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RESEARCH

WT1 mRNA in peripheral blood enables early prognostic stratification in AML patients receiving venetoclax and azacitidine therapy

Hiroyuki Sugiura[1] · Noboru Asada[2] · Takeru Asano[3] · Yasushi Hiramatsu[3] · Tomohiro Urata[4] · Toshi Imai[4] · Makoto Nakamura[5] · Kazuhiko Yamamoto[5] · Tatsunori Ishikawa[6] · Masanori Makita[6] · Takanori Yoshioka[7] · Kazutaka Sunami[7] · Kyosuke Saeki[8] · Yuichiro Nawa[8] · Maiko Kimura[9] · Makoto Takeuchi[9] · Masaya Abe[2] · Shoji Asakura[10] · Tomofumi Yano[10] · Yoshinobu Maeda[2]

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Abstract

Venetoclax and azacitidine (VEN/AZA), which target BCL-2 and DNA methylation, have demonstrated substantial efficacy, particularly in older or unfit patients with acute myeloid leukemia (AML). *Wilms' Tumor-1* (*WT1*) mRNA, measurable in peripheral blood (PB), is an established biomarker for monitoring treatment response and predicting AML prognosis. We conducted a retrospective analysis of patients with AML treated with VEN/AZA within the Okayama Hematology Study Group (OHSG). Patients who showed a marked reduction in PB *WT1* mRNA levels from baseline after the first or second treatment cycle had significantly improved overall survival (OS) and progression-free survival (PFS). Moreover, achieving PB *WT1* mRNA negativity-regardless of whether this occurred early or later in the therapy | was consistently associated with superior OS and PFS. These findings suggest that PB *WT1* mRNA is a sensitive and reliable biomarker for predicting treatment response and long-term outcomes in patients with AML receiving VEN/AZA.

Keywords *WT1* mRNA · Acute myeloid leukemia · Venetoclax · Azacitidine · Measurable residual disease

Introduction

Venetoclax, a selective B-cell lymphoma 2 (BCL-2) inhibitor, in combination with hypomethylating agents (HMAs), has become a standard regimen for patients with acute myeloid leukemia (AML) who are medically unfit for intensive chemotherapy. This combination venetoclax and azacitidine (VEN/AZA) has demonstrated substantial efficacy in both newly diagnosed and relapsed/refractory (R/R) AML, with high complete remission (CR) rates and improved survival reported in multiple prospective trials [1–5].

Despite these advances, the evaluation of the treatment response during VEN/AZA therapy remains clinically challenging. Bone marrow (BM) assessment is typically recommended 14–21 days after treatment initiation; however, profound myelosuppression and BM hypocellularity often

✉ Noboru Asada
nasada@okayama-u.ac.jp

1 Division of Internal Medicine, Fukuyama City Hospital, Hiroshima, Japan

2 Department of Hematology, Oncology and Respiratory Medicine, Dentistry and Pharmaceutical Sciences, Okayama University Graduate School of Medicine, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan

3 Department of Hematology and Oncology, Japanese Red Cross Society Himeji Hospital, Hyogo, Japan

4 Department of Hematology and Blood Transfusion, Kochi Health Sciences Center, Kochi, Japan

5 Department of Hematology, Okayama City Hospital, Okayama, Japan

6 Department of Hematology, Chugoku Central Hospital of Japan Mutual Aid Association of Public School Teachers, Hiroshima, Japan

7 Department of Hematology, NHO Okayama Medical Center, Okayama, Japan

8 Division of Hematology, Ehime Prefectural Central Hospital, Ehime, Japan

9 Department of Hematology, Japanese Red Cross Okayama Hospital, Okayama, Japan

10 Department of Internal Medicine, Okayama Rosai Hospital, Okayama, Japan

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hinder differentiation between residual disease and therapy-induced cytopenia. Therefore, reliable surrogate biomarkers for early response assessment are needed.

Recent studies have highlighted the prognostic value of measurable residual disease (MRD) monitoring using multiparametric flow cytometry of BM aspirates in patients with AML undergoing VEN/AZA therapy [6]. MRD negativity after treatment was associated with prolonged remission and improved EFS, and overall survival (OS). However, flow cytometry-based MRD assessment requires repeated BM aspirations and may not be feasible in all clinical settings.

The Wilm's tumor gene *WT1* mRNA expression in peripheral blood (PB) is an established tumor marker for monitoring disease status in myelodysplastic syndromes (MDS) and AML. The *WT1* gene, located on chromosome 11p13, encodes a transcriptional regulator with a 52–54 kDa zinc finger domain that modulates the expression of various growth factors and their receptors [7, 8]. Although initially identified as a tumor suppressor gene in pediatric Wilms' tumors, WT1 exerts oncogenic functions in hematopoietic malignancies, including AML [9].

Although MRD monitoring in the context of venetoclax and HMAs combination therapy has gained increasing attention [6, 10–12], the clinical utility of PB *WT1* mRNA kinetics during VEN/AZA therapy-particularly its prognostic and predictive significance | has remained unclear. To address this gap, we conducted a multicenter retrospective analysis of patients with AML treated with VEN/AZA to determine whether dynamic changes in PB *WT1* mRNA could serve as a surrogate marker of treatment response and survival.

Materials and methods

Study design

This retrospective study included 186 AML patients, excluding those with acute promyelocytic leukemia, who received VEN/AZA therapy between March 2021 and December 2022 at institutions affiliated with the Okayama Hematology Study Group (OHSG). Patients were followed until March 31, 2023. Clinical data were retrospectively collected from medical records.

Patients were excluded if they had acute promyelocytic leukemia, lacked at least one *WT1* mRNA measurement before and after VEN/AZA treatment, or had baseline *WT1* mRNA levels below the assay detection limit. Treatment responses were defined according to the 2017 European LeukemiaNet (ELN) guidelines [13]. Standard ELN2017 criteria were applied for CR, CR with incomplete hematologic recovery (CRi), morphologic leukemia-free state (MLFS), and partial remission (PR), whereas trial-specific ELN criteria were used for stable disease (SD) and progressive disease (PD) [13]. This study was approved by the Ethics Committee of Okayama University (No. 2208-012; June 24, 2022).

Dosing regimen

Venetoclax in combination with azacitidine (75 mg/m^2 administered subcutaneously or intravenously for seven consecutive days) was given in 28-day cycles. VEN was administered orally once daily. Dose ramp-up was performed over 3–4 days in accordance with the package insert to mitigate the risk of tumor lysis syndrome. Dose adjustments were made based on the package insert, particularly with the concomitant use of CYP3A-inhibiting antifungal agents. Dose reduction, temporary interruption, and shortening of treatment duration were permitted at the discretion of the treating physician.

WT1 mRNA expression analysis

Peripheral blood leukocyte *WT1* mRNA levels were quantified using the *WT1* mRNA Measurement Kit II "Otsuka" (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan). Total RNA was extracted from PB leukocytes and adjusted to 50 ng/μL prior to analysis.

The assay employed a multiplex one-step RT-qPCR method. A *WT1*-specific reverse primer initiated first-strand cDNA synthesis, followed by PCR amplification in the same reaction vessel. A TaqMan probe bound to the amplified cDNA and was cleaved by the 5'→3' exonuclease activity of the DNA polymerase, releasing fluorescence proportional to *WT1* cDNA levels during each cycle.

GAPDH mRNA was co-amplified as an endogenous control, with *WT1* and *GAPDH* probes labeled with FAM and HEX, respectively, enabling simultaneous duplex detection. Results were expressed as copies/μg RNA normalized to *GAPDH*. The lower limit of detection was 50 copies/μg RNA.

This assay has been reported to have a sensitivity of 86.7% and a specificity of 78.7% for PB samples using a cutoff value of 200 copies/μg RNA. For BM samples, a cutoff value of 1300 copies/μg RNA yields a sensitivity of 80.0% and specificity of 67.1% [14].

Statistical analysis

The Kruskal–Wallis test was used to compare PB *WT1* mRNA levels across response categories. Fisher's exact test was used to compare the CR/CRi rates between patients who achieved a reduction in PB *WT1* mRNA levels and those who did not.

OS and progression-free survival (PFS) were estimated using the Kaplan-Meier method, with group comparisons performed using the log-rank test. Except for receiver

operating characteristic (ROC) curve and multivariate analyses, statistical analyses were conducted using GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). ROC analysis, cutoff determination, and multivariate analyses were performed using R software (The R Foundation for Statistical Computing, Vienna, Austria).

Results

Patient characteristics

A total of 186 patients were included in this study. Among these, one was excluded because WT1 mRNA levels were not measured at any time point before or after VEN/AZA therapy. An additional 11 patients were excluded because their WT1 mRNA levels were below the assay detection limit. Consequently, 12 patients were excluded, and 174 patients were included in the final analysis (Supplementary Fig. S1).

Table 1 Patient characteristics

	Total (n = 174)
Age, median (range), year	74 (20-88)
Sex	
Female	75
Male	99
AML setting	
Newly diagnosed	73 (39.2)
Relapse/Refractory	101(58.0)
AML subtype	
De novo	96 (55.2)
Secondary	58 (33.3)
Therapy related	17 (10.5)
ECOG performance status	
0–1	142 (81.6)
2–3	32 (18.4)
Favorable-risk molecular abnormalities	
RUNX1::RUNX1T1	3 (2.2)
CBFB::MYH11	3 (2.2)
NPM1 mutation	6 (4.3)
AML, NOS	69 (39.7)
AML with MRC	71 (40.8)
AML with RGA	9 (5.2)
AML with mutated NPM1	6 (3.4)
Myeloid sarcoma	1 (0.6)
Therapy-related Myeloid neoplasm	16 (9.2)
Others	3 (1.6)
ELN2017 risk classification	
Favorable	14 (8.3)
Intermediate	83 (49.1)
Adverse	73 (42.0)

Abbreviation: *AML* acute myeloid leukemia, *AML NOS* acute myeloid leukemia not otherwise specified, *AML with MRC* acute myeloid leukemia with myelodysplasia related changes, *AML with RGA* acute myeloid leukemia with recurrent genetic abnormalities

Baseline characteristics were summarized in Table 1. The median age was 74 years (range. 20–88 years). More than half of the patients were treated for R/R AML ($n = 101$, 58.0%), 58 (33.3%) had secondary AML, and 17 (10.5%) had therapy-related AML. Eastern Cooperative Oncology Group (ECOG) performance status (PS) was 0–1 in 142 patients (81.6%) and 2–3 in 32 patients (18.4%). According to the fourth World Health Organization (WHO) classification, 69 patients (39.7%) had AML, not otherwise specified (NOS) and 72 (40.8%) had AML with myelodysplasia-related changes (AML-MRC). Based on ELN 2017 risk stratification, 8.3% of patients were classified as favorable risk, 49.1% as intermediate risk, and 42.0% as adverse risk. Core-binding factor AML (*RUNX1::RUNX1T1* and *CBFB::MYH11* rearrangements) was identified in three patients (2.2%), and *NPM1* mutations were detected in six patients (4.3%).

Treatment characteristics

Treatment details are summarized in Tables 2 and 3, and responses after the first cycle, second cycle, and at best response are shown in Fig. 1. Therapeutic efficacy improved with successive treatment cycles (Fig. 1A).

The best responses were CR in 71 (43.3%) patients, CRi in 35 (21.3%), MLFS in 8 (4.9%), PR in 16 (9.8%), SD in 14 (8.5%), and PD in 20 (12.2%); 106 patients (64.6%) achieved CR/CRi (Fig. 1B).

Best response was evaluated in 164 of 174 patients; however, the reasons for the lack of evaluation in the remaining

Table 2 Number of VEN/AZA cycles to response

WT1 mRNA reduction	Non-CR/CRi ($n = 59$)	CR/CRi ($n = 106$)	*P* value
≥1-log reduction after the first cycle	8 (16.3)	56 (56.0)	<0.001
≥2-log reduction after the first cycle	2 (4.1)	33 (33.0)	<0.001
≥1-log reduction after the second cycle	7 (25.9)	55 (64.0)	0.001
≥2-log reduction after the second cycle	2 (7.4)	40 (46.5)	0.001

Abbreviation: *VEN/AZA* Venetoclax and azacitidine combination therapy

Table 3 Best response during VEN/AZA therapy

	Total ($n = 164$)
Best response	
CR	71 (43.3)
CRi	35 (21.3)
MLFS	8 (4.9)
PR	16 (9.8)
SD	14 (8.5)
PD	20 (12.2)

Abbreviation: *CR* complete remission, *CRi* complete remission with incomplete blood count recovery, *MLFS* morphologic leukemia-free status, *PR* partial response, *SD* stable disease, *PD* progressive disease

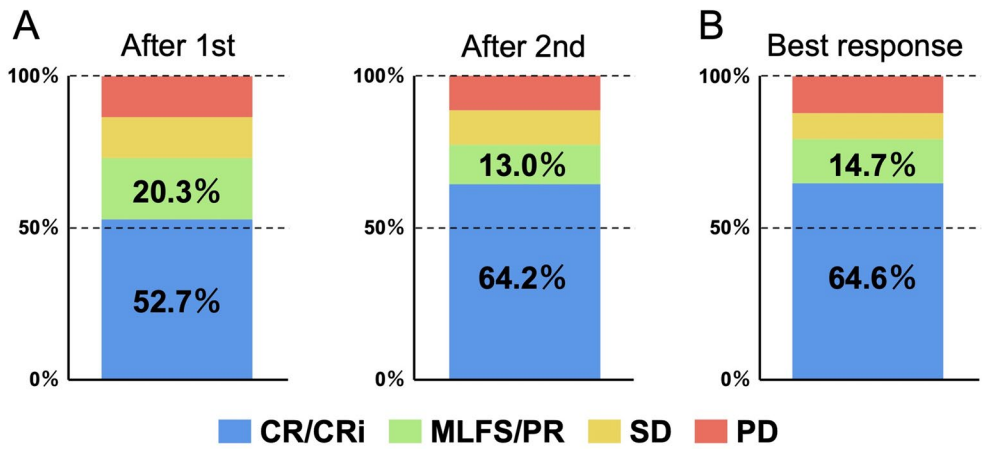

Fig. 1 Treatment responses to venetoclax plus azacitidine (VEN/AZA) therapy. (**A**) Treatment responses after the first cycle, second cycle, and at best response during VEN/AZA therapy. Therapeutic efficacy appeared to improve with successive treatment cycles. (**B**) Best responses achieved during VEN/AZA therapy. Complete remission (CR) was observed in 71 patients (43.3%), complete remission with incomplete blood count recovery (CRi) in 35 (21.3%), morphologic leukemia-free state (MLFS) in 8 (4.9%), partial response (PR) in 16 (9.8%), stable disease (SD) in 14 (8.5%), and progressive disease (PD) in 20 (12.2%). Overall, 106 patients (64.6%) achieved either CR or CRi. The median number of treatment cycles administered was four, with a median of one cycle from treatment initiation to best response and two cycles from best response to treatment discontinuation

10 patients were unavailable. The median number of treatment cycles was four. The median time from treatment initiation to best response was one cycle, and the median time from best response to treatment discontinuation was two cycles.

WT1 mRNA kinetics as a marker of therapeutic efficacy

Baseline PB *WT1* mRNA levels did not differ significantly among best response groups (Fig. 2A).Similarly, OS and PFS did not differ significantly between patients with high and low baseline *WT1* mRNA levels (cutoff: median value of 1400 copies/µgRNA; Supplementary Fig. S2).

After one cycle of VEN/AZA, PB *WT1* mRNA levels were significantly lower in patients who achieved CR than in those who achieved PR, SD, or PD (Fig. 2B). Patients who achieved CRi also exhibited significantly lower *WT1* mRNA levels than those with PD (Fig. 2B). Notably, patients with high *WT1* mRNA levels after the first cycle (cutoff: 580 copies/µgRNA) had significantly shorter OS and PFS (Fig. 2C and D).

After two cycles, PB *WT1* mRNA levels remained significantly lower in patients achieved CR or CRi than in those with PR or SD (Fig. 2E**)**. Patients with high *WT1* mRNA levels (cutoff: 210 copies/µgRNA) exhibited significantly worse OS and PFS (Fig. 2F and G).

Longitudinal analyses by best-response category showed that PB *WT1* mRNA levels consistently declined after both the first and second cycles in patients who achieved CR, CRi, or MLFS (Supplementary Fig. S3). In contrast, *WT1* mRNA levels increased after the second cycle in patients with PR or SD and increased as early as the first cycle in patients with PD (Supplementary Fig. S3).

Prognostic impact of *WT1* mRNA reduction during VEN/AZA treatment

After the first and second cycles of VEN/AZA therapy, a significantly higher proportion of patients who achieved a≥1-log or ≥2-log reduction in PB *WT1* mRNA levels ultimately attained CR or CRi as their best response (Table 4).

Consistent with previous reports showing that a≥1-log reduction in *WT1* mRNA levels after the second cycle of VEN/AZA is associated with favorable survival, we performed a similar analysis in our cohort. Consistent with earlier findings, a≥1-log reduction in *WT1* mRNA levels after the second cycle was significantly correlated with improved OS and PFS (Figs. 3A–D).

Furthermore, a≥2-log reduction after the first cycle was also associated with significantly better OS and PFS (Figs. 3E–H). In both univariate and multivariate analyses of OS and PFS, a≥2-log reduction in *WT1* mRNA after the second cycle emerged as an independent prognostic factor for OS and PFS (Tables 5 and 6).

ROC analysis demonstrated the predictive value of PB *WT1* mRNA levels after the first and second cycles of for achieving CR or CRi (Supplementary Figure S4). Optimal cutoff values were <2350 copies/µgRNA after the first cycle and <890 copies/µgRNA after the second cycle.

Collectively, these findings indicated that PB *WT1* mRNA is a early indicator of therapeutic efficacy of VEN/AZA.

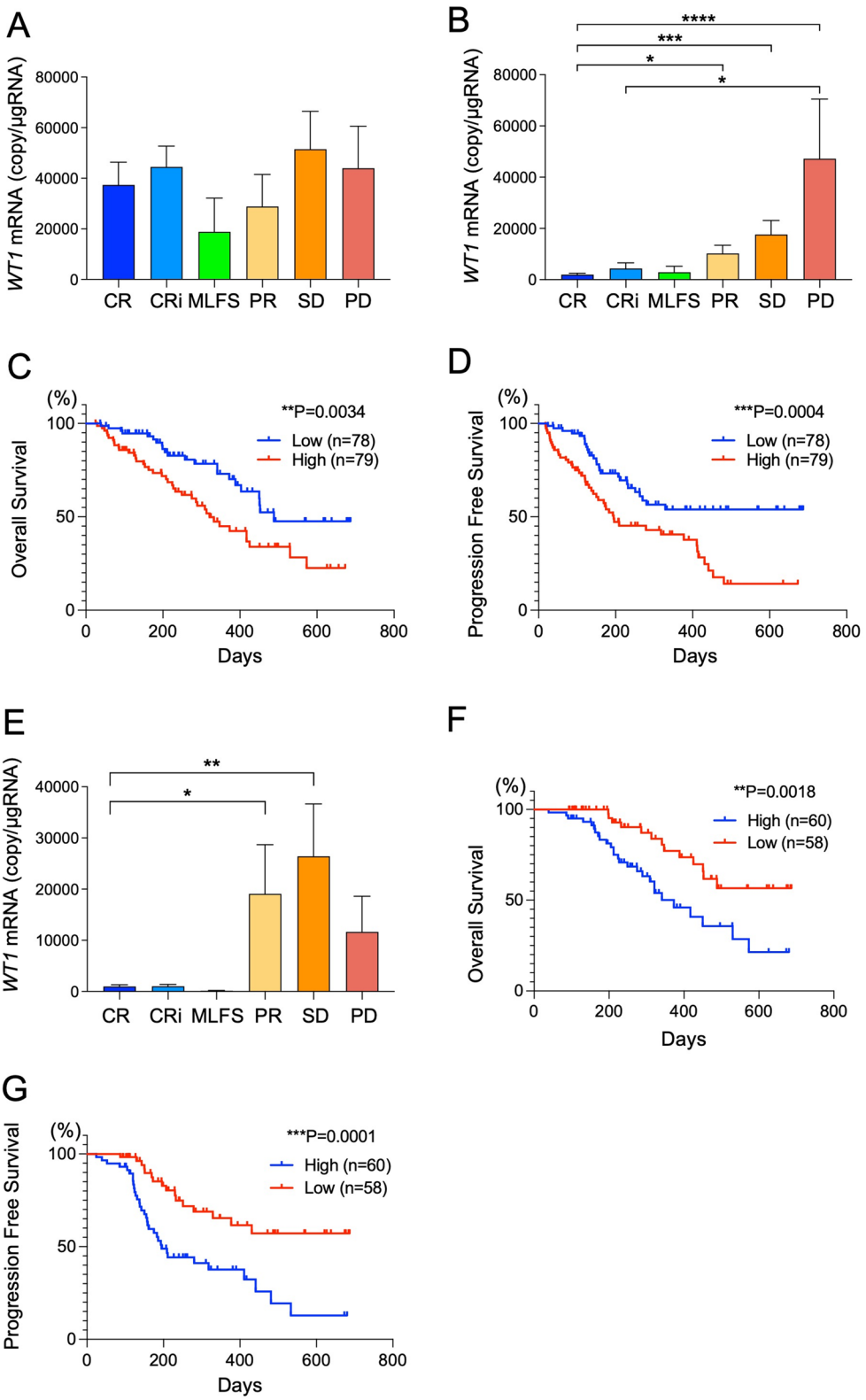

Fig. 2 Peripheral blood *WT1* mRNA levels in relation to treatment response and survival in patients receiving VEN/AZA therapy. *WT1* mRNA dynamics and survival outcomes according to treatment response to venetoclax and azacitidine (VEN/AZA). (**A**) Peripheral blood (PB) *WT1* mRNA levels before VEN/AZA therapy stratified by best response. (**B**) PB *WT1* mRNA levels after the first cycle of VEN/AZA treatment according to the treatment response. (**C**, **D**) Kaplan–Meier curves for overall survival (OS) (**C**) and progression-free survival (PFS) (**D**) stratified by PB *WT1* mRNA levels after the first cycle of VEN/AZA, divided into high and low groups using a cutoff of 580 copies/μg RNA. (**E**) PB *WT1* mRNA levels after the second cycle of VEN/AZA, stratified by treatment response. (**F**, **G**) Kaplan–Meier curves for OS (**F**) and PFS (**G**) according to PB *WT1* mRNA levels after the second cycle of VEN/AZA, divided into high and low groups using the median cutoff of 210 copies/μg RNA. Statistical analyses were performed using the Kruskal–Wallis test for panels A, B, and E, and the log-rank test for panels C, D, F, and G; $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, $^{****}p<0.0001$

Table 4 Proportion of patients achieving CR/CRi according to *WT1* mRNA reduction after the first and second cycles of VEN/AZA

WT1 mRNA reduction	Non-CR/CRi ($n=59$)	CR/CRi ($n=106$)	*P* value
≥1-log reduction after the first cycle	8 (16.3)	56 (56.0)	<0.001
≥2-log reduction after the first cycle	2 (4.1)	33 (33.0)	<0.001
≥1-log reduction after the second cycle	7 (25.9)	55 (64.0)	0.001
≥2-log reduction after the second cycle	2 (7.4)	40 (46.5)	0.001

Abbreviation: *CR* complete remission, *CRi* complete remission with incomplete blood count recovery

Prognostic impact of *WT1* mRNA negativity on OS and PFS

We further evaluated the prognostic significance of undetectable *WT1* mRNA levels. Patients who achieved *WT1* mRNA negativity in PB had significantly longer OS and PFS than those who remained *WT1* mRNA-positive (Figs. 4A, B).

No significant differences in OS or PFS were observed based on the timing of *WT1* mRNA negativity (first, second, third, or later cycles).However, all *WT1* mRNA-negative groups demonstrated superior outcomes compared with *WT1*-positive patients (Figs. 4C, D).

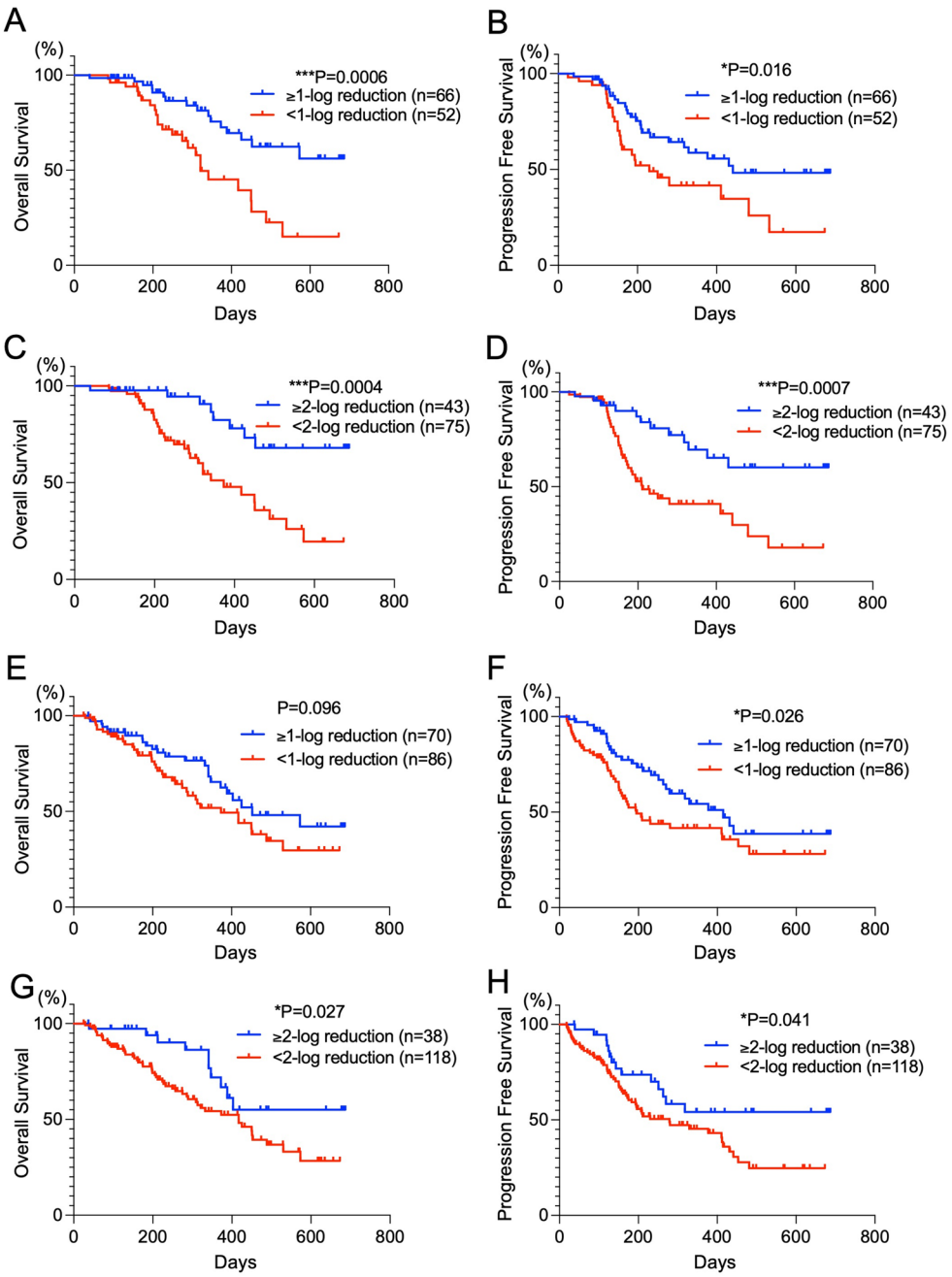

Fig. 3 Kaplan–Meier survival analyses according to *WT1* mRNA reduction after VEN/AZA therapy. (**A**, **B**) Overall survival (OS) and progression-free survival (PFS) in patients stratified by ≥1-log reduction in peripheral blood *WT1* mRNA levels after the second cycle of VEN/AZA therapy. A≥1-log reduction was significantly associated with improved OS (**A**) and PFS (**B**). (**C**, **D**) OS and PFS according to ≥2-log reduction in *WT1* mRNA levels after the second cycle of VEN/AZA. Patients achieving a≥2-log reduction exhibited significantly better outcomes (**C**, **D**). (**E**, **F**) OS and PFS comparisons between patients who did or did not achieve a≥1-log reduction in *WT1* mRNA after the first cycle of therapy. (**G**, **H**) OS and PFS comparisons according to the presence or absence of a≥2-log reduction after the first cycle. All statistical comparisons were performed using the log-rank test

Table 5 Univariate and multivariate analyses for overall survival

Variables	Univariate model			Multivariate model		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age, ≥75	1.40	0.88–2.23	0.15			
Sex, male	1.01	0.63–1.64	0.96			
ECOG PS, 2–4	1.60	0.91–2.79	0.10			
AML type, de-novo	0.42	0.26–0.68	0.00036	0.68	0.30–1.56	0.37
ELN2017, Adverse	1.83	1.147–2.909	0.011	1.12	0.48–2.61	0.79
≥2-log reduction after the second cycle	0.25	0.12–0.56	0.00061	0.25	0.096–0.67	0.0056

Abbreviation: *HR* hazard ratio, *95% CI* 95% confidence interval, *ECOG PS* ECOG Performance Status, *ELN* European LeukemiaNet, ≥2-log reduction after the second cycle; ≥2-log reduction of *WT1* mRNA after the second cycle of venetoclax azacitidine combination therapy

Table 6 Univariate and multivariate analyses for progression free survival

Variables	Univariate model			Multivariate model		
	HR	95%CI	*P* value	HR	95%CI	*P* value
Age, ≥75	0.87	0.56–1.34	0.52			
Sex, male	0.76	0.49–1.19	0.23			
ECOG PS, 2–4	1.46	0.87–2.46	0.16			
AML type, de-novo	0.63	0.41–0.97	0.037	0.81	0.41–1.63	0.56
ELN2017, Adverse	2.21	1.45–3.41	0.00039	1.77	0.89–3.51	0.10
≥2-log reduction after the second cycle	0.34	0.17–0.65	0.0012	0.34	0.155–0.77	0.0091

Abbreviation: *HR* hazard ratio, *95% CI* 95% confidence interval, *ECOG PS* ECOG Performance Status, *ELN* European LeukemiaNet, ≥2-log reduction after the second cycle; ≥2-log reduction of *WT1* mRNA after the second cycle of venetoclax azacitidine combination therapy

These findings suggest that the time required to achieve *WT1* mRNA negativity dose not affect prognosis, consistent with prior studies [6, 15]. Subgroup analyses stratified by disease status (newly diagnosed vs. relapsed/refractory AML) showed similar trends. Notably, *WT1* mRNA negativity remained significantly associated with improved OS and PFS in the relapsed/refractory subgroup (Supplementary Tables 1–2 and Supplementary Figs. S5–8).

Discussion

In patients receiving venetoclax in combination with HMAs, the achievement of MRD negativity by multiparameter flow cytometry has been reported as a significant prognostic factor for OS and PFS [6, 11]. More recently, molecular MRD assessment using mutation-specific reverse transcription quantitative polymerase chain reaction (RT-qPCR) has demonstrated strong predictive value in *NPM1*-mutated AML treated with venetoclax-based low-intensity therapy [15].

In contrast, *WT1* mRNA expression in PB has been investigated as an alternative MRD marker, particularly in the context of hematopoietic stem cell transplantation [16, 17]. In this study, we demonstrate the clinical utility of PB *WT1* mRNA dynamics as a practical biomarker for monitoring treatment efficacy and predicting long-term outcomes in patients with AML treated with VEN/AZA. Although the prognostic significance of MRD monitoring in AML is well established [12, 14, 18], our findings provide additional evidence supporting *WT1* mRNA as a broadly applicable, non-invasive surrogate marker that may complement–or, in some cases, substitute for–more invasive MRD assessment modalities.

In our cohort, patients who achieved *WT1* mRNA negativity in PB had significantly prolonged OS and PFS compared with those who remained positive. Notably, the timing of *WT1* mRNA negativity—whether after the first, second, or subsequent treatment cycles—did not significantly affect survival outcomes. This finding is consistent with previous reports suggesting that delayed molecular remission may confer survival benefits in patients treated with venetoclax-based low-intensity therapy [15, 19]. These results suggest that continued VEN/AZA therapy may remain beneficial even when *WT1* mRNA negativity is not achieved early, provided eventual clearance occurs.

Importantly, our findings extend previous observations by demonstrating that the prognostic relevance of *WT1* mRNA clearance is independent of the timing of its achievement, an aspect that has not been previously addressed. Additionally, ROC curve analysis identified specific PB *WT1* mRNA cutoff values (<2350 copies/μg RNA after cycle 1 and <890 copies/μg RNA after cycle 2) predictive of CR/CRi achievement. These thresholds may serve as clinically actionable benchmarks for early risk stratification and treatment optimization.

Furthermore, increased *WT1* mRNA levels after treatment cycles were frequently observed in patients with PR, SD, or PD, underscoring the potential of *WT1* mRNA kinetics as a dynamic indicator of leukemic activity and treatment resistance.

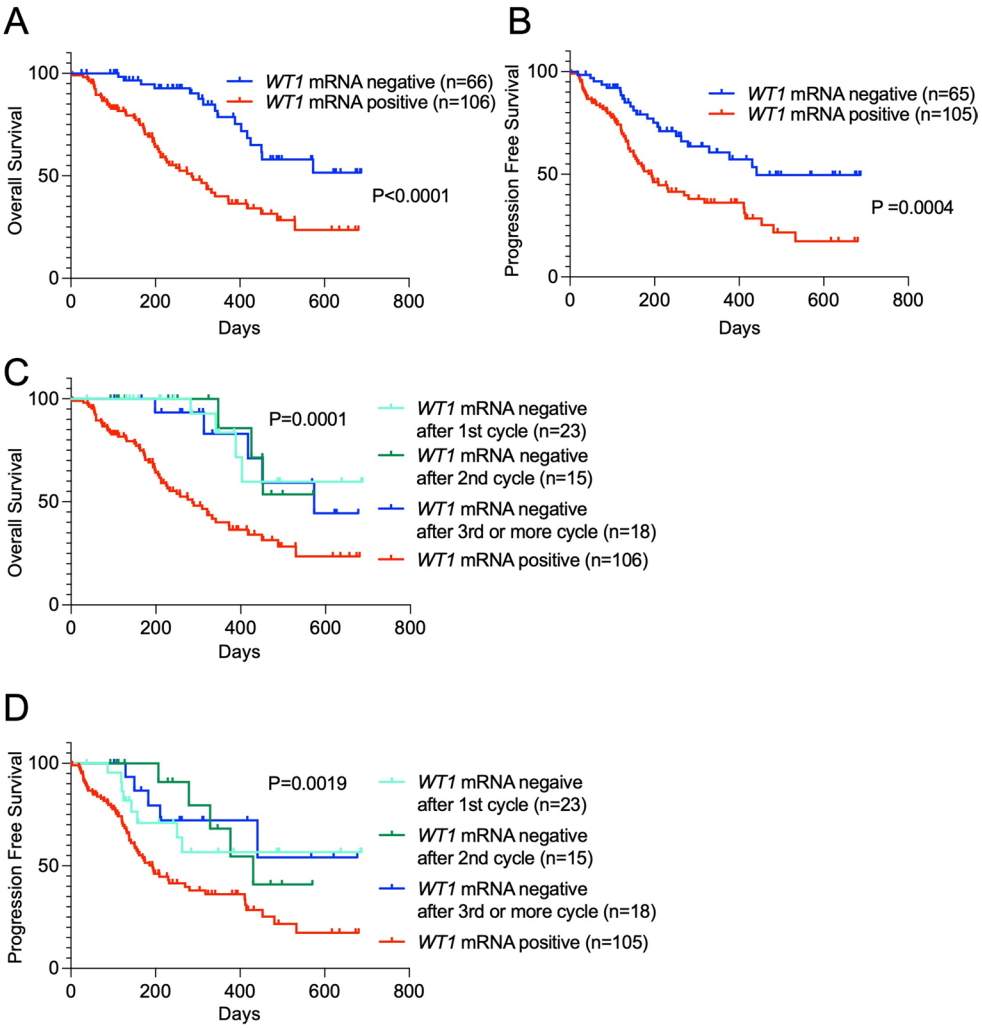

Fig. 4 Prognostic impact of *WT1* mRNA negativity in peripheral blood after VEN/AZA therapy. (**A**, **B**) Kaplan–Meier curves for overall survival (OS) (**A**) and progression-free survival (PFS) (**B**) stratified by *WT1* mRNA detectability in peripheral blood. Patients who achieved *WT1* mRNA negativity at any time during VEN/AZA treatment exhibited significantly improved OS and PFS compared with those who remained *WT1* mRNA-positive. (**C**, **D**) OS (**C**) and PFS (**D**) according to the timing of *WT1* mRNA negativity (after cycle 1, cycle 2, or cycle≥3). Although no significant differences were observed among the three negative groups, all demonstrated superior outcomes compared with patients who did not achieve *WT1* mRNA clearance. Statistical analyses were performed using the log-rank test

Although *WT1* mRNA lacks mutation specificity and is generally less sensitive than molecular assays, its broad applicability, standardized methodology, and non-invasiveness make it an attractive option for routine clinical monitoring. Unlike BM-based assessments, PB *WT1* monitoring reduces patient burden and enables more frequent evaluations of treatment response, which is an important advantage for elderly or frail patients who may not tolerate repeated BM examinations.

This study has several limitations. First, its retrospective study design introduces potential selection and information biases. Second, the cohort included both newly diagnosed and relapsed/refractory AML patients, and outcomes may have been influenced by post-remission therapies, including allogeneic stem cell transplantation. Third, patients with baseline *WT1* mRNA levels below the assay detection limit were excluded; thus, our findings are only applicable to patients with *WT1*-positive disease at baseline.

In addition, flow cytometry–based MRD and molecular MRD data were not available, precluding direct comparison with other established MRD assessment methods [20, 21]. The *WT1* mRNA assay used in this study is approved by the Japanese National Health Insurance System for monitoring MDS and AML. However, *WT1* mRNA levels were quantified using an assay-specific normalization method rather than the ABL1-based standardization used in internationally standardized RT-qPCR assays [20, 21]. Therefore, comparisons with previously reported *WT1* data should be interpreted with caution. Although *WT1* mRNA monitoring is widely implemented and standardized in Japan, its availability and adoption may vary across institutions and countries.

In conclusion, PB *WT1* mRNA is a clinically valuable biomarker for monitoring treatment response and predicting survival in patients with AML receiving VEN/AZA therapy. Both a≥2-log reduction after the second treatment cycle and the eventual achievement of *WT1* mRNA negativity were strongly associated with favorable outcomes. These findings support the integration of *WT1* mRNA kinetics into AML treatment algorithms and warrant prospective validation in larger, more homogeneous cohorts.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00277-026-07046-5.

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Author contributions H.S. and N.A. designed the study and drafted the manuscript. T.A., Y.M., T.U., T.I., M.N., K.Y., T.I., M.M., T.Y., K.S., K.S., Y.N., M.K., M.T., M.A., S.A. and T.Y. contributed data and provided clinical input. Y.M. supervised the study.

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Data availability The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval The study protocol was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of Okayama University Hospital (approved No. 2208-012; June 24, 2022). The requirement for written informed consent was waived due to the retrospective nature of the study, and an opt-out option was provided via the institutional website.

Competing interests Competing interests: KS has received research funding from ONO, BeiGene, AbbVie, Sanofi, Bristol Myers Squibb, GlaxoSmithKline, Chugai, Otsuka, Janssen, Novartis, Pfizer, Kyowa Kirin, Mitsubishi Tanabe, Incyte, and received honoraria from MS, Janssen, Sanofi, and Pfizer. YH has received honoraria from Chugai Pharmaceutical CO., Ltd and Sanofi K.K. All other authors declare no competing interests.

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