Clinical features and prognosis of normal karyotype acute myeloid leukemia pediatric patients with WT1 mutations: an analysis based on TCGA database

Jing Xu, Yaofang Zhang, Jinjun Hu, Yan Ren & Hongwei Wang

To cite this article: Jing Xu, Yaofang Zhang, Jinjun Hu, Yan Ren & Hongwei Wang (2020) Clinical features and prognosis of normal karyotype acute myeloid leukemia pediatric patients with WT1 mutations: an analysis based on TCGA database, Hematology, 25:1, 79-84, DOI: 10.1080/16078454.2020.1720102

To link to this article: https://doi.org/10.1080/16078454.2020.1720102

© 2020 The Author(s). Published by Informa UK Limited, trading as Taylor & Francis Group

Published online: 04 Feb 2020.

Article views: 301

View related articles

View Crossmark data
Clinical features and prognosis of normal karyotype acute myeloid leukemia pediatric patients with WT1 mutations: an analysis based on TCGA database

Jing Xu, Yaofang Zhang, Jinjun Hu, Yan Ren and Hongwei Wang

*The Second Clinical Medical College, Shanxi Medical University, Taiyuan, People’s Republic of China; bDepartment of Hematology, The Second Hospital of Shanxi Medical University, Taiyuan, People’s Republic of China

ABSTRACT

Objectives: To explore the clinical features and prognosis of normal karyotype acute myeloid leukemia (NK-AML) pediatric patients with WT1 mutations.

Methods: The clinical data and prognostic information of 220 NK-AML pediatric patients were selected from target-AML project of The Cancer Genome Atlas (TCGA) database. Survival analyses were performed for NK-AML pediatric patients with different combinations of mutations.

Results: We found that 28(12.7%) NK-AML patients harbored WT1 mutations. The positive rate of FLT3-ITD in the WT1-mutated group was higher than that in the WT1 wild-type group (P = 0.002). In contrast, WT1 mutation and NPM1 mutation were mutually exclusive (P = 0.013). Furthermore, the WT1-mutated group suffered lower rates of complete remission (CR) (P < 0.001 and P < 0.001, respectively) but higher rates of minimal residual disease (MRD) (P = 0.003 and P = 0.021, respectively) after both one and two courses of induction chemotherapy. Patients with WT1 mutations had significantly worse overall survival (OS) and event-free survival (EFS) in both univariate (P < 0.001 and P = 0.007, respectively) and multivariate survival analyses (P < 0.001 and P < 0.001, respectively). The stratification analysis showed that for FLT3-ITD positive patients, WT1 mutations predicted shorter OS (P = 0.003) and EFS (P < 0.001).

Conclusion: WT1 mutations conferred an independent poor prognosis for NK-AML pediatric patients.

1. Introduction

Acute myeloid leukemia (AML) is a group of heterogeneous diseases resulting from acquired somatic genetic lesions accumulated in hematopoietic progenitors [1]. The cytogenetic and molecular markers in the international risk stratification systems such as European Leukemia Network (ELN) [2] and National Comprehensive Cancer Network (NCCN) [3] can give guidance to the risk stratification of de novo normal karyotype AML (NK-AML) patients. Patients with NPM1 mutation and FLT3-ITD with a low allelic ratio (FLT3-ITD\textsuperscript{low}) have a similar favorable outcome as patients with an NPM1 mutation but no FLT3-ITD [4–6]. In contrast, AML with wild-type NPM1 and FLT3-ITD with a high allelic ratio (FLT3-ITD\textsuperscript{high}) has a poor prognosis and is considered as the adverse-risk patients [4]. RUNX1 and ASXL1 mutations [7,8] have also been added to the adverse-risk group when not accompanied by low-risk genetic alterations. But the prognostic significance of other gene mutations such as WT1 mutation is still unknown.

The WT1 gene, located on chromosome 11p13, encodes a zinc-finger protein [4]. It plays an important role in the development of genitourinary and hematopoietic systems and can act as a tumor suppress gene and an oncogene [9,10]. The truncated WT1 proteins, produced from frame-shift mutations, can induce the proliferation and block differentiation of stem cell, thereby contributing to leukemogenesis. WT1 mutations have been found in 10–20% of NK-AML patients [11–15], but its prognostic value remains controversial. Thus, it will be of major interest to explore the clinical features and prognosis of WT1-mutated NK-AML patients.

To further clarify the role of WT1 mutations in NK-AML pediatric patients, we compared the baseline data, treatment response and survival time between the WT1-mutated and WT1 wild-type NK-AML pediatric patients to evaluate its prognostic significance.

2. Material and methods

2.1. Patients

220 AML pediatric patients with normal karyotype selected from target-AML project (CCG-2961 [16], AAML0531 [17], AAML03P1 [18]) of The Cancer Genome Atlas (TCGA) database, were eligible for this study. Normal karyotype was defined by the
international system for human cytogenetic nomenclature (ISCN). Mutation analyses of FLT3-ITD, NPM1 and CEBPA were performed as previously described [19–21]. Information on WT1 mutations was available in all cases. Detail treatments and risk stratification of these studies have been previously described [22].

2.2. Statistical analysis
The statistical analyses were performed with the statistical software package SPSS (version 23.0; SPSS Inc.). The Mann–Whitney U test was applied for continuous variables. The χ² test or Fisher exact test was used to compare the frequencies of categorical data. CR1 and CR2 represent that complete remission (CR) was observed in patients after one course and two courses of induction chemotherapy, respectively. Overall survival (OS) was defined as the time from diagnosis until death or the last follow-up. Event-free survival (EFS) was defined as time between diagnosis and first event, including relapse, death, failure to achieve remission. 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 univariate and multivariate analyses for both OS and EFS.

3. Results
3.1. Patient characteristics
A total of 220 AML pediatric patients with normal karyotype, including 14 cases of CCG-2961, 183 cases of AAML0531 and 23 cases of AAML03P1, were analyzed. There were 121 males (55.0%) and 99 females (45.0%) with a median age of 12.4 (0.3–28.8) years. 179 (81.4%) were non-hispanic or non-latino, 32 (14.5%) were hispanic or latino, and 9 (4.1%) were unknown.

3.2. Comparison of baseline data and gene mutations
The clinical characteristics were compared between the WT1- mutated group and the WT1 wild-type group (Table 1). There were 28(12.7%) cases mutated in WT1 gene. There was no significant difference in age (P = 0.075), white blood cell (WBC) count at diagnosis (P = 0.169), percentage of bone marrow leukemic blast cells (P = 0.411) and percentage of peripheral blood blast cells (P = 0.565). What’s more, the WT1-mutated and WT1 wild-type AML patients were equally distributed over protocols (P = 0.240), gender (P = 0.061), race (P = 0.313), central nervous system (CNS) disease (P = 0.219) and chloroma (P = 0.743). However, the distribution of FAB type was different (P = 0.044) and WT1 mutations were more common in patients with French–American–British (FAB) class M4 (28.6% vs. 15.1%). And such mutations were also less frequent in a low-risk group (7.1% vs. 41.1%, P = 0.002). The positive rate of FLT3-ITD in the WT1-mutated group was higher than that in the WT1 wild-type group (67.9% vs. 36.5%, P = 0.002). In contrast, WT1 mutation and NPM1 mutation were negatively correlated (P = 0.013). There was no significant difference in the rate of FLT3 point mutation (7.1% vs. 8.9%, P = 1.000).

| Characteristic                              | WT1-mutated group (28) | WT1 wild-type group (192) | P    |
|--------------------------------------------|------------------------|---------------------------|------|
| Protocol [n (%)]                            | 3(10.7)                | 11(5.7)                   | 0.240|
| CCG-2961                                   | 1(3.6)                 | 22(11.5)                  |      |
| AAML03P1                                   | 24(85.7)               | 159(82.8)                 |      |
| Gender [n (%)]                              | 20(71.4)               | 101(52.6)                 | 0.061|
| Male                                       | 8(28.6)                | 91(47.4)                  |      |
| Female                                     | 12(42.9)               | 78(40.8)                  |      |
| Age [Median (range)]                       | 9.3(0.3–10.0)          | 12.7(9.0–14.0)            | 0.075|
| WBC at diagnosis [Median (range)]          | 51(21.5–470.0)         | 28.5(3.0–827.2)           | 0.169|
| Bone marrow leukemic blast percentage [Median (range)] | 69.2(20.0–100.0)      | 71.0(1.2–99.0)            | 0.411|
| Peripheral blast percentage [Median (range)] | 41.0(0.0–93.0)         | 44.0(0.0–99.0)            | 0.565|
| Ethnicity [n (%)]                           | 26(92.9)               | 153(79.7)                 | 0.313|
| Non-Hispanic or non-Latino                 |                        |                           |      |
| Hispanic or Latino                         | 0(0.0)                 | 90(47.4)                  |      |
| Unknown                                    | 0(0.0)                 | 94(41.4)                  |      |
| FAB type [n (%)]                            | 2(7.1)                 | 30(15.6)                  | 0.044|
| M0                                         | 0(0.0)                 | 5(2.6)                    |      |
| M1                                         | 2(7.1)                 | 38(21.4)                  |      |
| M2                                         | 3(11.7)                | 44(24.4)                  |      |
| M3                                         | 0(0.0)                 | 1(0.5)                    |      |
| M4                                         | 0(0.0)                 | 10(5.3)                   |      |
| M5                                         | 0(0.0)                 | 10(5.3)                   |      |
| M6                                         | 0(0.0)                 | 10(5.3)                   |      |
| M7                                         | 0(0.0)                 | 10(5.3)                   |      |
| NOS                                        | 8(28.6)                | 44(24.4)                  |      |
| CNS disease [n (%)]                         | 1(3.6)                 | 15(7.8)                   | 0.219|
| Yes                                        | 14(50.0)               | 52(2.8)                   |      |
| No                                         | 26(92.9)               | 187(97.6)                 |      |
| Chloroma [n (%)]                            | 2(7.1)                 | 52(2.8)                   | 0.743|
| Yes                                        | 14(7.3)                | 178(92.7)                 |      |
| No                                         | 27(96.4)               | 187(92.7)                 |      |
| Risk group [n (%)]                          | 9(32.1)                | 122(63.5)                 | 0.002|
| Low                                        | 7(25.9)                | 79(41.1)                  |      |
| Standard                                   | 28(93.8)               | 70(36.6)                  |      |
| High                                       | 11(39.3)               | 43(22.4)                  |      |
| SCT in 1st CR [n (%)]                       | 2(7.1)                 | 47(28.0)                  | 0.53 |
| Yes                                        | 2(7.1)                 | 17(8.9)                   | 1.000|
| No                                         | 26(92.9)               | 175(91.9)                 |      |
| FLT3-ITD [n (%)]                            | 19(67.9)               | 70(36.6)                  | 0.002|
| Positive                                   | 12(43.1)               | 182(96.3)                 |      |
| Negative                                   | 9(32.1)                | 7(3.7)                    |      |
| FLT3 point mutation [n (%)]                 | 2(7.1)                 | 52(2.8)                   | 0.743|
| Yes                                        | 14(7.3)                | 178(92.7)                 |      |
| No                                         | 27(96.4)               | 187(97.6)                 |      |
| NPM1 mutation [n (%)]                       | 1(3.6)                 | 37(19.4)                  | 0.073|
| Yes                                        | 14(7.3)                | 178(92.7)                 |      |
| No                                         | 26(92.9)               | 187(97.6)                 |      |
| CEBPA mutation [n (%)]                      | 9(32.1)                | 70(36.6)                  | 0.002|
| Yes                                        | 2(7.1)                 | 52(2.8)                   | 0.743|
| No                                         | 26(92.9)               | 187(97.6)                 |      |

Note: WBC: white blood cell; NOS: not otherwise specified; FAB: French–American–British; CNS: the central nervous system; SCT: stem cell transplantation; CR: complete remission.
between the two groups. In addition, patients with WT1 mutations had a lower mutation rate of CEBPA, but this difference was not statistically significant (3.6% vs. 19.4%, \( P = 0.073 \)).

### 3.3. Comparison of patients’ response to treatment

Lower rates of CR were observed in patients with WT1 mutation than WT1 wild-type group after both one (CR1) and two (CR2) course of induction chemotherapy (Table 2). 148(79.1%) WT1 wild-type cases achieved CR1 while the rate of CR1 of patients with WT1 mutations was only 39.3% \( (P < 0.001) \). Similarly, patients with WT1 mutations also had a lower CR2 (40.0% vs. 84.8%, \( P < 0.001 \)). There was no significant difference in the rate of stem cell transplantation (SCT) after CR1 (17.6% vs. 28.0%, \( P = 0.530 \)). Moreover, the WT1-mutated group had higher rates of minimal residual disease (MRD) \( (P = 0.003 \) and \( P = 0.021 \), respectively) after both one and two courses of induction chemotherapy. However, for the 119 and 117 patients obtained CR1 and CR2, the percentage of MRD between two groups had no statistical difference \( (P = 0.479 \) and \( P = 0.188 \), respectively). Details were shown in Table 3.

### 3.4. Survival analysis of WT1 mutated and WT1 wild-type patients

#### 3.4.1. Univariate analysis

The Log-rank method was used to compare the OS and EFS of pediatric patients in the WT1-mutated group and the WT1 wild-type group (Table 4 and Figure 1). The results showed that patients with WT1 mutations had significantly a shorter OS \( (HR = 2.861, 95\% CI: 1.669–4.903, P < 0.001, \text{Figure 1(A)) and EFS (HR = 3.430, 95\% CI: 2.155–5.459, P < 0.001, \text{Figure 1(B)) than patients without mutations. Meanwhile, the FLT3-ITD positive patients had an worse survival than the FLT3-ITD negative patients (shorter OS: \( P = 0.022, \text{Figure 1(C) and shorter EFS: \( P = 0.04, \text{Figure 1(D)) while patients with NPM1 mutations showed an improved survival than NPM1 wild-type patients (longer OS: \( P < 0.001, \text{Figure 1(E) and longer EFS: \( P < 0.001, \text{Figure 1(F))}.\)

#### 3.4.2. Stratification analysis

Given the results of univariate analysis, the stratification analysis (Figure 2) was performed to further explore the prognostic significance of WT1 mutations. WT1-mutated and FLT3-ITD positive patients had the worst OS \( (P < 0.001, \text{Figure 2(A)) and EFS \( (P < 0.001, \text{Figure 2(B))}, followed by patients with WT1 mutations but FLT3-ITD negative. WT1-mutated and FLT3-ITD positive patients suffered a worse survival than patients without WT1 mutations but FLT3-ITD positive (OS: \( P = 0.003, \text{Figure 2(A)); EFS: \( P < 0.001, \text{Figure 2(B))}.

#### 3.4.3. Multivariate analysis

COX proportional risk regression model was used to eliminate the effect of covariates FLT3-ITD and NPM1 mutations. Multivariate survival analysis suggested that WT1 mutation was an independent risk factor for OS \( (HR = 2.165, 95\% CI: 1.233–3.803, P = 0.007) \) and EFS \( (HR = 2.547, 95\% CI: 1.551–4.184, P < 0.001) \) in NK-AML patients (Table 4).

### 4. Discussion

In our study, we downloaded the high-quality clinical data and prognostic information of 220 NK-AML pediatric patients from TCGA database and compared the clinical features and prognosis between patients with WT1 mutations and patients without WT1 mutations.

The mutation rate of WT1 gene was 12.7% in our group, which was lower than that of Hollink et al. [15] (22%) but higher than that of Zidan et al. [23] (10.6%) and Virappane et al. [13] (10%). This difference may result from differences in race, sample size, and detection methods.

We found that WT1 gene mostly mutated in subtype M4 and standard risk group, which has not been previously reported. In addition, there was a substantial overlap between WT1 mutation and the class I mutation FLT3-ITD \( (P = 0.002) \). Ho et al. [24] also supported this result. However, they failed to explain the role of WT1 mutations in leukemogenesis because a positive correlation between WT1 mutation and CBF translocation, a classic class II event, was also observed.

### Table 2. Comparison of CR and MRD between WT1-mutated group and WT1 wild-type group in NK-AML pediatric patients.

| Variables                      | WT1-mutated group | WT1wild-type group | \( P \)  |
|--------------------------------|-------------------|--------------------|---------|
| CR status at end of course 1 \( [n (\%)] \)  | 11(39.3)          | 148(79.1)          | <0.001  |
| Yes                            | No                |                    |         |
| CR status at end of course 2 \( [n (\%)] \)  | 10(40.0)          | 156(84.8)          | <0.001  |
| Yes                            | No                |                    |         |
| MRD at end of course 1 \( [n (\%)] \)  | 15(60.0)          | 28(15.2)           |         |
| Yes                            | No                |                    |         |
| MRD at end of course 2 \( [n (\%)] \)  | 14(73.7)          | 56(37.6)           | 0.003   |
| Yes                            | No                |                    |         |
| Note: CR: complete remission; MRD: minimal residual disease. |

### Table 3. Comparison of MRD percentage after treatment between WT1-mutated group and WT1 wild-type group.

| Variables                                      | Total number | WT1-mutated group | WT1wild-type group | \( P \)  |
|------------------------------------------------|--------------|--------------------|--------------------|---------|
| MRD% for CR1 patients                         | 119          | 0(0–8.6)           | 0(0–44.0)          | 0.479   |
| MRD% for CR2 patients                         | 116          | 0(0–6.1)           | 0(0–10.0)          | 0.188   |

Note: MRD: minimal residual disease.
Generally speaking, our study was focused on NK-AML patients which was of less heterogeneity. WT1 mutation was inversely associated with NPM1 mutation in our study ($P = 0.013$), which was consistent with the result of Zidan et al. [23]. It has been reported that NPM1 mutation was a common synergistic mutation with FLT3-ITD in the process of leukemogenesis [25]. Based on the above results, we can speculate that WT1 mutation plays the role of class II mutation in the pathogenesis of leukemia.

We suggested that patients with WT1 mutations had an inferior response to induction chemotherapy compared with patients without mutations. Strikingly, WT1 mutation was an independent adverse prognostic factor for NK-AML pediatric patients in both univariate and multivariate analyses. Our results also confirmed that FLT3-ITD was a poor molecular marker and NPM1 mutation was a good molecular marker for NK-AML, which were in line with the previous reports [26–30]. So far, there were very few studies on the prognosis of WT1 mutations in NK-AML. The United Kingdom Medical Research Council Adult Leukemia Working Party [13] screened exons 7 and 9 of the WT1 gene of 470 young adult NK-AML using a

| Mutated genes | Overall survival | Event-free survival |
|---------------|-----------------|-------------------|
|               | HR (95%CI)      | PH R (95%CI)      | P     | HR (95%CI)    | P         |
| WT1           | 2.861(1.669–4.903) | <0.001            | 3.430(2.155–5.459) | <0.001 |
| FLT3-ITD      | 1.670(1.078–2.587) | 0.022             | 1.718(1.189–2.482) | 0.004  |
| NPM1          | 0.290(0.145–0.579) | <0.001            | 0.248(0.139–0.442) | <0.001 |

Univariate analysis

Multivariate analysis

WT1          | 2.165(1.233–3.803) | 0.007             | 2.547(1.531–4.184) | <0.001 |
FLT3-ITD     | 1.523(0.967–2.400) | 0.07              | 1.505(1.018–2.224) | 0.04   |
NPM1         | 0.311(0.154–0.623) | 0.001             | 0.263(0.147–0.471) | <0.001 |

Note: OS: overall survival; EFS: event-free survival; HR: hazard ratio.

Figure 1. Survival analysis of WT1, FLT3-ITD and NPM1 on NK-AML pediatric patients. A, C and E were the survival curves of OS in the WT1-mutated group, the FLT3-ITD positive group, the NPM1 mutated group and the corresponding wild-type group or negative group, respectively. B, D and F are the survival curves of corresponding EFS. OS: overall survival; EFS: event-free survival.
combination of direct sequencing and high-resolution capillary electrophoresis, its result was basically consistent with our study. Similarly, Egyptian research [23] showed that WT1 mutations were a negative prognostic indicator for disease-free survival (DFS) and OS in NK-AML patients. However, the report from the Japanese Childhood AML Cooperative Study Group [11] came to a different conclusion, which suggested that no significant differences were observed in the 3-year OS and DFS between patients with WT1 mutations and patients without. A comprehensive meta-analysis may be able to resolve this contradiction.

The prognosis of NK-AML pediatric patients with FLT3-ITD or NPM1 mutations still varies considerably. To provide further insight into the risk stratification, we further performed the stratification analysis according to their mutation status. Our result suggested that WT1 mutations predicted an even worse survival for FLT3-ITD positive NK-AML pediatric patients. In total, WT1 gene may function as an addition molecular marker for risk stratification.

In conclusions, WT1 mutation was an independent poor prognostic marker, which predicted a lower CR rate and worse OS and EFS for NK-AML pediatric patients. It may help to improve the risk stratification and prognostic evaluation of NK-AML pediatric patients in the future.

Acknowledgements

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Disclosure statement

No potential conflict of interest was reported by the author(s).

References

[1] Dohner H, Weisdorf DJ. Acute myeloid leukemia. N Engl J Med. 2015;373(12):1136–1152.

[2] Dohner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–447.

[3] O’Donnell MR, Tallman MS, Abboud CN, et al. Acute myeloid leukemia, Version 3. 2017, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw. 2017;15(7):926–957.

[4] Gale RE, Green C, Allen C, et al., The impact of FLT3 internal tandem duplication mutant level, number, size, and interaction with NPM1 mutations in a large cohort of young adult patients with acute myeloid leukemia. Blood. 2008;111(5):2776–2784.

[5] Pratacorona M, Brunet S, Nomdedeu J, et al. Favorable outcome of patients with acute myeloid leukemia harboring a low-allelic burden FLT3-ITD mutation and concomitant NPM1 mutation: relevance to post-remission therapy. Blood. 2013;121(14):2724–2738.

[6] Linch DC, Hills RK, Burnett AK, et al., Impact of FLT3/ITD mutant allele level on relapse risk in intermediate-risk acute myeloid leukemia. Blood. 2014;124(2):273–276.

[7] Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209–2221.

[8] Paschka P, Schlenk RF, Gaidzik VI, et al. ASXL1 mutations in younger adult patients with acute myeloid leukemia: a study by the German-Austrian acute myeloid leukemia study group. Haematologica. 2015;100(3):324–330.

[9] Yang L, Han Y, Suarez Saiz F, et al. A tumor suppressor and oncogene: the WT1 story. Leukemia. 2007;21(5):868–876.

[10] Haber DA, Buckler AJ, Glaser T, et al. An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms’ tumor. Cell. 1990;61(7):1257–1269.

[11] Sano H, Shimada A, Tabuchi K, et al. WT1 mutation in pediatric patients with acute myeloid leukemia: a report from the Japanese Childhood AML Cooperative study group. Int J Hematol. 2013;98(4):437–445.

[12] Paschka P, Marcucci G, Ruppert AS, et al. Wilms’ tumor 1 gene mutations independently predict poor outcome in adults with cytogenetically normal acute myeloid leukemia: a cancer and leukemia group B study. J Clin Oncol. 2008;26(28):4595–4602.

[13] Virappane P, Gale R, Hills R, et al. Mutation of the Wilms’ tumor 1 gene is a poor prognostic factor associated with chemotherapy resistance in normal karyotype acute myeloid leukemia: the United Kingdom Medical research Council adult Leukaemia Working Party. J Clin Oncol. 2008;26(33):5429–5435.

[14] Gaidzik VI, Schlenk RF, Moschyny S, et al. Prognostic impact of WT1 mutations in cytogenetically normal
Acute myeloid leukemia: a study of the German-Austrian AML study group. Blood. 2009;113(19):4505–4511.

[15] Hollink IH, van den Heuvel-Eibrink MM, Zimmermann M, et al. Clinical relevance of Wilms tumor 1 gene mutations in childhood acute myeloid leukemia. Blood. 2009;113(23):5951–5960.

[16] Lange BJ, Smith FO, Feusner J, et al. Outcomes in CCG-2961, a children’s oncology group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children’s oncology group. Blood. 2008;111(3):1044–1053.

[17] Gamis AS, Alonzo TA, Meshinchi S, et al. Gemtuzumab ozogamicin in children and adolescents with de novo acute myeloid leukemia improves event-free survival by reducing relapse risk: results from the randomized phase III Children’s Oncology group trial AAML0531. J Clin Oncol. 2014;32(27):3021–3032.

[18] Cooper TM, Franklin J, Gerbing RB, et al. AAML03P1, a pilot study of the safety of Gemtuzumab ozogamicin in combination with chemotherapy for newly diagnosed childhood acute myeloid leukemia: a report from the Children’s Oncology group. Cancer. 2012;118(3):761–769.

[19] Ho PA, Alonzo TA, Gerbing RB, et al. Prevalence and prognostic implications of CEBPA mutations in pediatric acute myeloid leukemia (AML): a report from the Children’s Oncology group. Blood. 2009;113(26):6558–6566.

[20] Meshinchi S, Alonzo TA, Stirewalt DL, et al. Clinical implications of FLT3 mutations in pediatric AML. Blood. 2006;108(12):3654–3661.

[21] Ho PA, Kutny MA, Alonzo TA, et al. Leukemic mutations in the methylation-associated genes DNMT3A and IDH2 are rare events in pediatric AML: a report from the Children’s Oncology group. Pediatr Blood Cancer. 2011;57(2):204–209.

[22] Vujkovic M, Attiyeh EF, Ries RE, et al. Genomic architecture and treatment outcome in pediatric acute myeloid leukemia: a Children’s Oncology group report. Blood. 2017;129(23):3051–3058.

[23] Zidan MA, Kamal Shaaban HM, Elghannam DM. Prognostic impact of Wilms tumor gene mutations in Egyptian patients with acute myeloid leukemia with normal karyotype. Hematology. 2014;19(5):267–274.

[24] Ho PA, Zeng R, Alonzo TA, et al. Prevalence and prognostic implications of WT1 mutations in pediatric acute myeloid leukemia (AML): a report from the Children’s Oncology group. Blood. 2010;116(5):702–710.

[25] Dovey OM, Cooper JL, Mupo A, et al. Molecular synergy underlies the co-occurrence patterns and phenotype of NPM1-mutant acute myeloid leukemia. Blood. 2017;130(17):1911–1922.

[26] Frohling S, Schlenk RF, Breitrick J, et al. Leukemia AML5GUAm: prognostic significance of activating FLT3 mutations in younger adults (16 to 60 years) with acute myeloid leukemia and normal cytogenetics: a study of the AML study group Ulm. Blood. 2002;100(13):4372–4380.

[27] Kottaridis PD, Gale RE, Frew ME, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical research Council AML 10 and 12 trials. Blood. 2001;98(6):1752–1759.

[28] Heath EM, Chan SM, Minden MD, et al. Biological and clinical consequences of NPM1 mutations in AML. Leukemia. 2017;31(4):798–807.

[29] Thiede C, Koch S, Creutzig E, et al. Prevalence and prognostic impact of NPM1 mutations in 1485 adult patients with acute myeloid leukemia (AML). Blood. 2006;107(10):4011–4020.

[30] Verhaak RG, Goudswaard CS, van Putten W, et al. Mutations in nucleophosmin (NPM1) in acute myeloid leukemia (AML): association with other gene abnormalities and previously established gene expression signatures and their favorable prognostic significance. Blood. 2005;106(12):3747–3754.