1	
Injection site reaction	5		
Malaise	1		
Purpura	1		
Constipation	1		
Nausea	1		
Urticaria	1		
Fever	1		
Oral hemorrhage	1		
ALT increased	1		
Edema limbs	1		
Skin infection	1		
Mental disturbance	1		

The adverse events were classified according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0

to be unrelated with AZA-5. As a result, 4 patients discontinued AZA-5 treatment before 4 cycles were completed due to nonhematological toxicities.

4 | Discussion

In this prospective trial, we evaluated the safety and efficacy of AZA-5 for untreated MDS patients with lower-risk MDS (RA and RARS based on the FAB classification). A total of 45/51 (88.2%) patients had 4 courses of therapy. The most common toxicities were hematological toxicities. However, they were all manageable and no patients dropped out of the study due to hematological toxicities.

Hypomethylating agents, including AZA, improve survival in patients with higher-risk MDS[2] but are less well-studied in lower-risk patients. In 2011 when this trial started, darbepoetin (DPO), which may be effective for anemic lower-risk MDS patients,[14] was not approved in Japan. Therefore, there were no patients treated with DPO at entry in our trial.

The HI and HR rates in the present study were 47.1% and 21.6%, respectively, which are similar to results of the previous phase I/II study of AZA in Japan[3] (AZA-7 in Japan) conducted for all-risk MDS patients. In the AZA-7 study in Japan, the HI rates in lower-risk MDS patients were 57.9% (11/19) for RA and RARS, and 60.9% (14/23) for intermediate-1 of IPSS. In our study, the HI rates were 5/8 for low (62.5%) and 18/36 (50%) for intermediate-1. This result indicates that AZA-5 is not inferior to AZA-7 in lower-risk MDS patients. Furthermore, in the AZA-7 study, two-thirds of the lower-risk MDS patients who were blood transfusion-dependent at baseline became transfusion independent during the study period. Compared with the results of the AZA-7 study, the amelioration rates were rather low in our study (for RBC 10/21 [47.6%] and for PC 1/7 [14.3%], respectively). Recently, a study of a 3-day administration regimen of azacitidine (AZA-3) in lower-risk MDS reported HI and HR rates of 49% and 25%, respectively,[15] which are similar to the results in the present study. However, the transfusion independency rate was 16%, which is inferior to that in the present study. These findings together demonstrate that AZA treatment not only reduces the risk of infection and hemorrhage due to cytopenia, but also improves quality of life by eliminating the need for blood transfusions in lower-risk MDS

patients. Furthermore, these data suggest that the hematological and cytogenetical response would be obtained with low-dose azacitidine, but that the improvement of transfusion dependency might be related to the azcitidine dose. Although treatment with AZA-5 may be suitable for transfusion-dependent (RBC and/or PC) patients with a history of thrombosis or hypertension who are not indicated for DPO, further study is necessary to determine the appropriate dose of azacitidine in lower-risk MDS.

As shown in Table 3, 7 patients were Int-2 according to IPSS risk classification, and 10 patients were in the higher-risk (High and Very high) group based on IPSS-R. However, subgroup analyses according to IPSS classification (Low + Int-1 vs Int-2, $P = .4247$) or IPSS-R classification (Low + Int vs High + very High, $P = .0805$) showed no marked difference in response to AZA-5 between these risk groups. Similarly, using the MDACC score, there was no significant difference in the HI rate among 3 categories (data not shown). Furthermore, response rates to AZA-5 were hardly affected by poor karyotypes based on IPSS (Good vs Int + Poor, $P = .1602$) or IPSS-R (Good vs Int + Poor + Very Poor, $P = .2588$). These results indicate that AZA-7 is superior to AZA-5 in lower-risk MDS with poor karyotype based on IPSS or IPSS-R[16]

In addition to our study, a prospective phase 2 study using AZA-5 for erythropoietin-unresponsive patients with lower-risk MDS has already been reported.[5] The overall response rate was 15/32 (47%), which was similar to the HI rate observed in our study. This study reported that some patients completing 8 cycles obtained better response than those with 4 cycles. In contrast, we planned the 4 cycle AZA-5 study in the present study according to the phase 1/2 AZA-7 study in Japan.[3] Further study to determine the appropriate treatment duration for lower-risk MDS is also necessary.

It has been reported that WT1 mRNA expression can be a useful marker for diagnosis and risk evaluation of MDS.[17,18] Therefore, we measured WT1 mRNA expression levels to evaluate disease progression during AZA treatment. However, because there was no correlation between WT1 mRNA expression levels before treatment and therapy responses in the present study, WT1 may not be useful as a prediction marker for AZA response.

Recently, in a multicenter retrospective cohort of patients with non-del(5q) lower-risk MDS treated with erythropoietin-stimulating agents (ESA), none of the commonly used second-line treatments (HMA and lenalidomide) significantly improved OS. Early failure of ESA was associated with a higher risk of AML progression.[19] These results indicate the benefit of early treatment with AZA for lower-risk MDS.

In conclusion, AZA-5 was effective in a substantial proportion of lower-risk MDS patients. In addition, toxicities of AZA-5 were well tolerated and clinically manageable. These results indicate that AZA-5 is a promising therapeutic option for lower-risk MDS.

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CONFLICT OF INTEREST

I. Matsumura received research funding from Nippon Shinyaku Pharmaceutical Co., Ltd. All other authors declare no conflict of interest.

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