Leukemia Research Reports 15 (2021) 100231

Contents lists available at ScienceDirect

Leukemia Research Reports

journal homepage: www.elsevier.com/locate/lrr

Prognostic impact of peripheral blood *Wilms' tumour 1* mRNA expression levels in response to azacytidine in MDS: A single-centre analysis

Tomoya Maeda, Methodology;Investigation [a,*], Akira Matsuda [a], Chie Asou [a], Daisuke Okamura [a], Ken Tanae [a], Mika Kohri [a], Maho Ishikawa [a], Naoki Takahashi [a], Kunihiro Tsukasaki [a], Nobutaka Kawai [a], Norio Asou [a], Masami Bessho [a,b]

[a] *Department of Haemato-Oncology, Saitama International Medical Centre, Saitama Medical University, Hidaka, Saitama, Japan*
[b] *Department of Haematology, Saitama Medical University Hospital, Saitama Medical University, Iruma-gun, Saitama, Japan*

ARTICLE INFO

Keywords:
Myelodysplastic Syndromes
Wilms' tumour 1
WT1
azacytidine
hypomethylating agent

ABSTRACT

To determine the impact of peripheral blood (PB) *Wilms' tumour 1* (*WT-1*) mRNA levels in patients with primary myelodysplastic syndromes (MDS), we analysed the relationships between several clinical variables at the time of diagnosis and the haematological response of patients treated with azacytidine. We observed overall responses in 20 (63%) patients; there were no significant differences in clinical variables, including bone marrow blast counts, IPSS scores and IPSS-R risk scores, between responders and non-responders. The responders' PB *WT-1* mRNA levels were significantly lower than those of non-responders ($P = 0.03$). PB *WT-1* mRNA expression could be a marker for predicting the response to azacytidine in patients with *de novo* MDS.

1. Introduction

Myelodysplastic syndromes (MDS) are heterogeneous disorders characterised by cytopenia with dysplasia and a propensity to progress to acute myeloid leukaemia [1, 2]. Several prognostic scoring systems for MDS have been reported, including the International Prognostic Scoring System (IPSS), the World Health Organisation (WHO) Prognostic Scoring System (WPSS) and the revised IPSS (IPSS-R) [3–5]. Azacytidine, a hypomethylating agent, has been demonstrated to induce responses, delay leukaemic transformation and improve survival in higher-risk MDS. Because haematological response rates are not as high in patients treated with azacytidine, it is important to identify patients with MDS who respond to azacytidine. However, the predictors of haematologic response in patients with MDS taking azacytidine have yet to be fully determined.

The survival of patients with MDS has been associated with the expression of several genes, including *LEF1, CDH1, Wilms' tumour 1* gene (*WT-1*) and *MN1*, and the expression levels of *WT-1* were higher in patients with MDS with poor survival [6]. Although *WT-1* was initially identified as a tumour suppressor gene in patients with Wilms' tumour, recent results indicated that *WT-1* acts as an oncogene in various solid tumours and haematological malignancies [7]. *WT-1* levels in bone marrow (BM) could be useful for predicting the survival of patients with myeloid neoplasms treated with azacytidine [8]. *WT-1* expression levels in peripheral blood (PB) have proven useful for assessing the risk in patients with MDS [9]. PB sampling has multiple distinct benefits over BM sampling such as more frequent PB collection from the same patient than BM. *WT-1* levels in PB reflect the disease progression of patients with MDS treated with hypomethylating agents [10]. However, the relationship between *WT-1* levels in PB and the response to azacytidine treatment is unclear; moreover, the prognostic role of PB *WT-1* levels in primary MDS has not been fully established.

To obtain a more complete insight into the prognostic value of PB *WT-1* levels in primary MDS, we analysed the relationships between several clinical variables (including *WT-1* mRNA expression levels) at the time of diagnosis (baseline) and the haematological response (best response) of patients with MDS to azacytidine and elucidated the impact of this response on their overall survival.

2. Materials and methods

2.1. Patients

Patients with MDS (according to the French-American-British classification) who were referred to Saitama International Medical Centre (Saitama Medical University, Saitama, Japan) between July 2011 and

* Corresponding author. ORCID Number: 0000-0002-3107-5991
E-mail address: maedat@saitama-med.ac.jp (T. Maeda).

https://doi.org/10.1016/j.lrr.2020.100231
Received 19 September 2020; Received in revised form 22 November 2020; Accepted 6 December 2020
Available online 8 December 2020

June 2019 were included in this study. All chemotherapy-naïve patients who were diagnosed with primary MDS were enrolled. We excluded all patients with disorders other than primary MDS (e.g., therapy-related myeloid neoplasm, chronic myelomonocytic leukaemia, low BM blast count [20%–30% blasts] acute myeloid leukaemia). Of these patients, one patient with MDS and myelofibrosis was successfully treated with azacytidine [11].

2.2. Wilms' tumour 1 gene mRNA measurement method

We extracted total RNA from PB nucleated cells and measured the amount of *WT-1* mRNA by a real-time quantitative reverse transcription polymerase chain reaction assay using a *WT-1* mRNA Assay Kit (Otsuka Pharmaceutical Co., Tokyo, Japan) [8].

2.3. Treatment schedule

Azacytidine was subcutaneously or intravenously administered at a daily dose of 75 mg/m^2 for five or seven consecutive days every four weeks. The azacytidine administration route and dosing schedule were selected at the treating physician's discretion based on the patients' bleeding tendency, feasibility for drug administration and Eastern Cooperative Oncology Group (ECOG) performance status score. In the azacytidine 001 study and the Cancer and Leukaemia Group B (CALGB) 9221 study, azacytidine-dosing cycles could be delayed and/or modified by 7–14 days as required because of haematologic toxicity until the patients had haematologically recovered [12, 13]. The treatment schedule for this study was performed in accordance with those of azacytidine-001 and CALGB 9221 studies. Azacytidine treatment was continued until disease progression or the onset of unacceptable adverse events. Patients were then allowed to receive additional supportive care, including antifungal prophylaxis and standard antibiotics, as per our division's policy. Granulocyte-colony stimulating factor was used for life-threatening infections.

2.4. Response criteria

We conducted response assessment using the 2006 International Working Group (IWG) response criteria for MDS [14], a modified version of previously published standardized IWG MDS response criteria [15]. Each patient's haematologic response was evaluated after each cycle. Overall response was defined as a complete response (CR), marrow CR, partial response or any haematologic improvement (HI).

2.5. Statistical analysis

We performed statistical comparisons using the nonparametric Mann–Whitney U test for continuous data and compared patient characteristics with the chi-squared test (or Fisher's exact test when the expected values <5). We then analysed survival times using the Kaplan–Meier method and statistically compared the prognosis using the log-rank test. Overall survival was defined as the time from the date of initial diagnosis to that of death as a result of any cause, haematopoietic stem cell transplantation (HSCT), or the last contact for surviving patients. A P-value of <0.05 was considered to be significant. To explain the relationship between the azacytidine therapeutic response and *WT-1* levels in PB, we plotted receiver operating characteristic (ROC) curves for each parameter using the nearest point to the top-left corner of the plot as the cut-off value. We then evaluated the associations between *WT-1* levels and myeloblast ratios in BM using Spearman's rank correlation test. All statistical analyses were performed using EZR version 1.40 (Saitama Medical Centre, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria). Importantly, EZR is a modified version of R Commander designed to frequently add statistical functions used in biostatistics [16].

3. Results

3.1. Patient characteristics

In this study, a total of 32 patients were included, and their baseline characteristics before azacytidine treatment are summarised in Table 1. The median age was 71 years (range, 31–85 years). Moreover, 30 (94%) of the included patients had an ECOG performance status of 0–1. The diagnoses were MDS with multilineage dysplasia, MDS with excess blasts type 1 (MDS-EB-1), MDS with excess blasts type 2 (MDS-EB-2) and unclassifiable MDS in 4, 8, 18 and 1 patient, respectively. One case could not be diagnosed as per the WHO classification because of an unevaluated blast ratio as a result of hypocellular BM with fibrosis. MDS with fibrosis is not recognised as a distinct entity in WHO classification. Among the included patients, 12 displayed normal cytogenetics (38%), two exhibited complex cytogenetics including monosomy 7, eight had complex cytogenetics excluding monosomy 7 and 10 had other chromosomal abnormalities. Accordingly, the IPSS cytogenetic risk score was "good", "intermediate" and "poor" for 14 (44%), 8 (25%) and 10 patients (31%), respectively. We calculated the IPSS score as per the criteria described [3]. Furthermore, 24 patients (75%) were classified as intermediate-2 or high risk in the IPSS risk group, and eight patients (25%) were classified as intermediate-1 risk. There were no patients with low risk.

3.2. Treatment response

The median number of cycles of azacytidine treatment was five (range, 1–87 cycles). We observed an overall response in 20 of 32 patients (63%) who were administered azacytidine (Table 1). Seven patients (22%) achieved a complete response, including a BM complete response, and 19 patients (59%) achieved HI. Seven patients (22%) achieved HI in platelets (HI-P), HI in erythrocytes (HI-E) and HI in neutrophils (HI-N). Two patients (6%) achieved HI-P and HI-E, one (3%) achieved HI-P and HI-N, three (9%) achieved HI-E and HI-N, and six (19%) achieved HI-E. The median follow-up period after starting the first cycle of azacytidine was 15.1 months (range, 2.5–99.0 months). Six patients (19%) underwent allogeneic HSCT after a median of five cycles of azacytidine (range, 2–12 cycles). The median number of azacytidine cycles to the first response and to the best response was two (range, 1–11) and four (range, 2–20) cycles, respectively. Note that 18 (90%) of the 20 patients who had a first response did so in four cycles, while the other two patients achieved a response at cycles 7 and 11, respectively. In these early responders (defined as a first response within four cycles), continued azacytidine therapy improved response category in 13 of the patients (72%). For seven of the 20 responders (35%), the first response was the best response. Interestingly, in this study, two of the seven were late responders and there were no other response profiles.

3.3. Differences in clinical data between responders and non-responders

Table 1 shows the comparison of clinical data from responders and non-responders. The median number of azacytidine cycles administered to responders and non-responders was eight (range 1–87) and three (range 1–13), respectively. The distribution of applied azacytidine dosing schedules for 5 days or 7 days did not differ between these groups. There were no significant differences in sex, age, performance status, white blood cell counts, absolute neutrophil counts, haemoglobin levels, platelet counts, lactate dehydrogenase levels, the percentage of blasts in BM, IPSS cytogenetic risk, IPSS risk or IPSS-R risk between responders and non-responders.

The *WT-1* mRNA levels in PB in the non-responders were significantly higher than those in the responders ($P = 0.03$). In primary MDS, although the BM blast percentage is one of the strongest prognostic indicators, the rate among responders did not significantly differ between the BM blast 0–9% (73.3%) and BM blast 10–19% groups (52.9%, $P =$

Table 1
Patient characteristics: Responders vs. non-responders to Azacytidine

	Total	Responders	Non-responders	*P*-value*
Patients, n (%)	32	20 (62.5%)	12 (37.5%)	
Females, n (%)	8 (25%)	5 (25%)	3 (25%)	1.00
Age, years	71 (31–85)†	71 (59–83)	73.5 (31–85)	0.51
ECOG performance status, n	1 (0–3)†	1 (0–3)	1 (0–2)	0.42
WBC, $\times 10^9$/L	2.16 (0.61–9.26)†	2.21 (0.61–7.19)	1.89 (0.62–9.26)	0.63
ANC, $\times 10^9$/L	0.64 (0.01–5.37)†	0.67 (0.04–5.14)	0.46 (0.01–5.37)	0.72
Haemoglobin, g/dL	8.0 (4.8–10.9)†	7.9 (4.8–10.8)	8.4 (7.4–10.9)	0.13
Platelets, $\times 10^9$/L	46 (7–342)†	62 (7–342)	32 (12–342)	0.08
LDH, U/L	215 (130–591)†	209 (130–335)	230 (136–591)	0.55
BM blasts, %	10.2 (0.1–18.2)†	9.0 (0.1–18.2)	11.9 (2.8–17.6)	0.76
Patients with <10% marrow blasts, n (%)	15	11 (73.3%)	4 (26.7%)	0.29
Patients with ≥10% marrow blasts, n (%)	17	9 (52.9%)	8 (47.1%)	
WT-1 in PB, copies/μg RNA	2650 (<50–50,000)†	2050 (<50–44,000)	7550 (170–50,000)	0.03
Patients with *WT-1* ≤ 2600 copies/μg RNA, n (%)	17	15 (88.2%)	2 (11.8%)	0.003
Patients with *WT-1* > 2600 copies/μg RNA, n (%)	15	5 (33.3%)	10 (66.7%)	
WHO classification				0.84
MDS-MLD	4 (12.5%)	2 (10.0%)	2 (16.7%)	
MDS-EB-1	8 (25.0%)	6 (30.0%)	2 (16.7%)	
MDS-EB-2	18 (56.3%)	10 (50.0%)	8 (66.7%)	
MDS-U with SLD and pancytopenia	1 (3.1%)	1 (5.0%)	0	
Others (MDS with fibrosis)	1 (3.1%)	1 (5.0%)	0	
IPSS cytogenetic risk				0.24
Good	14 (43.7%)	10 (50.0%)	4 (33.3%)	0.47**
Intermediate	8 (25.0%)	6 (30.0%)	2 (16.7%)	
Poor	10 (31.3%)	4 (20.0%)	6 (50.0%)	
IPSS risk				0.74
Low	0	0	0	
Intermediate-1	8 (25.0%)	6 (27.2%)	2 (16.7%)	
Intermediate-2	15 (46.9%)	9 (40.9%)	6 (50.0%)	
High	9 (28.1%)	5 (22.7%)	4 (33.3%)	
IPSS-R risk				0.22
Very low	0	0	0	
Low	1 (3.1%)	0	1 (8.3%)	
Intermediate	5 (15.6%)	4 (20.0%)	1 (8.3%)	
High	10 (31.3%)	8 (40.0%)	2 (16.7%)	
Very high	16 (50.0%)	8 40.0%)	8 (66.7%)	
Interval from diagnosis to AZA treatment, months	1.3 (0.3–12.0)†	1.4 (0.3–5.0)	1.1 (0.5–12.0)	0.95
AZA schedule 7 days/5 days	21 (66%)/ 11 (34%)	14 (70%)/6 (30%)	7 (58%)/5 (42%)	0.52
Total number of AZA cycles until final observation	5 (1–87)†	8 (1–87)	3 (1–13)	0.004

Abbreviations: ANC, absolute neutrophil count. AZA, azacytidine; BM, bone marrow; EB, excess blasts; ECOG, Eastern Cooperative Oncology Group; IPSS, International Prognostic Scoring System; IPSS-R, Revised International Prognostic Scoring System; LDH, lactate dehydrogenase; MDS, myelodysplastic syndromes; MLD, multilineage dysplasia; ND, not determined; PB, peripheral blood; RNA, ribonucleic acid; U, unclassifiable; SLD, single lineage dysplasia; WBC, white blood cell; WHO, World Health Organisation.

† Median (range)

* Responders vs. non-responders.

** Statistical analysis between good and not good.

0.29). The threshold BM blast value was 10% as per the median value of the upper boundary of 20% blast cells defined as MDS in the WHO classification. We then examined two parameters (*WT-1* mRNA expression levels in PB and the myeloblast ratio in BM) for patients with MDS but identified no correlation between these two parameters ($r = 0.04$, $P = 0.84$; Fig. 1A). We then calculated the cut-off *WT-1* value in PB via ROC analysis and identified an optimal cut-off of 2600 copies/μg RNA for predicting a response to azacytidine treatment in patients with MDS (sensitivity, 75.0%; specificity, 83.3%; Fig. 1B). The responder rate was significantly higher in the group with ≤2600 copies/μg RNA (88.2%) than in the group with >2600 copies/μg RNA (33.3%; $P = 0.003$).

3.4. Overall survival

The median follow-up time for the azacytidine responders was 24.1 months (range, 8.8–99.0 months). There was a significant difference in overall survival between azacytidine responders and non-responders ($P < 0.0001$) (Fig. 2A). By comparing the patients based on *WT-1* mRNA levels, we observed that patients with low *WT-1* levels (≤2600 copies/μg RNA) tended to have better overall survival than those with high *WT-1* levels without reaching significance (>2600 copies /μg RNA, $P = 0.18$; Fig. 2B). The patients with low *WT-1* levels in PB (≤2600 copies/μg RNA) and low blast percentages in BM (0–9%), who we thought would be responders, did not have a significantly better prognosis than those in the other groups (group B vs. groups A, C and D; $P = 0.33$; Fig. 2C, D).

4. Discussion

In clinical practice, the treatment algorithm for higher-risk patients with MDS (corresponding to IPSS intermediate-2 or higher) of the European LeukemiaNet recommendation is extensively employed [17]. The treatment outcomes of our study were comparable to those of previous studies in terms of the overall response rate of 60% and HI of 58%. The CALGB 9221 study and AZA-001 study reported response rates of 60% and ~50%, respectively. The identification of reliable and consistent clinical variables and biomarkers that predict clinical benefits from azacytidine therapy in patients with MDS is highly desirable.

In the azacytidine treatment of patients with MDS, various clinical factors (e.g., ECOG performance score, PB blasts, cytogenetics, transfusion dependence, lactate dehydrogenase levels, *TET2* mutations, *DNMT3A* mutations, *PARP1* mRNA levels, increased platelet counts after the initial administration of azacytidine) have been reported to predict the haematological response and clinical usefulness of azacytidine [18-23]. Using BM samples, Pellagatti et al. demonstrated that the expression of several genes that included *WT-1* was significantly associated with the survival of patients with MDS [6]. These molecular genetic biomarkers can add to existing risk models and refine the prognostic prediction. However, the BM sampling method is more invasive than that of PB and does not allow for more frequent check-ups, which in turn enables close monitoring for better treatment outcomes. Because of their technical limitations and lack of clinical access, these genetic tests using BM samples generally have not been adapted as routine elements of care in clinical settings. Kitamua et al. found that WT-1 mRNA expression levels in BM were favourably correlated with those in PB in patients with AML and MDS using the same assay kit applied in our study [24]. Furthermore, the sample sets obtained multiple times from the same patients during the course of disease indicated that the change in expression levels in BM reflected the disease status. These changes in expression in BM closely related to those in PB. Thus,

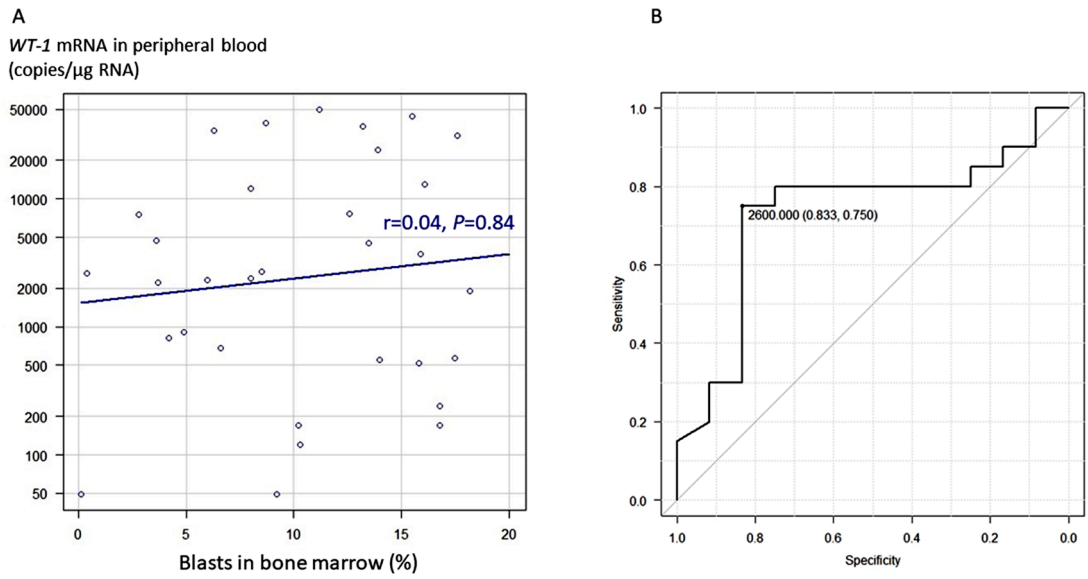

Fig. 1. Peripheral blood *WT-1* mRNA levels and bone marrow (BM) blast ratios in patients with primary MDS treated with azacytidine (n = 32). A, Relationship between peripheral blood *WT-1* mRNA expression levels and BM blast ratios. B, ROC curve of *WT-1* mRNA expression levels in PB and cut-off value (sensitivity, specificity) for predicting azacytidine treatment response in the patients with MDS.

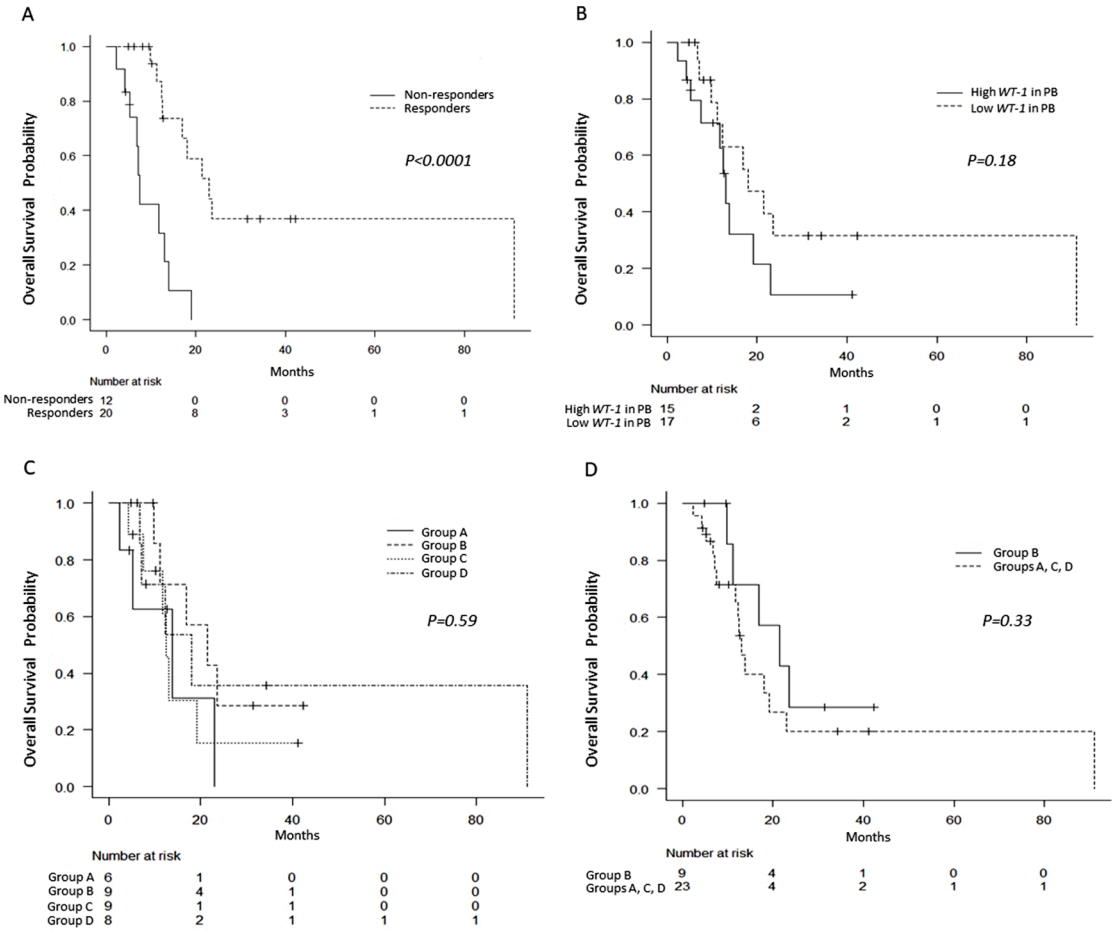

Fig. 2. Kaplan–Meier curves for overall patient survival (n = 32). A, Overall survival by response status to azacytidine. B, Overall survival by baseline *WT-1* status. All patients were divided into four groups (Group A to D cf. Fig. 3: According to *WT-1* in PB and BM blast ratio). C, Overall survival of patients in each group. D, Overall survival of the patients with low *WT-1* levels in PB (≤2600 copies/μg RNA) and low percentage of blasts in BM (0–9%) (Group B cf. Fig. 3.) compared with others.

PB samples could be useful for assessing disease status, particularly in patients with MDS receiving bridging treatment with AZA before HSCT.

In the WHO classification, MDS subtypes are primarily diagnosed by the percentage of BM blasts. In our study, the rate of azacytidine responders tended to be higher in the low BM blast group (<10%) than in the high group (≥10%). We reported that the azacytidine responders had significantly lower *WT-1* mRNA levels in PB than those of non-responders. *WT-1* mRNA levels in PB could be a predictor of response to azacytidine treatment. Using the cut-off value of the ROC analysis, MDS should be divided into high and low *WT-1* mRNA expression levels. As per these two parameters, the patients were therefore divided into four groups. The threshold values for BM blasts and *WT-1* were 10% and 2600 copies/μg RNA, respectively. The low BM blast group was divided into high (Group A) and low *WT-1* (Group B) groups. The high BM blast group was divided into high (Group C) and low *WT-1* (Group D) groups. The response rate was significantly higher in Group B than in Group A (100% vs. 33.3%, $P = 0.01$). The response rate tended to be higher in Group D than in Group C (75.0% vs. 33.3%, $P = 0.15$). The response rate was highest in Group B and lowest in both Groups A and C (Fig. 3). Conclusively, patients classified into Group B should be treated with azacytidine. Moreover, we extended our data to reveal relationships between *WT-1* mRNA levels in PB and IPSS-R risk scores rather than BM blasts. There was no correlation between these two parameters ($r = 0.02$, $P = 0.41$; Fig. S1). Pfeilstöcker et al. reported that a cut-off of 3.5 points in the IPSS-R scoring system was optimal for segregating patients into lower-risk and higher-risk MDS groups [25]. However, almost all patients (97%) in this study had IPSS-R risk scores exceeding 3.5 points. Then, we performed the same analyses using IPSS-R score cut-offs of 4.5, 6.0 and 6.5 points. Using a cut-off of 4.5 points, the response rate was highest in Group B (100%) and lowest in Groups A and C (33.3%; Fig. S2A). This result was similar to that obtained using BM blasts. Conversely, using cut-offs of 6.0 and 6.5 points, the response rates were lower in Group B (83.3 and 84.6%, respectively) than in Group D (both 100%; Fig. S2B, S2C). Comparing the difference between these two parameters (BM blasts and IPSS-R scores), the response rate in Group A tended to be lower using BM blasts (33.3%) than using IPSS-R risk scores (50.0% at cut-offs of 6.0 and 6.5). Overall, it was not more suitable to use IPSS-R scores than BM blasts as the parameter for these analyses regarding the response to azacytidine in the present study.

We reported that the *WT-1* mRNA level in PB was a predictor of response to azacytidine by comparing the characteristics of responders and non-responders. Based on this result, we considered the possibility that *WT-1* mRNA levels in PB could predict the prognosis of patients with MDS treated with azacytidine. There was a significant difference in overall survival between azacytidine responders and non-responders. However, comparing patients based on *WT-1* mRNA expression levels demonstrated that patients with low *WT-1* (≤2600 copies/μg RNA) did not significantly improve but tended have better overall survival than those with high *WT-1* (>2600 copies/μg RNA). In this study, *WT-1* mRNA levels in PB were significantly associated with haematological response but not overall survival in the patients with MDS. This study had certain limitations. The most obvious limitation associated with this survival analysis was its small sample size. Other limitations included the retrospective and single-institution nature of the study, the lack of tests for BM samples and the inclusion of patients who received HSCT, which affected the interpretation of the prognostic and predictive analyses. Consequently, additional study is warranted.

In conclusion, *WT-1* mRNA levels in non-responders were significantly higher than those in responders. Our results suggest that the *WT-1* mRNA expression level in PB could be a simple predictive factor for azacytidine treatment for patients with MDS. Treatment strategies using the *WT-1* mRNA expression level in PB might help improve the prognosis of patients with MDS.

Statements

Ethical approval

This study was approved by the Institutional Review Board of Saitama International Medical Centre, Saitama Medical University (IRB approval no. 14-039).

Funding

This work was supported in part by the Practical Research for Innovative Cancer Control Program from the Japan Agency for Medical Research and Development, AMED (Grant Number JP19ck0106251).

CRediT authorship contribution statement

Tomoya Maeda: Data curation, Writing - original draft. **Akira Matsuda:** Conceptualization, Methodology, Writing - review & editing. **Chie Asou:** Resources. **Daisuke Okamura:** Resources. **Ken Tanae:** Resources. **Mika Kohri:** Resources. **Maho Ishikawa:** Resources, Validation. **Naoki Takahashi:** Resources. **Kunihiro Tsukasaki:** Resources. **Nobutaka Kawai:** Resources. **Norio Asou:** Writing - review & editing,

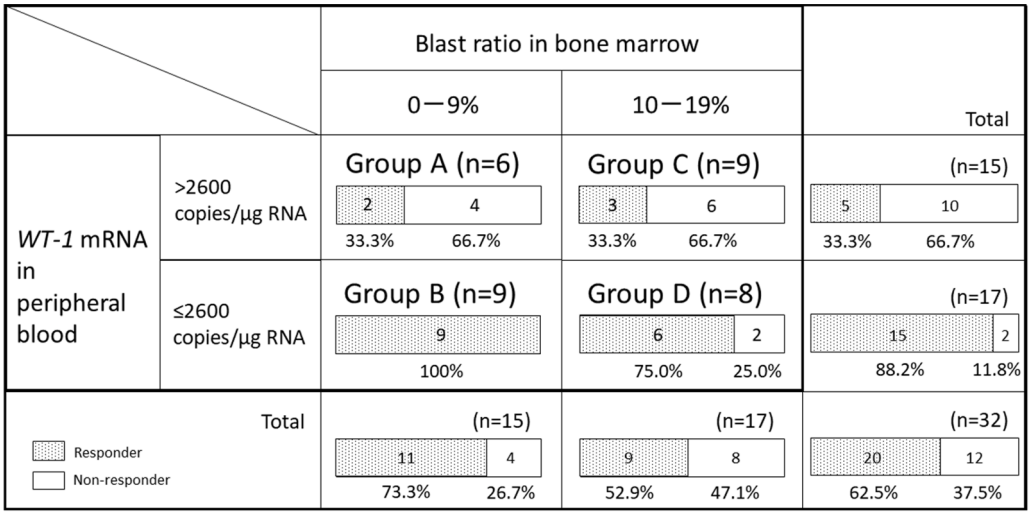

Fig. 3. Number and rates of azacytidine treatment response in patients with MDS. These patients were divided into four groups by their *WT-1* levels in PB and blast ratios in BM. Group B had the most favourable response to azacytidine.

Funding acquisition. **Masami Bessho:** Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Tomoya Maeda has received honoraria from Nippon Shinyaku and advisory boards for Janssen. Akira Matsuda has received honoraria from GlaxoSmithKline, Kyowa Kirin, Nippon Shinyaku, Celgene, Alexion, Sanofi, Beckman Coulter, Siemens Healthineers and Shire Japan. Maho Ishikawa has received honoraria from Bristol-Myers Squibb and Pfizer. Kunihiro Tsukasaki has a consultancy in Daiich-Sankyo, Ono Pharma, and HUYA, has received research funding from Bayer and Celgene, and honoraria from Chugai Pharma, Celgene, Mundy and Kyowa Kirin. Norio Asou has received scholarships from Chugai Pharma, Astellas, Sumitomo Dainippon and Eisai and research funding from Novartis and Otsuka Pharma, has consulted for SRL, Nippon Shinyaku, and Novartis and has received honoraria from Kyowa Kirin, Sumitomo Dainippon, Asahi Kasei and Fuji Pharma. Masami Bessho has received honoraria from Chugai Pharma and GlaxoSmithKline. These sponsors had no control over the interpretation, writing or publication of this study. The other authors have no declaration of conflicts of interest to declare in relation to this manuscript.

Acknowledgement

The authors would like to thank Enago (www.enago.jp) for the English language review.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.lrr.2020.100231.

References

[1] A Tefferi, JW Vardiman, Myelodysplastic syndromes, N. Engl. J. Med. 361 (19) (2009 Nov) 1872–1885, https://doi.org/10.1056/NEJMra0902908.
[2] JW Vardiman, J Thiele, DA Arber, RD Brunning, MJ Borowitz, A Porwit, et al., The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes, Blood 114 (5) (2009) 937–951, https://doi.org/10.1182/blood-2009-03-209262. Jul 30.
[3] P Greenberg, C Cox, MM LeBeau, P Fenaux, P Morel, G Sanz, et al., International scoring system for evaluating prognosis in myelodysplastic syndromes, Blood 89 (6) (1997) 2079–2088. Mar.
[4] L Malcovati, U Germing, A Kuendgen, MG Della Porta, C Pascutto, R Invernizzi, et al., Time-dependent prognostic scoring system for predicting survival and leukemic evolution in myelodysplastic syndromes, J. Clin. Oncol. 25 (23) (2007) 3503–3510, https://doi.org/10.1200/JCO.2006.08.5696. Aug.
[5] PL Greenberg, H Tuechler, J Schanz, G Sanz, G Garcia-Manero, F Solé, et al., Revised international prognostic scoring system for myelodysplastic syndromes, Blood 120 (12) (2012) 2454–2465, https://doi.org/10.1182/blood-2012-03-420489. Sep.
[6] A Pellagatti, A Benner, KI Mills, M Cazzola, A Giagounidis, J Perry, et al., Identification of gene expression-based prognostic markers in the hematopoietic stem cells of patients with myelodysplastic syndromes, J. Clin. Oncol. 31 (28) (2013) 3557–3564, https://doi.org/10.1200/JCO.2012.45.5626. Oct.
[7] A Essafi, ND. Hastie, WT1 the oncogene: a tale of death and HtrA, Mol. Cell. 37 (2) (2010) 153–155, https://doi.org/10.1016/j.molcel.2010.01.010. Jan.
[8] M Santaliestra, A Garrido, M Carricondo, E Bussaglia, M Pratcorona, ML Blanco, et al., Bone marrow WT-1 levels in patients with myeloid neoplasms treated with 5-azacytidine: Identification of responding patients, Eur. J. Haematol. 103 (3) (2019) 208–214, https://doi.org/10.1111/ejh.13275. Sep.
[9] Y Ueda, C Mizutani, Y Nannya, M Kurokawa, S Kobayashi, J Takeuchi, et al., Clinical evaluation of WT-1 mRNA expression levels in peripheral blood and bone marrow in patients with myelodysplastic syndromes, Leuk Lymphoma 54 (7) (2013) 1450–1458, https://doi.org/10.3109/10428194.2012.745074. Jul.
[10] X Du, S Geng, J Weng, C Deng, Z Lu, M Li, et al., WT-1 mRNA level reflects disease changes and progression of myelodysplastic syndromes patients with 'stable disease', Br. J. Haematol. 184 (3) (2019) 447–450, https://doi.org/10.1111/bjh.15098. Feb.
[11] D Okamura, A Matsuda, M Ishikawa, T Maeda, K Tanae, M Kohri, et al., Hematologic improvements in a myelodysplastic syndromes with myelofibrosis (MDS-F) patient treated with azacitidine, Leuk Res. Rep. 3 (1) (2014) 24–27, https://doi.org/10.1016/j.lrr.2014.03.002. Apr.
[12] P Fenaux, GJ Mufti, E Hellstrom-Lindberg, V Santini, C Finelli, A Giagounidis, et al., Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study, Lancet Oncol 10 (3) (2009) 223–232. Mar10.1016/S1470-2045(09)70003-8.
[13] LR Silverman, EP Demakos, BL Peterson, AB Kornblith, JC Holland, R Odchimar-Reissig, et al., Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the Cancer and Leukemia Group B, J. Clin Oncol 20 (10) (2002) 2429–2440, https://doi.org/10.1200/JCO.2002.04.117. May.
[14] BD Cheson, PL Greenberg, JM Bennett, B Lowenberg, PW Wijermans, SD Nimer, et al., Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia, Blood 108 (2) (2006) 419–425, https://doi.org/10.1182/blood-2005-10-4149. Jul.
[15] BD Cheson, JM Bennett, H Kantarjian, A Pinto, CA Schiffer, SD Nimer, et al., Report of an international working group to standardize response criteria for myelodysplastic syndromes, Blood 96 (12) (2000) 3671–3674. Dec.
[16] Y. Kanda, Investigation of the freely available easy-to-use software "EZR" for medical statistics, Bone Marrow Transplant 48 (3) (2013) 452–458, https://doi.org/10.1038/bmt.2012.244. Mar.
[17] L Malcovati, E Hellström-Lindberg, D Bowen, L Adès, J Cermak, C Del Cañizo, et al., Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet, Blood 122 (17) (2013) 2943–2964, https://doi.org/10.1182/blood-2013-03-492884. Oct.
[18] R Itzykson, S Thépot, B Quesnel, F Dreyfus, O Beyne-Rauzy, P Turlure, et al., Prognostic factors for response and overall survival in 282 patients with higher-risk myelodysplastic syndromes treated with AZA, Blood 117 (2) (2011) 403–411, https://doi.org/10.1182/blood-2010-06-289280. Jan.
[19] JH Moon, SN Kim, BW Kang, YS Chae, JG Kim, JH Baek, et al., Predictive value of pretreatment risk group and baseline LDH levels in MDS patients receiving azacitidine treatment, Ann. Hematol 89 (7) (2010) 681–689, https://doi.org/10.1007/s00277-010-0921-5. Jul.
[20] R Itzykson, O Kosmider, T Cluzeau, V Mansat-De Mas, F Dreyfus, O Beyne-Rauzy, et al., Impact of TET2 mutations on response rate to azacitidine in myelodysplastic syndromes and low blast count acute myeloid leukemias, Leukemia 25 (7) (2011) 1147–1152, https://doi.org/10.1038/leu.2011.71. Jul.
[21] F Traina, V Visconte, P Elson, A Tabarroki, AM Jankowska, E Hasrouni, et al., Impact of molecular mutations on treatment response to DNMT inhibitors in myelodysplasia and related neoplasms, Leukemia 28 (1) (2014) 78–87, https://doi.org/10.1038/leu.2013.269. Jan.
[22] PT Diamantopoulos, CN Kontandreopoulou, A Symeonidis, I Kotsianidis, V Pappa, A Galanopoulos, et al., Bone marrow PARP1 mRNA levels predict response to treatment with 5-azacytidine in patients with myelodysplastic syndrome, Ann. Hematol. 98 (6) (2019) 1383–1392, https://doi.org/10.1007/s00277-019-03650-w. Jun.
[23] AM Zeidan, JW Lee, T Prebet, P Greenberg, Z Sun, M Juckett, et al., Platelet count doubling after the first cycle of azacitidine therapy predicts eventual response and survival in patients with myelodysplastic syndromes and oligoblastic acute myeloid leukaemia but does not add to prognostic utility of the revised IPSS, Br. J. Haematol. 167 (1) (2014) 62–68, https://doi.org/10.1111/bjh.13008. Oct.
[24] K Kitamura, T Nishiyama, K Ishiyama, S Miyawaki, K Miyazaki, K Suzuki, et al., Clinical usefulness of WT1 mRNA expression in bone marrow detected by a new WT1 mRNA assay kit for monitoring acute myeloid leukemia: a comparison with expression of WT1 mRNA in peripheral blood, Int. J. Hematol. 103 (1) (2016) 53–62, https://doi.org/10.1007/s12185-015-1882-1. Jan.
[25] M Pfeilstöcker, H Tuechler, G Sanz, J Schanz, G Garcia-Manero, F Solé, et al., Time-dependent changes in mortality and transformation risk in MDS, Blood 128 (7) (2016) 902–910, https://doi.org/10.1182/blood-2016-02-700054. Aug 18.