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Research Article

Peripheral Blood Wilms' Tumor Gene mRNA as a Parameter to Predict Hematological Responses and Prognoses in Patients with Myelodysplastic Syndromes Treated with Azacitidine - a

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ABSTRACT

Azacitidine (AZA), a hypomethylating agent, effectively achieves Hematological Improvements (HI) or improves Overall Survival (OS) in patients with Myelodysplastic Syndromes (MDS). However, predictive factors, particularly hematological markers, of responses to AZA remain largely unknown. We retrospectively analyzed Overall Response Rates (ORR) based on HI and OS in 70 MDS patients who were treated with AZA in our institute. Peripheral Blood Wilms' Tumor Gene (PB-WT1) mRNA levels for each cycle of the treatment were measured in all patients. Although pre-treatment PB-WT1 mRNA levels were not significantly different between responders and non-responders, reductions in PB-WT1 mRNA levels within 3 cycles were identified as an effective predictor for the achievement of HI. Furthermore, PB-WT1 mRNA levels slightly increased a few months before HI were lost during the treatment. PB-WT1 mRNA levels greater than 3000 copies/μg RNA were associated with inferior OS in a univariate analysis. Our results suggest that PB-WT1 mRNA provides useful information on hematological responses, disease progression, and risk assessments in patients being treated with AZA.

Keywords: Azacitidine; Overall Survival; Prognostic Factors; Peripheral Blood Wilms' Tumor Gene m-RNA; Hematological Improvement

INTRODUCTION

Myelodysplastic Syndromes (MDS) are hematopoietic stem cell disorders that are characterized by ineffective hematopoiesis and have a high risk of progressing to Acute Myeloid Leukemia (AML) [1]. Azacitidine (AZA), a pyrimidine analogue, is an antineoplastic agent that acts mainly by causing hypomethylation of cytosine residues in newly replicated DNA and has shown efficacy in the treatment of MDS [2]. Overall survival (OS) was previously reported to be significantly better in high-risk MDS patients treated with AZA than in those receiving conventional treatments [3]. Furthermore, several studies have demonstrated the clinical benefits of AZA in patients with AML [4] and Chronic Myelomonocytic Leukemia (CMML)[5]. Performance Status (PS), cytogenetic risk, transfusion dependence, and Peripheral Blood (PB) blast counts have been identified as prognosis factors for AZA [6]. Platelet doubling after the first AZA treatment has also been shown to influence patient prognoses [7]. However, there is currently no simple biomarker that predicts the clinical courses of patients with such myeloid malignancies who are being treated with AZA.

Wilms' Tumor Gene (WT1) is a useful marker for Minimal Residual Disease (MRD) in AML [8]. PB-WT1 mRNA expression levels, which are strongly associated with the percentage of blasts in Bone Marrow (BM), increase with disease progression in MDS patients [9]. Moreover, WT1 mRNA is a useful independent prognostic marker in elderly patients with MDS [10]. Nevertheless, there has been no report analyzed a significance of WT1 mRNA in a population of AZA-treated cases.

Based on these findings, we herein investigated the clinical significance of WT1 mRNA as a clinical parameter in 70 MDS patients being treated with AZA.

PATIENTS AND METHODS

We retrospectively analyzed 70 MDS patients who were treated with AZA in our institute between 03/2011 and 07/2017. MDS were diagnosed based on cytological examinations of Bone Marrow (BM) smears according to the WHO 2016 criteria. These patients were risk stratified using the Revised International Prognostic Scoring System (IPSS-R) [11]. WT1 mRNA was measured by one step real-time reverse transcription polymerase chain reaction. All patients received AZA subcutaneously or intravenously at a dose of 75 mg/m^2 for seven or five days in each cycle, which was repeated every 28 days. The treatment was continued in all patients until disease progression or a development of severe complications, such as pneumoniae. An unpaired *t*-test was performed to compare PB-WT1 mRNA levels between responders, those who achieved Hematological Improvements (HI) based on the IWG 2006 criteria [12], and non-responders, those who did not achieve HI. A paired *t*-test was performed to examine changes in PB-WT1 mRNA levels before and after a minimum of 3 cycles. Survival curves were estimated by the Kaplan-Meier method and the Log-rank test was used to compare survival curves. OS was measured from the date of the initiation of AZA. Univariate and multivariate analyses were performed with the Log-rank test and Cox's proportional hazards model. Statistical analyses were performed using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) software. Values of $p < 0.05$ were considered significant in all analyses.

RESULTS

Baseline patient characteristics (Table 1)

The median age of patients was 73.5 old years (range 58-85), and 18.6% of patients were women. The diagnosis of MDS based on the WHO criteria was as follows: 20 MDS with Multilineage Dysplasia (MDS-MLD), 27 MDS with Excess Blasts-1 (EB-1), 22 MDS with Excess Blasts-2 (EB-2), and 1 case not determined due to an

Table 1: Baseline patient characteristics based on WHO 2016 criteria.

	***n* = 70**
age(median)	58-85(73.5)
M:F	57:13
BM-Blast(median)	0-19(5.3)
BM-Blast unknown	5
PB-WT1 mRNA(median)	50-82000(2300)
PB-WT1 mRNA unknown	0
karyotype-Very good	1
karyotype-Good	21
karyotype-Intermediate	3
karyotype-Poor	11
karyotype-Very poor	30
karyotype-unknown	4
WHO-MLD	20
WHO-EB-1	27
WHO-EB-2	22
unknown	1
IPSS-R-Very low	0
IPSS-R-Low	2
IPSS-R-Int	8
IPSS-R-High	23
IPSS-R-Very high	33
IPSS-R-unknown	4

unsuccessful BM aspiration. IPSS-R was assessed: 0 Very low, 2 low, 8 Intermediate, 23 High, 33 Very high, and 4 unknown.

PB-WT1 mRNA levels before and during the AZA treatment

Median WT1 mRNA levels were 2300 (range 50-82000) copies/μg RNA. No significant differences were observed in pre-treatment PB-WT1 mRNA levels between responders and non-responders (Figure 1). PB-WT1 mRNA levels significantly decreased within 3 cycles of the AZA treatment in responders who achieved hematological improvement (range pre 50-28000 copies/μg RNA, post 50-18000 copies/μg RNA, $p = 0.00548$), but not in non-responders (range pre 64-82000 copies/μg RNA, post 50-80000 copies/μg RNA, $p = 0.798$) (Figure 2).

PB-WT1 mRNA levels increased in median of 2 (range 1-7) months before HI were lost. Representative case series are shown in Figure 4.

Prognostic factors of OS

49 deaths occurred after a median follow-up of 9 months (range 1-43), and median OS was 10 months. Figure 3 shows OS curves according to karyotype, IPSS-R, PB-WT1 mRNA at pretreatment, achievement of HI, blast percentages in BM.

We selected potential predictors for OS that were suggested to be important in previous studies. In the univariate analysis, BM blasts exceeding 15% ($p = 0.0471$), very poor karyotypes ($p = 0.00124$), no achievement of HI (HI negative, $p = 0.0172$), and PB-WT1 mRNA ≥ 3000 copies/μg RNA ($p = 0.0119$), Very high risk in IPSS-R ($p = 0.000124$) were identified as poor prognostic factors for OS. In the multivariate analysis, HI negative ($p = 0.0008703$) and Very high in IPSS-R ($p = 0.006529$) correlated with shorter survival (Table 2).

DISCUSSION

In the present study, we examined sequential changes in PB-WT1 mRNA levels in patients treated with AZA. Several factors such as BM blast percentages or karyotypes were previously found to be informative for predicting hematological responses [13]. Therefore, repeated BM aspiration is recommended, but impossible in daily clinical settings. Previous studies demonstrated that WT1 mRNA expression levels correlated between PB and BM samples, and PB-

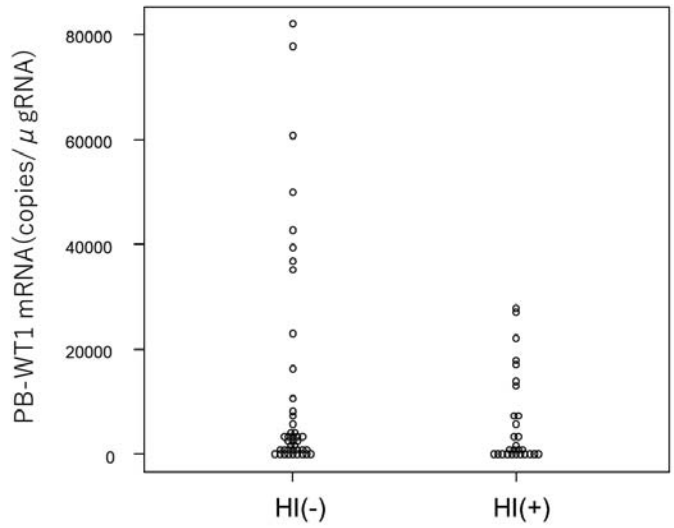

Figure 1: Pre-treatment PB-WT1 mRNA levels with Hematological Improvements (HI) PB-WT1 mRNA levels were not significantly different, $p = 0.128$.

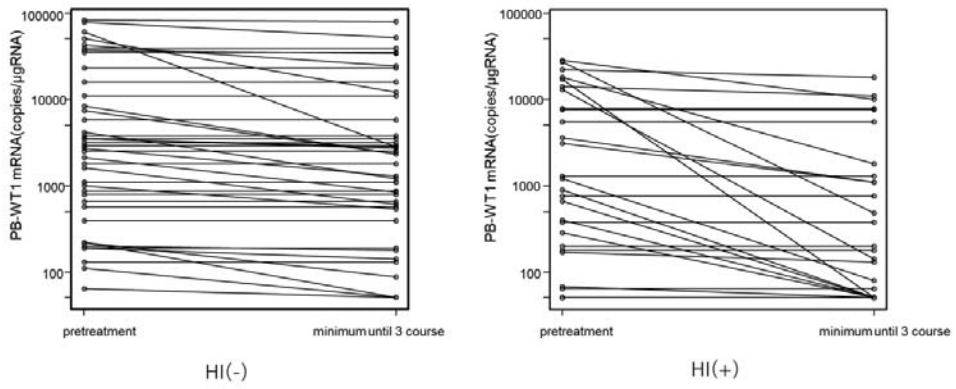

Figure 2: Comparison of PB-WT1 mRNA levels before and after a minimum of 3 cycles.
Hematological Improvement (HI)-positive patients showed significantly decreased PB-WT1 mRNA levels within 3 cycles of AZA ($p = 0.00548$), whereas HI-negative patients did not ($p = 0.798$).

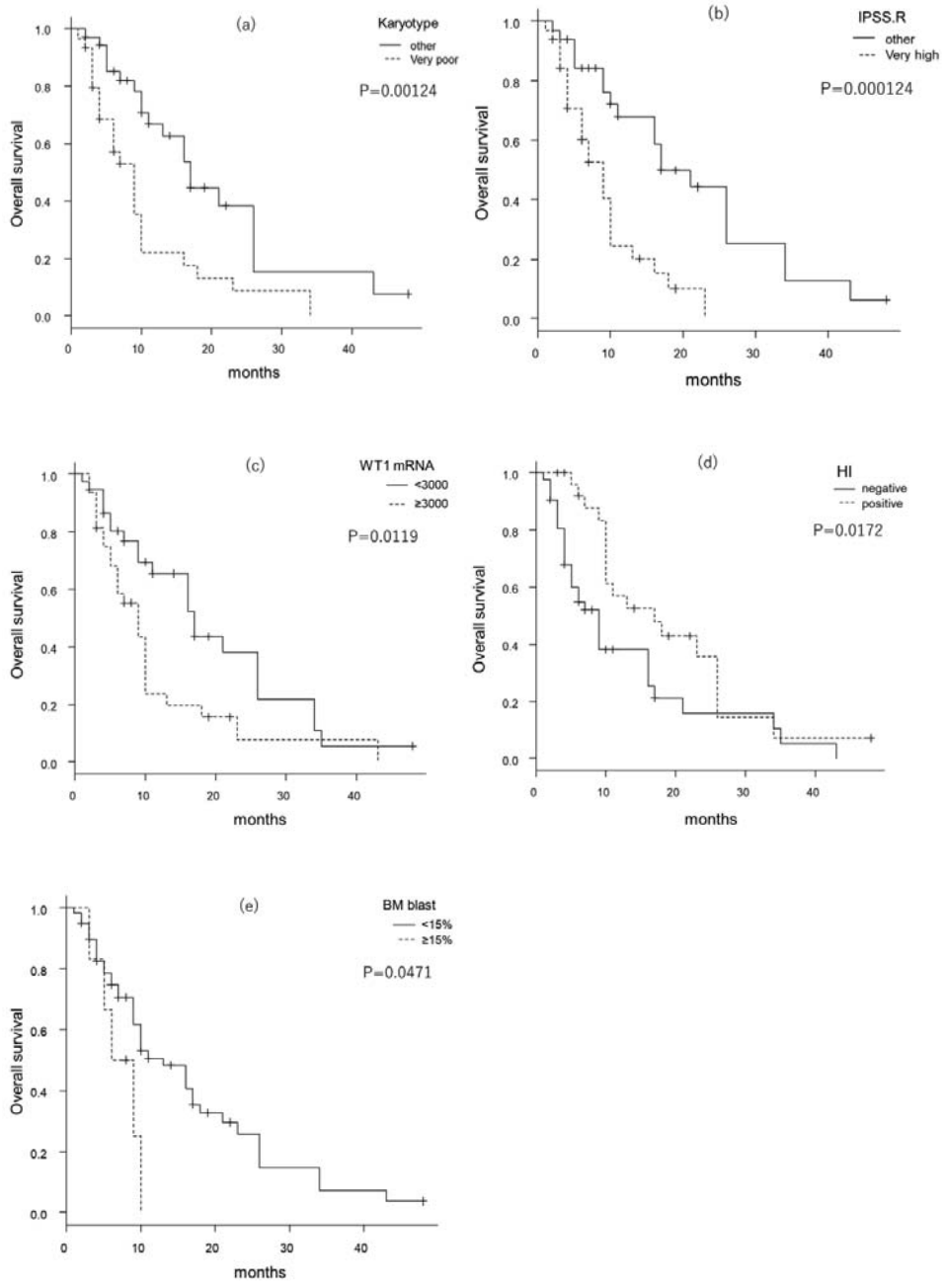

Figure 3: Overall survival according to (a) karyotype (Very poor and other), (b) IPSS-R (Very high and other), (c) PB-WT1 mRNA levels at pretreatment (< 3000 copies/μg RNA and ≥ 3000 copies/μg RNA), (d) achievement of HI, (e) blast percentages in bone marrow (<15% and ≥15%).

WT1 mRNA levels correlated with the percentage of blasts in BM smears from MDS patients [9,14]. Therefore, PB-WT1 mRNA was proposed to be a useful marker for predicting the prognosis of MDS [9,15].

The optimal duration of AZA therapy has not yet been clarified, particularly in patients who did not achieve HI. The AZA-001 study revealed that although almost 90% of HI were obtained within 6 cycles, the delayed appearance of HI was noted in several cases [3]. Previous studies, including ours, found that patients with HI had better OS than those without HI [3,6]. Therefore, it is important to assess decreases in PB-WT1 mRNA levels after early treatment cycles, which may be a useful predictor for the achievement of HI. The results of the present study showed that decreased levels after the AZA treatment, but not pre-treatment levels predicted hematological responses based on the achievement of HI. Nevertheless, the duration of this response only lasts for several months and patients exhibit poor clinical courses once HI is lost. Ommen et al. reported that a median interval from WT-1 positivity to clinical relapse was 44 days when bimonthly peripheral blood sampling was performed in acute myeloid leukemia cases [16]. In the present study, PB-WT1 mRNA levels increased in a median of 2 cycles before the loss of HI. Thereafter, PB-WT1 mRNA levels continued to increase during disease progression. Therefore, we could prepare subsequent treatment options as soon as slight increases were detected in PB-WT1 mRNA levels.

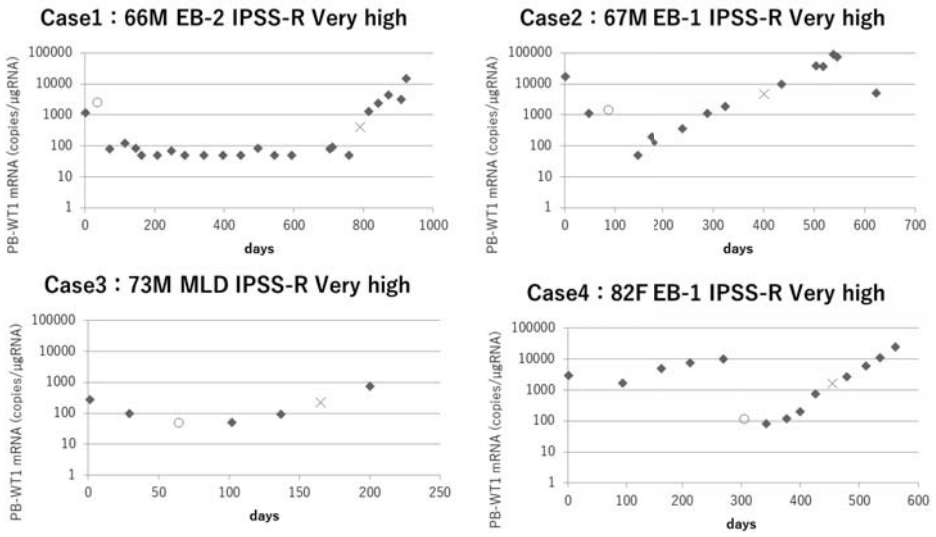

Figure 4: Time course of PB-WT1 mRNA. Representative cases which obtained Hematological Improvements (HI) are shown. The circle means the point of HI and the cross means the point of failure (loss of HI).

Table 2: Prognostic factors for overall survival.

		Univariate analysis		Multivariate analysis	
		Median OS(95% CI)	*p*	HR(95%CI)	*P*
BM-blast	< 15	13.09(9-17)	0.0471	1.483(0.526-4.179)	0.4558
	≥ 15	7.5(3-NA)			
Karyotype	other	17(11-26)	0.00124	1.248(0.576-2.703)	0.5747
	Very poor	9(4-10)			
IPSS-R	other	17(11-26)	0.000124	3.811(1.453-9.995)	0.006529
	Very high	9(6-10)			
Hematological improvement	positive	17(10-26)	0.0172	0.324(0.167-0.629)	0.0008703
	negative	9(5-16)			
PB-WT1 mRNA (Copies/ μg RNA)	< 3000	17(11-26)	0.0119	1.12(0.551-2.280)	0.7532
	≥ 3000	9(5-10)			

Patients with lower pre-treatment levels of WT1 (< 3000 copies/μg RNA) had slightly longer survival durations, while those with high PB-WT1 mRNA levels showed an inferior OS. Tamura et al. have shown that a high WT-1 mRNA level was a strong predictor of OS or a time to AML transformation in MDS patients [17] or Kobayashi et al. have proven that a survival probability of MDS patients could be divided into 3 groups according to their PB WT1 mRNA levels [18]. Nevertheless, these results were obtained before AZA treatment era or treatment options were not limited to AZA, respectively.

Kobayashi et al. furthermore emphasized that patients with a WT1 levels over 10^4 copies/μg RNA in the BM had a poor prognosis and also mentioned that a measurement of BM was superior to that of PB samples. Nevertheless, BM WT1 mRNA measurement was not performed in our institute from a reason that BM sampling is hard to be repeated, therefore is not adequate to pursue a clinical course.

Itzkson et al. reported that besides PS and red blood cell transfusion dependency, cytogenetics and the presence of circulating blasts may independently predict OS in patients with higher-risk MDS including low BM blast counts (21-30%) [6]. A multivariate analysis performed in the AZA-001 study demonstrated that the benefits of AZA were apparent when high-risk MDS patients achieved HI [6]. The present study identified an achievement of HI together with Very high risk in IPSS-R as independent prognostic factors in a multivariate analysis, whereas PB-WT1 mRNA levels had a significant impact on survival, at least in a univariate analysis.

In conclusion, either a sequential change or the pre-treatment level of PB-WT1 mRNA was shown to be important for predicting an achievement or a loss of HI and survival duration in patients treated with AZA. The measurement of PB-WT1 mRNA levels may become more common as a useful marker in the near future.

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