Impact of Nagasaki Atomic Bomb Exposure on Myelodysplastic Syndrome Patients Who Are Treated with Azacitidine

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Abstract. *Background/Aim: High-dose radiation exposure greatly increases the risk of myelodysplastic syndromes (MDS), however the clinical characteristics of MDS among atomic bomb survivors have not been thoroughly investigated to date. We designed this study to identify these characteristics. Patients and Methods: We retrospectively evaluated data from 13 atomic bomb survivors with MDS and 15 elderly patients with de novo MDS who were diagnosed between April 2011 and April 2013 at the Nagasaki Genbaku Hospital. All patients were treated with azacitidine (AZA; a hypomethylating agent) and overall survival rates were estimated. Results: No clear difference was observed in the clinical response to AZA between the two groups. However, atomic bomb survivors had a survival disadvantage, independent of their karyotype. Conclusion: Minute genetic alterations caused by exposure to atomic radiation can adversely affect the response to AZA, even 66 years after the exposure. Further studies are required to clarify the mechanisms underlying this phenomenon.*

Myelodysplastic syndromes (MDS) are a group of malignant clonal hematological stem cell disorders that are characterized by the presence of cytopenia. In addition, patients with MDS are at a high risk of progressing to acute myeloid leukemia (AML) (1). MDS may arise *de novo* after chemotherapy and/or radiation therapy for other cancers or rarely as a result of environmental exposures. The pathogenesis of MDS remains elusive, although the current models explaining the mechanisms leading to MDS and, eventually to AML, describe a complex multi-step process. The initial genetic insult to the hematopoietic stem cells may be caused by chemicals, radiation, cytotoxic drugs or random endogenous mutations and the accumulation of several genetic alterations that affect cell-cycle control, transcription or tumor suppressor genes may eventually result in the expansion of an MDS clone.

Ionizing radiation is known to be carcinogenic in humans and numerous studies have assessed the effect of ionizing radiation of various doses on the development of cancer. Based on a results, it has been established that numerous solid cancers, including oesophageal, stomach, liver, lung, breast and thyroid cancers (2-4), as well as leukemias (5), occur more frequently after exposure to radiation. For example, exposure to high-dose radiation from an atomic bomb (A-bomb) explosion or nuclear reactor accident is known to greatly increase the risk of developing MDS for at least 45 years after the acute exposure; therefore, this exposure also increases the relative risk of AML mortality (6, 7). However, the clinical characteristics of A-bomb-related MDS have not been thoroughly investigated and an effective treatment is lacking for these patients.

Azacitidine (AZA) is a DNA hypomethylating agent that was recently approved in Japan as the first therapeutic agent for treating patients with MDS. One multi-centre study found that AZA increased the survival of patients with MDS and prevented the progression of MDS to AML (8) and a similar increased survival would be expected in high-risk Japanese patients with MDS (9). Therefore, considering the current lack of data, we conducted this retrospective cohort study to investigate the efficacy of AZA in A-bomb survivors with MDS (AB-MDS).

Patients and Methods

Patients. Thirteen patients with AB-MDS and 15 elderly patients with *de novo* MDS (A-bomb-naïve) were diagnosed at the Nagasaki Genbaku Hospital between April 2011 and April 2013 and included in this study. The ethical review board of our institution approved this retrospective analysis, which was conducted according to the 1975 Declaration of Helsinki and its revision in 1983; informed consent was obtained from each patient. A-bomb survivors were defined as patients who were recognized by the Japanese government for surviving the Nagasaki attack. MDS was diagnosed according to the World Health Organization criteria (10) and subclassified according to the International Prognostic

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Key Words: Atomic bomb, high-dose radiation exposure, myelodysplastic syndrome, azacitidine, hypomethylating agents.

Table I. *Clinical and biological characteristics of the myelodysplastic syndrome patients (N=28).*

	Total	Atomic bomb survivor		*p*-Value
	(N=28)	Yes (N=13)	No (N=15)	
Sex, male/female	17/11	9/4	8/7	0.460
Age (years)	70.5 (61-87)	74 (68-84)	71 (61-87)	0.188
WHO classification				
RCMD	16 (57)	7 (54)	9 (60)	0.259
RAEB-1	7 (25)	2 (15)	5 (33)	
RAEB-2	5 (17)	4 (31)	1 (6)	
IPSS				
Intermediate-1	10 (35)	5 (38)	5 (33)	1.00
Intermediate-2	14 (50)	6 (46)	8 (53)	
High	4 (14)	2 (15)	2 (13)	
IPSS-R				
Very low	4 (14)	2 (15)	2 (13)	0.707
Low	4 (14)	3 (23)	1 (6)	
Intermediate	2 (7)	0 (0)	2 (13)	
High	11 (39)	5 (38)	6 (40)	
Very High	7 (25)	3 (23)	4 (26)	
Karyotype risk				
Good+intermediate	16 (57)	10 (77)	6 (40)	0.067
Poor	12 (42)	3 (23)	9 (60)	
Transfusion dependent	22 (78)	10 (77)	12 (80)	1.00
Baseline blood counts				
Hemoglobin (g/dL)	8.25 (6.4-13.4)	9.0 (6.4-10.9)	8.2 (6.6-13.4)	0.369
ANC (×109/L)	0.87 (0.04-12)	0.8 (0.04-2.9)	1.0 (0.08-12)	0.259
Platelets (×109/L)	6.5 (0.4-33)	6.4 (0.4-30)	6.6 (1.8-33)	0.980
Number of azacitidine cycles	4.5 (1-21)	4 (1-19)	8 (1-21)	0.227
Days of follow-up from initiation of treatment	389 (91-1022)	(91-1009)	(141-1009)	0.205

Data are presented as number (%) or median (range). WHO, World Health Organization; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, refractory anemia with excess blasts; IPSS, international prognostic scoring system; IPSS-R, revised international prognostic scoring system; ANC, absolute neutrophil count.

Scoring System (IPSS) (11) and the revised IPSS (12). Moreover, the karyotype risk was analyzed according to the IPSS.

Treatment. AZA was administered intravenously at a dose of 75 mg/m^2/day in 28-day cycles. For the first cycle, AZA was first administered for 7 days in the hospital, whereas AZA was administered for 5 days in an outpatient unit for all subsequent cycles. At the beginning of each cycle, the dose of AZA could be adjusted (either delayed or decreased) based on the hematological laboratory test results, such as nadir counts and the occurrence of Grade 3/4 non-hematologic toxicity. The response to AZA was evaluated based on blood counts and bone marrow aspirates. Complete remission (CR), partial remission (PR), marrow CR and stable disease with hematological improvement (HI; the correction of one or more cytopenias in the absence of CR or PR) were defined according to the International Working Group 2006 criteria (13). Safety data were recorded during each cycle and classified according to the National Cancer Institute Common Terminology Criteria version 4.0 (Ref).

Table II. *Response to azacitidine according to the International Working Group 2006 criteria.*

	Total (%)	Atomic bomb survivor		*p*-Value
	(N=28)	Yes (N=13)	No (N=15)	
Overall response	13 (46)	5 (38)	8 (53)	0.476
CR	9 (27)	2 (15)	7 (35)	0.289
mCR	2 (6)	1 (8)	1 (5)	
PR	2 (6)	2 (15)	0 (0)	
SD	20 (61)	8 (62)	12 (60)	
Hematological improvement				
Any–HI	11/26 (42)	3/13 (23)	8/13 (61)	0.111
HI–E	10/25 (40)	2/13 (15)	8/12 (66)	0.015
HI–N	2/14 (14)	0/8 (0)	2/6 (33)	0.165
HI–P	4/18 (22)	1/9 (11)	3/9 (33)	0.576

Data are presented as number (%). CR, Complete remission; mCR, marrow complete remission; PR, partial remission; SD, stable disease; HI, hematologic improvement, E, erythroid; N, neutrophil; P, platelet.

Statistical analysis. The Kaplan–Meier method was used to estimate patient survival, while the difference in survival between the two groups was analyzed using the Gehan-Breslow-Wilcoxon test. Using Cox regression models, multivariate analyses were used to estimate the effects of age at exposure, distance at exposure, higher IPSS score, transfusion dependency, poor-risk karyotype and any response to AZA in the A-bomb survivor group. Similar multivariate analyses were used to estimate the effect of exposure to the A-bomb, age, higher IPSS score, transfusion dependency, higher IPSS score, poor-risk karyotype and any response to AZA among all patients with MDS. All analyses were performed using the EZR software (14).

Results

Patients' characteristics. The patients' characteristics are shown in Table I. The characteristics of the AB-MDS and *de novo* MDS groups were not significantly different. No specific chromosomal abnormalities were detected in the AB-MDS group.

Responses and outcomes for AZA therapy. The hematological and overall responses to AZA therapy are shown in Table II. The overall response did not differ significantly between the

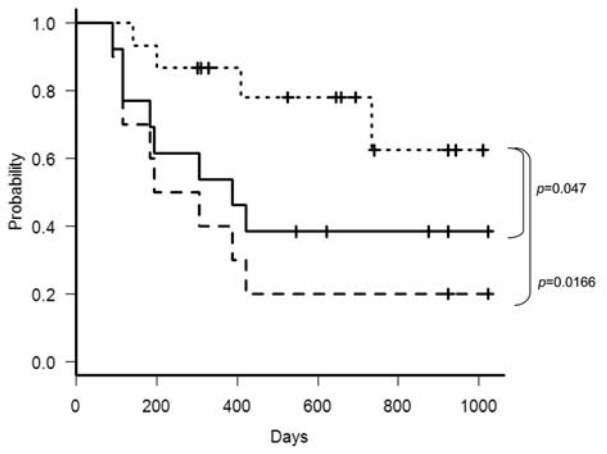

Figure 1. *Overall survival probability for Nagasaki atomic bomb survivors with myelodysplastic syndrome (AB-MDS) compared to that of elderly patients with de novo MDS. Patients with AB-MDS who were exposed at an age of >10 years, continuous line; patients with AB-MDS who were exposed at an age of <10 years, dashed line; patients with de novo MDS, dotted line.*

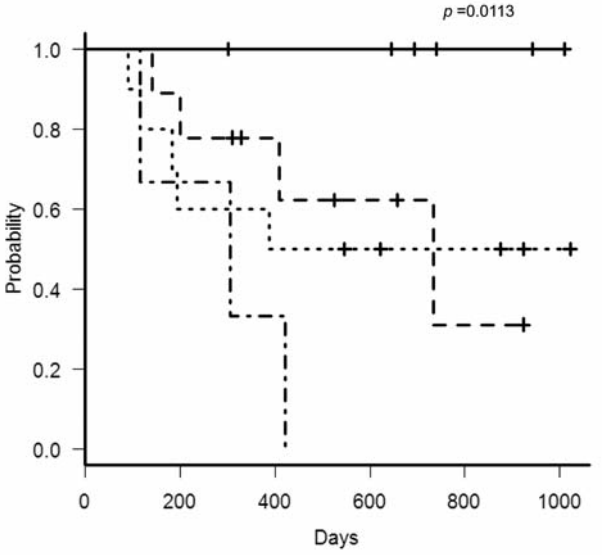

Figure 2. *Overall survival probability for Nagasaki atomic bomb survivors with myelodysplastic syndrome (AB-MDS) compared to that of elderly patients with de novo MDS, stratified according to karyotype risk. Patients with de novo MDS and without the poor-risk karyotype, continuous line; patients with de novo MDS and the poor-risk karyotype, dashed line; patients with AB-MDS and without the poor-risk karyotype, dotted line; patients with AB-MDS and the poor-risk karyotype, dash-dotted line.*

AB-MDS and *de novo* MDS groups. However, all HIs tended to be inferior in the AB-MDS group, although only erythroid HI exhibited a statistically significant difference.

The overall survival (OS) was significantly shorter in the AB-MDS than that in the *de novo* MDS group, especially in the subgroup of patients who were exposed before the age of 10 (Figure 1).

In the multivariate analysis, A-bomb exposure (hazard ratio (HR)=4.842; 95% confidence interval (CI)=1.340-17.49; p=0.016) and poor-risk karyotype (HR=3.796; 95% CI=1.089-13.23; p=0.036) were found to be significant independent risk factors for OS. Furthermore, when the survival was stratified according to the poor-risk karyotype, a significant difference was still observed between the groups (p=0.011, Figure 2).

Safety outcomes. The Grade 3/4 hematologic adverse events (AEs) and all Grade AEs that occurred in ≥10% of patients are shown in Table III. Regarding the Grade 3/4 hematologic AEs, 67%, 53% and 35% of patients experienced neutropenia, thrombocytopenia and anaemia, respectively. The incidence of the hematologic AEs was comparable between the AB-MDS and *de novo* MDS groups.

Grade 3/4 non-hematologic AEs occurred in <10% of the patients. Common non-hematologic AEs included fatigue, anorexia, pyrexia, oedema, constipation, diarrhoea and rash; the incidence of non-hematologic AEs was comparable between the AB-MDS and *de novo* MDS groups. However, Grade 1/2 increases in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (γ-GTP) were observed more frequently in the AB-MDS group compared to in the *de novo* MDS group, although these differences did not reach statistical significance.

Discussion

It has been well-established that exposure to A-bomb radiation is associated with the occurrence of several malignancies, including MDS (6). However, little is currently known regarding the effectiveness of various anticancer agents for A-bomb-related malignancies. To the best of our knowledge, the present study is the first to examine the response to AZA therapy in AB-MDS patients.

In the present study, we found that exposure to A-bomb radiation adversely affected hematological improvement and outcomes for AZA therapy in MDS patients, especially in A-bomb survivors who were exposed to radiation at a younger age (<10 years old). Generally, survivors who are exposed to radiation at a younger age are more likely to develop cancer (6, 15). In addition, the hazard ratio for A-bomb radiation exposure in our multivariate analysis was comparable to that associated with the poor-risk karyotype. Therefore, it appears that AB-MDS shares clinical characteristics with therapy-related MDS (tMDS), which is caused by artificial mutagenic exposure for treatment purposes, including therapeutic

Table III. *Adverse events occurring in at least 10% of the patients administered azacitidine.*

	Total	Atomic bomb survivor	
	(N=28)	Yes (N=13)	No (N=15)
Hematologic			
Leukocytopenia	19 (67)	9 (69)	10 (66)
Neutropenia	19 (67)	9 (69)	10 (66)
Thrombocytopenia	15 (53)	7 (54)	8 (53)
Anemia	10 (35)	5 (38)	4 (26)
Non-hematologic			
Fatigue	25 (89)	10 (77)	15 (100)
Anorexia	18 (64)	10 (77)	8 (53)
Pyrexia	17 (60)	9 (69)	8 (53)
Edema	18 (64)	9 (69)	9 (60)
Constipation	16 (57)	8 (62)	8 (53)
Diarrhea	14 (50)	6 (46)	8 (53)
Rash	14 (50)	8 (62)	6 (40)
Dizziness	12 (42)	3 (23)	9 (60)
Mucosa	10 (35)	4 (31)	6 (40)
Itching	9 (32)	2 (15)	7 (46)
Headache	8 (28)	2 (15)	6 (40)
Vomiting	6 (21)	2 (15)	4 (26)
Nausea	6 (21)	1 (8)	5 (33)
AST increase	3 (10)	3 (23)	0 (0)
ALT increase	3 (10)	3 (23)	0 (0)
γ-GTP increase	3 (10)	3 (23)	0 (0)

Data are presented as number (%). AST, Aspartate aminotransferase; ALT, alanine aminotransferase; γ-GPT, gamma-glutamyl transpeptidase.

radiation exposure *via* radiotherapy aimed at treating other cancers. However, although a previous study of tMDS reported a worse prognosis for patients with tMDS compared to that for patients with *de novo* MDS, only the poor-risk karyotype remained a significant risk factor for poor prognosis in the multivariate analysis (16). Hence, in tMDS, the mutation spectrum in MDS cells may be more similar to that in *de novo* MDS cells, rather than in AB-MDS cells.

In contrast to tMDS, A-bomb exposure itself was found to be a risk factor for prognosis in the present study and this effect was independent of the presence or absence of the poor-risk karyotype. Furthermore, we observed a tendency towards a lower ratio of the poor-risk karyotype in the patients with AB-MDS (Table I). Therefore, our results suggest that there may exist additional mutations in the genes that determine the outcome of AZA therapy in these patients, other than the poor-risk karyotype. A-bomb radiation is characterized by immediate, high-intensity, full-body radiation that includes neutron radiation; thus, we speculate that the non-clastogenic genomic alterations caused by this unique radiation may be associated with the poor response to AZA therapy observed in the patients with AB-MDS.

A high incidence of point-mutations in the *AML1/RUNX1* (17) and p53 (18) genes has previously been reported in patients with nuclear weapon-related radiation-associated MDS, although the influence of these mutations on the effectiveness of AZA therapy, if any, remains unclear. Moreover, the precise mechanisms for AZA therapy in MDS have yet to be elucidated. Therefore, comprehensive genomic analysis of AB-MDS, including application of next-generation sequencing technology, may provide clues to the mechanisms for AZA therapy in MDS and help identify novel prognostic markers for AZA therapy.

The MDS incidence rate is significantly and inversely related to the exposure distance from the hypocentre of an atomic bomb explosion (6) and there may be a relationship between the exposure distance from the hypocentre and the treatment responses in A-bomb survivors. In the present study, we did not observe a difference in this distance when the living and deceased A-bomb survivors were compared, although the number of cases analyzed in the study may not be sufficient to evaluate this relationship.

Lastly, high-dose radiation exposure may damage both hematopoietic stem cells and the stem cells of other organs. In this study, there were no differences in the baseline characteristics, including liver and renal functions, between the AB-MDS and *de novo* MDS groups (data not shown). However, AEs related to liver function occurred more frequently in the AB-MDS group. Specifically, increased AST, ALT and γ-GTP levels were only observed in the AB-MDS group, although these increases were not statistically significant. Based on these findings, we speculate that it is possible that there is latent liver function weakness in A-bomb survivors.

In summary, our findings provide initial data demonstrating that the OS for survivors of the Nagasaki atomic bomb who have MDS is significantly shorter than that for A-bomb-naïve MDS patients. Most of the previous studies have examined the frequency of cancer occurrence according to age and radiation. Thus, to our knowledge, this is the first study to report a decline in survival rate after treatment with AZA for MDS in A-bomb survivors. Although these results suggest that exposure to an atomic explosion may play a role in the response to treatment, even >66 years after the exposure, further studies are required to elucidate the mechanism(s) of this phenomenon.

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Conflicts of Interest

The Authors report no conflicts of interest related to this manuscript.

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