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±5.5% vs 48.9±1.8%, $P<0.001$) and overall survival (41.4±6.6% vs 64.3±1.7%, $P<0.001$). 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 \textit{WT1} mutations had no independent prognostic significance in predicting outcome.\textsuperscript{14} Recently, in a cohort of 353 pediatric patients with AML, Niktoreh \textit{et al.}\textsuperscript{15} have found that \textit{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 \textit{WT1} mutations remains unknown.

To determine the clinical implication of \textit{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 \textit{WT1} mutations are independent poor prognostic factors in pediatric AML in terms of 5-year EFS and OS. Patients with both \textit{WT1} and FMS-like tyrosine kinase 3/internal tandem duplication (\textit{FLT3}/ITD) mutations had a dismal prognosis. Moreover, HSCT might be an effective strategy for patients with \textit{WT1} mutations.

\textbf{Methods}

\textbf{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 \textit{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 \textit{WT1}, \textit{FLT3}/ITD, \textit{NPM1} and \textit{CEBPA} were performed as previously described.\textsuperscript{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.\textsuperscript{19} Written informed consent of all patients in these studies were in accordance with the Helsinki Declaration.

\textbf{Statistical analysis}

The data were analyzed with the Statistical Package for the Social Sciences (SPSS®) version, 20.0 (IBM Corporation, Armonk, NY, USA). The \(\chi^2\) test was used to compare the frequencies of mutations. Fischer's exact test was used when data were sparse. The nonparametric Mann–Whitney \textit{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 $56.9 \times 10^9/L$) than in WT1 wild-type patients (median $30.8 \times 10^9/L$; $P = 0.041$). 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.
| Characteristic                               | All patients | WT1-mutated case | WT1 wildtype case | P-value |
|---------------------------------------------|-------------|-----------------|------------------|---------|
| Number (%)                                 | 870         | 58 (6.7%)       | 812 (93.3%)      |         |
| Age, median (year)                         | 9.6         | 11.0            | 9.5              | 0.221   |
| < 3 years, n (%)                           | 21 (24.3%)  | 6 (10.3%)       | 205 (25.2%)      | 0.011   |
| 3 ≤ Age < 10 years, n (%)                  | 23 (27.2%)  | 19 (32.8%)      | 218 (26.8%)      | 0.329   |
| 10 ≤ Age < 18 years, n (%)                 | 422 (48.5%) | 33 (56.9%)      | 389 (47.9%)      | 0.186   |
| Sex                                        |             |                 |                  |         |
| male, n (%)                                | 454 (52.2%) | 36 (62.1%)      | 418 (51.5%)      | 0.119   |
| female, n (%)                              | 416 (47.8%) | 22 (37.9%)      | 394 (48.5%)      |         |
| 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 (%)                  |             |                 |                  |         |
| 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%)         |         |
| M3                                         | 193 (27.0%) | 21 (41.2%)      | 172 (25.9%)      | > 0.999 |
| M4                                         | 160 (22.4%) | 3 (5.9%)        | 157 (23.7%)      |         |
| M5                                         | 11 (1.5%)   | 4 (7.8%)        | 7 (1.1%)         | 0.018   |
| M6                                         | 39 (5.5%)   | 1 (2.0%)        | 38 (5.7%)        | 0.003   |
| M7                                         |             |                 |                  | 0.351   |
| Risk group: n (%)                          |             |                 |                  | < 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, *FLT3/ITD* internal tandem duplication of the FLT3 gene, HSCT hematopoietic stem cell transplantation, *NPM1* Nucleophosmin, WBC white blood cell count.
|                      | All patients | WT1-mutated case | WT1 wildtype case | P value |
|----------------------|--------------|------------------|-------------------|---------|
| **FLT3/ITD**         |              |                  |                   |         |
| Positive, n (%)      | 147 (16.9%)  | 28 (48.3%)       | 119 (14.7%)       | <0.001  |
| Negative, n (%)      | 722 (83.1%)  | 30 (51.7%)       | 692 (85.3%)       |         |
| **FLT3/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   |
| **NPM1**             |              |                  |                   |         |
| Positive, n (%)      | 66 (7.6%)    | 3 (5.3%)         | 63 (7.8%)         | 0.794   |
| Negative, n (%)      | 802 (92.4%)  | 63 (94.7%)       | 748 (92.2%)       |         |
| **CEBPA**            |              |                  |                   |         |
| Positive, n (%)      | 49 (5.7%)    | 1 (1.7%)         | 48 (5.9%)         | 0.245   |
| Negative, n (%)      | 817 (94.3%)  | 57 (98.3%)       | 760 (94.1%)       |         |
| **Cytogenetic status** |            |                  |                   | <0.001  |
| Normal (n, %)        | 196 (23.7%)  | 23 (44.2%)       | 173 (22.3%)       |         |
| Abnormal (n, %)      | 631 (76.4%)  | 29 (55.8%)       | 602 (77.7%)       | 0.317   |
| inv(16)(n, %)        | 106 (12.8%)  | 9 (17.3%)        | 97 (12.5%)        | 0.046   |
| t(8;21) (n, %)       | 128 (15.5%)  | 3 (5.8%)         | 125 (16.1%)       |         |
| **HSCT in 1st CR**   |              |                  |                   | 0.906   |
| No (n, %)            | 663 (83.8%)  | 38 (84.4%)       | 625 (83.8%)       |         |
| Yes (n, %)           | 128 (16.2%)  | 7 (15.6%)        | 121 (16.2%)       |         |
| **Protocol**         |              |                  |                   | 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   |

*CEFPA* 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 | WT1-mutated case | WT1 wildtype case | P value |
|----------------------|--------------|-------------------|-------------------|--------|
| CR status at end of course 1 |              |                   |                   |        |
| CR, n (%)            | 656 (76.3%)  | 35 (60.3%)        | 621 (77.4%)       | 0.002  |
| Not CR, n (%)        | 189 (22.0%)  | 20 (34.5%)        | 169 (21.1%)       | 0.003  |
| Death, n (%)         | 15 (1.7%)    | 3 (5.2%)          | 12 (1.5%)         | 0.017  |
| CR status at end of course 2 |              |                   |                   |        |
| CR, n (%)            | 736 (87.2%)  | 38 (69.1%)        | 698 (88.5%)       | < 0.001|
| Not CR, n (%)        | 88 (10.4%)   | 14 (25.5%)        | 74 (9.4%)         | < 0.001|
| Death, n (%)         | 20 (2.4%)    | 3 (5.5%)          | 17 (2.2%)         | < 0.001|

CEBPAA 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

### 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 ± 5.5%) compared with WT1 wild-type patients (48.9 ± 1.8%; P < 0.001). Moreover, patients with WT1 mutations had worse 5-year OS (41.4 ± 6.6%) than those without WT1 mutations (64.3 ± 1.7%; P < 0.001) (Fig. 1b). When analyses were restricted to patients having normal cytogenetics, there were significantly differences in outcome between patients with and without WT1 mutations (Fig. 1c, d) (5-year EFS: 15.2 ± 7.8% vs 51.8 ± 3.8%, P < 0.001; 5-year OS: 34.4 ± 10.4% vs 66.1 ± 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 ± 8.6% vs 48.3 ± 2.1%, P = 0.027) and OS (48.0 ± 9.3% vs 64.6 ± 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 ± 4.0% vs 49.7 ± 1.9% for FLT3/ITD-negative; P < 0.001). Moreover, the FLT3/ITD positive group had worse 5-year OS (51.5 ± 4.3%) than the FLT3/ITD-negative group (65.0 ± 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 ± 6.5% vs 38.4 ± 4.5% for WT1 wild-type patients, HR: 2.179 [1.364–3.482], P = 0.001; 5-year OS = 27.5 ± 8.8% vs 57.0 ± 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 P-value | OS hazard ratio (95% CI) | OS P-value |
|---------------------------------|---------------------------|-------------|--------------------------|------------|
| FLT3/ITD(-):                   |                           |             |                          |            |
| WT1 wildtype vs WT1 mutant     | 1.861(1.197–2.892)        | 0.006       | 1.600(0.933–2.744)       | 0.088      |
| FLT3/ITD(+)                    |                           |             |                          |            |
| WT1 wildtype vs WT1 mutant     | 2.179(1.364–3.482)        | 0.001       | 2.225(1.305–3.796)       | 0.003      |
| WT1 wildtype:                  |                           |             |                          |            |
| FLT3/ITD(-) vs FLT3/ITD(+)     | 1.386(1.075–1.788)        | 0.012       | 1.305(0.961–1.771)       | 0.088      |
| WT1 mutant:                    |                           |             |                          |            |
| FLT3/ITD(-) vs FLT3/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 cures 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 ± 9.0% vs 16.7 ± 15.2%; P = 0.584) and OS (34.5 ± 12.3% vs 33.3 ± 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 ± 18.7% vs 22.3 ± 7.0% for chemotherapy-only; P = 0.316) and OS (57.1 ± 18.7% vs 43.6 ± 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 ± 19.2%) and OS (50.0 ± 20.4%) were higher in children with HSCT than those with chemotherapy-only (EFS: 0.0 ± 0.0%, P = 0.152; OS: 17.3 ± 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 × 10⁹/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 × 10⁹/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     | WT1        | 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 x 10^9/L | 1.499(1.220–1.841) | < 0.001 |
| OS      | WT1        | 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 x 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.\textsuperscript{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%.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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., 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. 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. 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. 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. 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. 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. Recently, Pronier et al. 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. 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. Recently, Sinha et al. 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. Recently, Eisfeld et al. 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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