Wilms Tumor 1 Mutations are Independent Poor Prognostic Factors in Pediatric Acute Myeloid Leukemia

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Research

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Abstract

Background: The role of *Wilms tumor 1* (*WT1*) mutations remains controversial for patients with acute myeloid leukemia (AML) with regard to the prognostic impact. Here, we aimed to determine the clinical implication of *WT1* mutations in a large cohort of pediatric AML.

Methods: The clinical data of 870 pediatric patients with AML were downloaded from the therapeutically applicable research to generate effective treatment (TARGET) dataset. We analyzed the prevalence, clinical profile and prognosis of *WT1* mutations in these patients.

Results: *WT1* mutations were founded in 6.7% of total patients. *WT1* mutations were closely associated with normal cytogenetics ($P<0.001$), FMS-like tyrosine kinase 3/internal tandem duplication (*FLT3*/ITD) mutations ($P<0.001$), and low complete remission induction rates ($P<0.01$). Compared to patients without *WT1* mutations, patients with *WT1* mutations had worse 5-year event-free survival ($21.7\pm5.5\%$ vs $48.9\pm1.8\%$, $P<0.001$) and overall survival ($41.4\pm6.6\%$ vs $64.3\pm1.7\%$, $P<0.001$). Moreover, patients with both *WT1* and *FLT3*/ITD mutations had a dismal prognosis. Compared to chemotherapy alone, hematopoietic stem cell transplantation had a tendency to improve prognoses of *WT1*-mutated patients. In multivariate analysis, *WT1* mutations conferred an independent adverse impact on event-free survival (hazard ratio 1.910, $P = 0.001$) and overall survival (hazard ratio 1.709, $P = 0.020$).

Conclusion: Our findings demonstrate that *WT1* mutations are independent poor prognostic factors in pediatric AML.

Background

Acute myeloid leukemia (AML) is a type of blood cancer that originates in the bone marrow from immature white blood cells known as myeloblasts. About 20% of all children with leukemia have AML.^{1,2} In the last years, collaborative studies have joined efforts to link the degree of genetic heterogeneity of AML to clinical outcome, allowing risk stratification before therapy and guiding post-induction treatment.³ The *Wilms tumor 1* (*WT1*) gene, located on chromosome 11p13, encodes a zinc-finger protein that exists in multiple isoforms. It has been implicated in regulation of cell survival, proliferation and differentiation, and may function both as a tumor suppressor and as an oncogene.^{4,5} Various mutations across *WT1* gene have been reported in solid tumors and AML.^{6,7} However, the role of *WT1* mutations remains controversial for patients with AML with regard to the prognostic impact.⁸

The *WT1* mutations have been shown to be independent predictors of worse clinical outcome in some but not all adult AML studies.^{9–11} Recently, *WT1* mutations are proposed to be prognostic markers of risk stratification for adult AML.¹² However, the prognostic implications of *WT1* mutations have not been clarified in pediatric AML. Moreover, large cohort studies on the clinical significance of *WT1* mutations in pediatric AML are paucity. A pediatric study of 298 patients with AML found that *WT1* mutations conferred an independent poor prognostic significance.¹³ However, another study of 842 pediatric AML

revealed that the presence of *WT1* mutations had no independent prognostic significance in predicting outcome.¹⁴ Recently, in a cohort of 353 pediatric patients with AML, Niktoreh *et al.*¹⁵ have found that *WT1* mutations significantly increased the chance of relapse or treatment failure and reduced the probability of 3-year overall survival (OS), but had no significant impact on 3-year probability of event-free survival (EFS). On the other hand, hematopoietic stem cell transplantation (HSCT) is an important treatment modality for patients with AML. However, the role of HSCT for patient with *WT1* mutations remains unknown.

To determine the clinical implication of *WT1* mutations, independent large cohort study of pediatric AML is needed. Therefore, we analyzed the clinical data of 870 pediatric patients with AML from the therapeutically applicable research to generate effective treatment (TARGET) dataset. We found that *WT1* mutations are independent poor prognostic factors in pediatric AML in terms of 5-year EFS and OS. Patients with both *WT1* and FMS-like tyrosine kinase 3/internal tandem duplication (*FLT3*/ITD) mutations had a dismal prognosis. Moreover, HSCT might be an effective strategy for patients with *WT1* mutations.

Methods

Clinical data on pediatric AML

Clinical data on patients with AML were downloaded from the TARGET dataset (<https://ocg.cancer.gov/programs/target/data-matrix>). In total, 870 pediatric patients younger than 18 years old with the information of *WT1* mutations were included in our study. Year of diagnosis ranged from 1996 to 2010. Year of last follow up ranged from 1997 to 2015. The diagnosis of pediatric AML and risk stratification were defined according to the Children's Oncology Group (COG) guidelines. Subtype classifications of AML were assigned according to the French–American–British (FAB) classifications. Mutation analyses of *WT1*, *FLT3*/ITD, *NPM1* and *CEBPA* were performed as previously described.^{14, 16–18} Treatment protocols for AML included AAML03P1, AAML0531 and CCG-2961. HSCT was considered for high-risk patients in the first complete remission. Detail treatments and risk stratification of these studies have been previously described.¹⁹ Written informed consent of all patients in these studies were in accordance with the Helsinki Declaration.

Statistical analysis

The data were analyzed with the Statistical Package for the Social Sciences (SPSS®) version, 20.0 (IBM Corporation, Armonk, NY, USA). The χ^2 test was used to compare the frequencies of mutations. Fischer's exact test was used when data were sparse. The nonparametric Mann–Whitney *U*-test was applied for continuous variables. Complete remission (CR) was defined as bone marrow aspirate containing < 5% blasts by morphology. EFS was defined as time between diagnosis and first event, including induction failure, relapse or death of any cause. OS was defined as time between diagnosis and death from any cause. The survival curves were estimated using the Kaplan–Meier method and compared using the log-

rank test. Cox proportional hazard models were used to estimate hazard ratios (HR) for multivariate analyses. A two-sided *P*-value less than 0.05 was considered statistically significant for all statistical analyses.

Results

Relationship between *WT1* mutations and clinical characteristics

The patients' clinical characteristics are shown in Table 1. Overall, among the 870 pediatric patients with AML, 58 patients (6.7%) were identified with *WT1* mutations. The white blood cell count (WBC) at diagnosis was significantly higher in *WT1*-mutated patients (median $56.9 \times 10^9/\text{L}$) than in *WT1* wild-type patients (median $30.8 \times 10^9/\text{L}$; $P = 0.041$). In *WT1*-mutated group, the FAB subtypes were mainly M1, M2, and M4. A higher proportion of *WT1*-mutated patients had M4 morphology in comparison with *WT1* wild-type patients (41.2% vs 25.9%; $P = 0.018$). We also evaluated the associations between *WT1* mutations and cytogenetic and molecular alterations. In terms of cytogenetics, *WT1* mutations were found more frequently in the normal cytogenetics subset (44.2% of *WT1*-mutated patients had normal cytogenetics compared with 22.3% of those without *WT1* mutations; $P < 0.001$). Regarding molecular alterations, there was also a substantial overlap between *WT1* mutations and *FLT3*/ITD, as shown in Table 1, 48.3% of those carrying a *WT1* mutation were *FLT3*/ITD positive as opposed to 14.7% of patients without *WT1* mutations ($P < 0.001$). Moreover, *WT1*-mutated patients more frequently showed high risk (40.7% vs 12.6%; $P < 0.001$). The treatment protocols for pediatric AML were equally distributed between these two groups ($P = 0.058$). However, there were no significant differences in the median age, the median of *FLT3*/ITD allelic ratio, *NPM1*, and *CEBPA* mutations between *WT1*-mutated group and *WT1* wild-type group.

Table 1
Characteristics of pediatric patients with or without *WT1* mutations

	All patients	<i>WT1</i> -mutated case	<i>WT1</i> wildtype case	<i>P</i> value
Number (%)	870	58 (6.7%)	812(93.3%)	
Age, median (year)	9.6	11.0	9.5	0.221
< 3years, n (%)	21(24.3%)	6 (10.3%)	205 (25.2%)	0.011
3 ≤ Age < 10years, n (%)	23(27.2%)	19 (32.8%)	218 (26.8%)	0.329
10 ≤ Age < 18years, n (%)	422(48.5%)	33 (56.9%)	389 (47.9%)	0.186
Sex	454 (52.2%)	36 (62.1%)	418 (51.5%)	0.119
male, n (%)	416 (47.8%)	22 (37.9%)	394 (48.5%)	
female, n (%)				
WBC, ×10 ⁹ /L, Median (range)	31.7(0.2–610)	56.9(1.1–446)	30.8(0.2–610)	0.041
FAB classification: n (%)	20 (2.8%)	1 (2.0%)	19 (2.9%)	0.001
M0	96 (13.4%)	10 (19.6%)	86 (13.0%)	> 0.999
M1	193 (27.0%)	11 (21.6%)	182 (27.5%)	0.181
M2	2 (0.3%)	0 (0.0%)	2 (0.3%)	0.362
M3	193 (27.0%)	21 (41.2%)	172 (25.9%)	> 0.999
M4	160 (22.4%)	3 (5.9%)	157 (23.7%)	0.018
M5	11 (1.5%)	4 (7.8%)	7 (1.1%)	0.003
M6	39 (5.5%)	1 (2.0%)	38 (5.7%)	0.005
M7				0.351
Risk group: n (%)	328 (39.0%)	15 (27.8%)	313 (39.8%)	< 0.001
Low risk	391 (46.5%)	17 (31.5%)	374 (47.6%)	0.079
Standard risk	121 (14.4%)	22 (40.7%)	99 (12.6%)	0.022
High risk				< 0.001
CEBPA CCAAT enhancer binding protein alpha, CR complete remission, FAB French–American–British morphology classification, <i>FLT3</i> /ITD internal tandem duplication of the <i>FLT3</i> gene, HSCT hematopoietic stem cell transplantation, <i>NPM1</i> Nucleophosmin, WBC white blood cell count				

	All patients	<i>WT1</i> -mutated case	<i>WT1</i> wildtype case	<i>P</i> -value
<i>FLT3</i> /ITD	147 (16.9%)	28 (48.3%)	119 (14.7%)	< 0.001
Positive, n (%)	722(83.1%)	30 (51.7%)	692 (85.3%)	
Negative, n (%)				
<i>FLT3</i> /ITD allelic ratio, Median (range)	0.54 (0.03–9.50)	0.55 (0.03–5.19)	0.54 (0.03–9.50)	0.865
<i>NPM1</i>	66(7.6%)	3(5.3%)	63(7.8%)	0.794
Positive, n (%)	802(92.4%)	63(94.7%)	748(92.2%)	
Negative, n (%)				
<i>CEBPA</i>	49(5.7%)	1(1.7%)	48(5.9%)	0.245
Positive, n (%)	817(94.3%)	57(98.3)	760(94.1%)	
Negative, n (%)				
Cytogenetic status	196(23.7%)	23(44.2%)	173(22.3%)	< 0.001
Normal (n, %)	631 (76.4%)	29 (55.8%)	602 (77.7%)	
Abnormal (n, %)	106(12.8%)	9(17.3%)	97(12.5%)	0.317
inv(16)(n, %)	128(15.5%)	3(5.8%)	125(16.1%)	0.046
t(8;21) (n, %)				
HSCT in 1st CR	663 (83.8%)	38 (84.4%)	625 (83.8%)	0.906
No (n, %)	128 (16.2%)	7 (15.6%)	121 (16.2%)	
Yes (n, %)				
Protocol	91 (10.5%)	7 (12.1%)	84 (10.3%)	0.058
AAML03P1 (n, %)	732 (84.1%)	44 (75.9%)	688 (84.7%)	0.679
AAML0531 (n, %)	47(5.4%)	7 (12.1%)	40 (4.9%)	0.074
CCG-2961 (n, %)				0.031

CEBPA CCAAT enhancer binding protein alpha, CR complete remission, FAB French–American–British morphology classification, *FLT3*/ITD internal tandem duplication of the *FLT3* gene, HSCT hematopoietic stem cell transplantation, *NPM1* Nucleophosmin, WBC white blood cell count

	All patients	<i>WT1</i> -mutated case	<i>WT1</i> wildtype case	<i>P</i> -value
CR status at end of course 1	656 (76.3%)	35 (60.3%)	621 (77.4%)	0.002
CR, n (%)	189 (22.0%)	20 (34.5%)	169 (21.1%)	0.003
Not CR, n (%)	15 (1.7%)	3 (5.2%)	12 (1.5%)	0.017
Death, n (%)				0.074
CR status at end of course 2	736 (87.2%)	38 (69.1%)	698 (88.5%)	< 0.001
CR, n (%)	88 (10.4%)	14 (25.5%)	74 (9.4%)	< 0.001
Not CR, n (%)	20 (2.4%)	3 (5.5%)	17 (2.2%)	< 0.001
Death, n (%)				< 0.001
				0.136
<i>CEBPA</i> CCAAT enhancer binding protein alpha, CR complete remission, FAB French–American–British morphology classification, <i>FLT3</i> /ITD internal tandem duplication of the <i>FLT3</i> gene, HSCT hematopoietic stem cell transplantation, <i>NPM1</i> Nucleophosmin, WBC white blood cell count				

Clinical outcome and prognostic effect of *WT1* mutations

The CR rate was determined for all patients after the first and second course of induction therapy. At the end of the first course of therapy, patients with *WT1* mutations had a lower rate of CR (60.3%) compared with those without *WT1* mutations (77.4%), and the difference was statistically significant ($P = 0.002$). At the end of the second course of therapy, 38(69.1%) of the 55 patients with *WT1* mutations achieved a CR compared with 698 (88.5%) of 789 patients without *WT1* mutations ($P < 0.001$). Taken together, *WT1* mutations were significantly associated with low induction CR rates.

Next, we evaluated the survival data for all the 870 pediatric patients. The median follow-up time for the survivors was 5.6 years. As shown in Fig. 1a, *WT1*-mutated patients had a significantly worse 5-year EFS ($21.7 \pm 5.5\%$) compared with *WT1* wild-type patients ($48.9 \pm 1.8\%$; $P < 0.001$). Moreover, patients with *WT1* mutations had worse 5-year OS ($41.4 \pm 6.6\%$) than those without *WT1* mutations ($64.3 \pm 1.7\%$; $P < 0.001$) (Fig. 1b). When analyses were restricted to patients having normal cytogenetics, there were significant differences in outcome between patients with and without *WT1* mutations (Fig. 1c, d) (5-year EFS: $15.2 \pm 7.8\%$ vs $51.8 \pm 3.8\%$, $P < 0.001$; 5-year OS: $34.4 \pm 10.4\%$ vs $66.1 \pm 3.7\%$, $P < 0.001$). In the subgroup of abnormal cytogenetics (Fig. 1e, f), *WT1*-mutated patients also had worse survival time compared with *WT1* wild-type patients in terms of 5-year EFS ($31.0 \pm 8.6\%$ vs $48.3 \pm 2.1\%$, $P = 0.027$) and OS ($48.0 \pm 9.3\%$ vs $64.6 \pm 2.0\%$, $P = 0.048$).

Prognostic impact of *WT1* and *FLT3*/ITD mutations

Survival data for patients with *FLT3*/ITD positive and negative were also explored. As shown in Fig. S1a, *FLT3*/ITD positive was significantly associated with inferior EFS (5-year EFS = $33.5 \pm 4.0\%$ vs $49.7 \pm 1.9\%$ for *FLT3*/ITD-negative; $P < 0.001$). Moreover, the *FLT3*/ITD positive group had worse 5-year OS ($51.5 \pm 4.3\%$) than the *FLT3*/ITD-negative group ($65.0 \pm 1.8\%$; $P = 0.003$) (Fig. S1b).

Given the overlap between *WT1* and *FLT3*/ITD status, subset analysis was performed to assess the relative influence of *WT1* and *FLT3*/ITD on the prognosis of children with AML (Fig. 2a, b; Table 2). In the *FLT3*/ITD-positive subgroup, *WT1*-mutated patients had an extremely dismal prognosis (5-year EFS = $12.5 \pm 6.5\%$ vs $38.4 \pm 4.5\%$ for *WT1* wild-type patients, HR: 2.179 [1.364–3.482], $P = 0.001$; 5-year OS = $27.5 \pm 8.8\%$ vs $57.0 \pm 4.7\%$ for *WT1* wild-type patients, HR: 2.225[1.305–3.796], $P = 0.003$). When restricted to the *FLT3*/ITD-negative subgroup, *WT1* mutations had an adverse impact on 5-year EFS (HR: 1.861[1.197–2.892], $P = 0.006$) instead of 5-year OS (HR: 1.600[0.933–2.744], $P = 0.088$). Similarly, for the *WT1* wild-type patients, *FLT3*/ITD positive had reduced 5-year EFS (HR: 1.386[1.075–1.788], $P = 0.012$) but not 5-year OS (HR: 1.305[0.961–1.771], $P = 0.088$). However, *FLT3*/ITD mutations had no significantly negative influence on the outcome of *WT1*-mutated patients (EFS HR: 1.605[0.886–2.906], $P = 0.118$; OS HR: 1.748[0.870–3.514], $P = 0.117$).

Table 2
Statistical comparison of survival data according to both *WT1* and *FLT3*/ITD status

Comparison	EFS hazard ratio (95% CI)	EFS <i>P</i> value	OS hazard ratio (95% CI)	OS <i>P</i> value
<i>FLT3</i> /ITD(-): <i>WT1</i> wildtype vs <i>WT1</i> mutant	1.861(1.197–2.892)	0.006	1.600(0.933–2.744)	0.088
<i>FLT3</i> /ITD(+): <i>WT1</i> wildtype vs <i>WT1</i> mutant	2.179(1.364–3.482)	0.001	2.225(1.305–3.796)	0.003
<i>WT1</i> wildtype: <i>FLT3</i> /ITD(-)vs <i>FLT3</i> /ITD(+)	1.386(1.075–1.788)	0.012	1.305(0.961–1.771)	0.088
<i>WT1</i> mutant: <i>FLT3</i> /ITD(-) vs <i>FLT3</i> /ITD(+)	1.605(0.886–2.906)	0.118	1.748(0.870–3.514)	0.117
CI confidence interval, EFS event-free survival, FLT3/ITD internal tandem duplication of the FLT3 gene, OS overall survival.				

Similar results were found in the subgroup of cytogenetically normal AML patients according to the combined *WT1* and *FLT3*/ITD status (Fig. S2). Of note, the survival curves showed that there were no

significant differences between *WT1*-mutated patients with *FLT3*/ITD-positive ($n = 17$) and *FLT3*/ITD negative ($n = 6$), in terms of 5-year EFS ($14.1 \pm 9.0\%$ vs $16.7 \pm 15.2\%$; $P = 0.584$) and OS ($34.5 \pm 12.3\%$ vs $33.3 \pm 19.2\%$; $P = 0.665$).

The effect of SCT in patients with *WT1* mutations

As shown in Table 1, there was no significant difference on the proportion of HSCT in *WT1*-mutated group and *WT1* wild-type group (15.6% vs 16.2% , $P = 0.906$). The survival analysis, after HSCT stratification, showed that for *WT1*-mutated pediatric patients, HSCT conferred a favorable prognostic impact on the trend of better 5-year EFS ($42.9 \pm 18.7\%$ vs $22.3 \pm 7.0\%$ for chemotherapy-only; $P = 0.316$) and OS ($57.1 \pm 18.7\%$ vs $43.6 \pm 8.2\%$ for chemotherapy-only; $P = 0.483$) (Fig. 3a, b).

To further evaluate the role of HSCT in patient with co-occurring *WT1* and *FLT3*/ITD mutations, we explored the impact of HSCT on those patients. As shown in Fig. 3c, d, for AML patients with both *WT1* mutations and *FLT3*/ITD positive, 5-year EFS ($33.3 \pm 19.2\%$) and OS ($50.0 \pm 20.4\%$) were higher in children with HSCT than those with chemotherapy-only (EFS: $0.0 \pm 0.0\%$, $P = 0.152$; OS: $17.3 \pm 11.1\%$, $P = 0.205$), respectively, although the differences between the two groups were not statistically significant.

Multivariate analysis of prognostic factors

Cox regression analyses were then performed to evaluate *WT1* mutation status as a predictor of EFS and OS alongside other prognostic factors: age (utilizing 10 years of age as the cutoff value), white blood cell count at diagnosis (utilizing $50 \times 10^9 /L$ as the cutoff value), high risk, standard risk, and HSCT. We identified *WT1* mutations as an independent prognostic factor for both EFS and OS in pediatric patients with AML (Table 3). *WT1* mutations were significantly associated with inferior EFS (HR: 1.910, 95% CI: 1.297–2.812, $P = 0.001$) and OS (HR: 1.709, 95% CI: 1.090–2.679, $P = 0.020$). In addition, age older than 10 years, white blood cell count greater than $50 \times 10^9/L$ at first diagnosis, high-risk and standard-risk were significantly related to poor EFS and OS, while HSCT was related to better survival prognosis (HR: 0.431, 95% CI: 0.313–0.593, $P < 0.001$) and OS (HR: 0.594, 95% CI: 0.419–0.843, $P = 0.004$).

Table 3
Cox regression analysis of *WT1* mutations and other prognostic factors

Outcome	Variable	Hazard ratio (95% CI)	P-value
EFS	<i>WT1</i>	1.910(1.297–2.812)	0.001
	High risk	3.136(2.235–4.400)	< 0.001
	Standard risk	2.581(2.207–3.286)	< 0.001
	HSCT	0.431(0.313–0.593)	<0.001
	Age > 10 years	1.300(1.053–1.607)	0.015
	WBC > 50 × 10 ⁹ /L	1.499(1.220–1.841)	<0.001
OS	<i>WT1</i>	1.709(1.090–2.679)	0.020
	High risk	3.991(2.653–6.004)	<0.001
	Standard risk	3.413(2.494–4.670)	<0.001
	HSCT	0.594(0.419–0.843)	0.004
	Age > 10 years	1.496(1.158–1.933)	0.002
	WBC > 50 × 10 ⁹ /L	1.307(1.018–1.677)	0.036
CI confidence interval, EFS event-free survival, HSCT hematopoietic stem cell transplantation, OS overall survival, WBC white blood cell count.			

Discussion

The TARGET program is a collaborative COG-national cancer institute (NCI) project with the aim of comprehensively characterizing the mutational, transcriptional and epigenetic landscapes of a large, well-annotated cohort of pediatric cancer.²⁰ Using such a large cohort of subjects, we tried to investigate the clinical implication of *WT1* mutations in pediatric AML. Our findings showed that the frequency of *WT1* mutations was 6.7% for total 870 pediatric patients. This result was similar with adult studies. In a large cohort of adult study, the frequency of *WT1* mutations among 3157 patients was 5.5%.²¹ Next, we found that *WT1* mutations were significantly associated with FAB subtypes of M4, high white blood cell counts at first diagnosis, normal cytogenetics and *FLT3*/ITD mutations. However, no association was found between *WT1* mutations and *CEBPA* mutations. These results were different with some other studies. A report from the COG, Ho *et al.*¹⁴ also found that *WT1* mutations was related to normal cytogenetics and *FLT3*/ITD mutations, but they found no correlation between *WT1* mutations and white blood cell counts or M4 subtype. Another pediatric report from the Europe, Hollink *et al.*¹³ showed that *WT1* mutations clustered significantly in the subgroup with normal cytogenetics and were associated with *FLT3*/ITD and *CEBPA* mutations.

The prognostic impact of *WT1* mutations have not been clarified in pediatric AML. In our study, we found that patients with *WT1* mutations had lower CR induction rates, worse EFS and OS rates when comparing with patients without *WT1* mutations. Patients with both *WT1* and *FLT3/ITD* mutations had a dismal prognosis. The multivariate analysis showed that *WT1* mutations were an independent adverse impact factor. These results are consistent with the findings by Hollink *et al.*¹³, though they found the CR induction rates did not differ significantly between patients with *WT1*-mutated and *WT1* wild-type AML. A report from the French study group confirmed that *WT1* mutations were an independent prognostic factor for pediatric AML.²² However, a report from the Japanese study group showed that *WT1* mutations were related to a poor prognosis in patients with normal cytogenetics, excluding those with *FLT3/ITD* and those younger than 3 years.²³ By contrast, a report from the Nordic Society of Pediatric Hematology and Oncology (NOPHO) revealed that no significant correlation with survival was seen for *WT1* mutations.²⁴ Notably, they found that patients with *WT1* mutations and *FLT3/ITD* negative had superior EFS compared with patients with *WT1* wildtype with or without concurrent *FLT3/ITD*.²⁴ These discrepant results may be due to the differences in sample size, exon of *WT1* mutations and treatment protocols across studies.

The mechanism of *WT1* mutations in leukemogenesis remains elusive. Several different *WT1* mutations have been described in AML, which occur primarily in exons 1, 7 and 9. *WT1* mutations may result in the loss of DNA binding ability due to loss of the zinc-finger domain, or result in loss of expression of the *WT1* protein altogether.²⁵⁻²⁷ *WT1* mutations fail to properly direct the ten-eleven translocation-2 to its target sites, either by disruption of the interaction itself or by failing to bind to DNA.^{28,29} Recently, Pronier *et al.*³⁰ have found that *WT1* heterozygous loss enhances stem cell self-renewal, *WT1* depletion cooperates with *FLT3/ITD* mutation to induce fully penetrant AML. Mutational analysis of a large cohort of AML cases revealed that *WT1* may play an important role in epigenetic pathway.^{31,32} Given the epigenetic alterations catalogued in *WT1* mutant, epigenetic-targeted therapy has been explored as a potential mechanism to deal with this subgroup of leukemia.³³ Recently, Sinha *et al.*³⁴ have found that mutant *WT1* is associated with DNA hypermethylation of polycomb repressor complex 2 targets in AML, and inhibitor of enhancer of zeste homolog 2 (EZH2) may be more effective active for this AML subtype.

Alternately, HSCT is one of the most effective treatments for AML. However, it is unknown whether *WT1*-mutated patients will benefit from SCT or not. Our studies showed that compared to chemotherapy alone, HSCT had a tendency to improve prognoses of *WT1*-mutated patients, and for patients with both *WT1* and *FLT3/ITD* mutations as well. These results are in agreement with a previous pediatric report.¹⁴ Recently, Eisfeld *et al.*¹² have found that co-occurrence of *WT1* and *NPM1* mutations confers especially poor outcome in a large cohort of 863 adult AML. They proposed that mutated *WT1* co-occurrence with mutated *NPM1* would be an adverse marker for risk stratification, indicating patients with both *WT1* and *NPM1* mutations might be considered for HSCT. However, since *NPM1* mutation is relatively rare in children, we wouldn't make such conclusion due to the small numbers of patients with both *WT1* and *NPM1* mutation. Thus, whether *WT1* mutation is an indication for HSCT in pediatric AML requires further investigation.

Conclusion

In conclusion, we analyzed the clinical implication of *WT1* mutations in a largest pediatric AML cohort. Our findings showed that *WT1* mutations are independent poor prognostic factors in pediatric AML. Patients with co-occurring *WT1* and *FLT3*/ITD mutations had a dismal prognosis. Moreover, HSCT might be an effective strategy for patients with *WT1* mutations. These results have important implications to contribute in refining risk stratification of pediatric AML and show the need for further validations in independent pediatric cohorts.

Abbreviations

CEBPA, CCAAT/enhancer binding protein-alpha; CI, confidence interval; CR, complete remission; EFS, event-free survival; FAB, French-American-British morphology classification; *FLT3*/ITD, internal tandem duplication of the *FLT3* gene; HR, Hazard ratio; HSCT, hematopoietic stem cell transplantation; *NPM1*, nucleophosmin; OS, overall survival; SCT, stem cell transplantation; WBC, white blood cell count.

Declarations

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Authors' contributions

L.C. and L.X. participated in project design, data collection, analysis, interpretation and manuscript drafting; Y.W. participated in data interpretation and manuscript drafting; W.W. participated in data collection and analysis; D.Z. and J.F. participated in project design, data interpretation and manuscript drafting. All authors contributed to drafting and reviewing the manuscript and approved the submitted and final version.

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Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Data availability

The datasets generated and/or analyzed during the current study are available from the TARGET website: <https://ocg.cancer.gov/programs/target/data-matrix>

Ethics approval and consent to participate

The written informed consent of all patients in this study was consistent with the Helsinki Declaration.

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Figures

Figure 1

Survival curves of pediatric AML patients with and without WT1 mutations. Probability of EFS (a) and OS (b) for all patients with and without WT1 mutations, respectively. Probability of EFS (c) and OS (d) for cytogenetically normal patients with and without WT1 mutations, respectively. Probability of EFS (e) and OS (f) for cytogenetically abnormal patients with and without WT1 mutations, respectively.

Figure 2

Survival curves of all pediatric AML patients according to the combined WT1 and FLT3/ITD status. Probability of EFS (a) and OS (b) for patients according to the combined WT1 and FLT3/ITD status, respectively.

Figure 3

Survival curves of pediatric AML patients according to WT1 and hematopoietic stem cell transplantation (HSCT) status. Probability of EFS (a) and OS (b) for patients with WT1 mutations according to HSCT status, respectively. Probability of EFS (c) and OS (d) for patients with WT1 mutations and FLT3/ITD positive according to HSCT status, respectively.

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