BCL2 overexpression: clinical implication and biological insights in acute myeloid leukemia

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Abstract

Background: BCL2 protein inhibitor venetoclax (ABT-199) has been authorized by Food and Drug Administration for relapsed/refractory chronic lymphoid leukemia with 17p deletion. Although venetoclax/ABT-199 also caused cell death in acute myeloid leukemia (AML), whether it could be applied to clinical treatment needs further studies. Here, we revealed clinical implication of BCL2 overexpression in de novo adult AML, and may provide theoretical basis for targeted therapy using venetoclax.

Methods: BCL2 expression was analyzed in adult AML patients from public datasets The Cancer Genome Atlas (TCGA) and confirmed by another independent cohort from our own data.

Results: BCL2 expression showed up-regulated in AML patients among TCGA data and confirmed by our own data. BCL2 overexpression was correlated with FAB-M0/M1, whereas BCL2 under-expression was related to FAB-M5. However, BCL2 expression has no effect on overall survival (OS) and leukemia-free survival (LFS) of AML patients (determined in BCL2low and BCL2high groups). Interestingly, in the BCL2low group, patients undergoing autologous or allogeneic hematopoietic stem cell transplantation (auto/allo-HSCT) had significantly better OS and LFS compared with patients only received chemotherapy, whereas, no significant difference was found in OS and LFS between chemotherapy and auto/allo-HSCT patients in the BCL2high group. BCL2 expression was found positively correlated with HOX family gene, and negatively correlated with tumor suppressor microRNA such as miR-195, miR-497, and miR-193b.

Conclusions: BCL2 overexpression identified specific FAB subtypes of AML, but it did not affect prognosis. Patients with BCL2 overexpression did not benefit from auto/allo-HSCT among whole-cohort-AML and cytogenetically normal AML.

Keywords: BCL2, Expression, HSCT, ABT-199/venetoclax, AML
midostaurin for AML with FLT3 mutations, which accounts for approximately 30% of AML patients [6]. Moreover, the approval of enasidenib, an IDH2 inhibitor, has also approved by FDA for IDH2-mutated AML as another breakthrough in AML therapy [7].

Located on chromosome 18q21.33, BCL2 gene is found in human B-cell lymphomas, which is first identified through cloning the breakpoint of a translocation of t(14;18) [8]. It has proven to be major negative regulator in apoptosis, playing key roles in neoplastic transformation and leukemogenesis [9]. BCL2 protein plays crucial role in inhibiting the influx of adenine nucleotides through the outer mitochondrial membrane, resulting in reducing ATP hydrolysis and inhibiting cytochrome-C release [10]. Based on its oncogenic role in cancer, a highly potent and selective inhibitor of BCL2, ABT-199, presents antitumor activity while sparing platelets [11]. In 2016, venetoclax (ABT-199) has been authorized by FDA for relapsed/refractory chronic lymphoid leukemia (CLL) with 17p deletion. Although ABT-199 also induced cell death in AML [12], whether it can be applied to clinical treatment needs further studies. Notably, the FDA granted accelerated approval to venetoclax in combination with hypomethylating agents azacitidine or decitabine or low-dose cytarabine for the treatment of newly-diagnosed AML in adults who are age 75 years or older, or who have comorbidities that preclude use of intensive induction chemotherapy [7]. Herein, we revealed clinical implication of BCL2 overexpression in de novo adult AML, and may provide theoretical basis for targeted therapy using BCL2 inhibitor venetoclax.

**Patients and methods**

**Patients and ethics**

A first cohort of 173 adult AML patients with BCL2 expression data from TCGA were identified through cloning the breakpoint of a translocation of t(14;18) and included in this study [13]. A total of 73 patients accepted auto/allo-HSCT for consolidation treatment, and the remaining 100 patients only received chemotherapy. The main clinical and laboratory features of the AML patients were presented in Table 1. The study protocol was approved by the Washington University Human Studies Committee, and informed consents were obtained from all patients.

A second cohort of 154 AML patients and 35 healthy donors was also enrolled in the study. The main clinical and laboratory features of the AML patients were presented in Additional file 1. All participants provided informed consents, and the study was approved by the Institutional Review Board of the Affiliated People's Hospital of Jiangsu University.

**Samples preparation, RNA isolation, and reverse transcription**

Bone marrow (BM) aspirate specimens were collected from 35 controls, 154 AML patients at diagnosis time, 48 AML patients at complete remission (CR) time, and 23 AML patients at relapse time. BM mononuclear cells were separated using Lymphocyte Separation Medium (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China). Total RNA was extracted from BMNCs using Trizol reagent (Invitrogen, Carlsbad, CA). Reverse transcription was performed to synthesize cDNA using random primers as our previous reports [14–17].

**RT-qPCR**

Real-time quantitative PCR (RT-qPCR) was performed to examine BCL2 mRNA using AceQ qPCR SYBR Green Master Mix (Vazyme Biotech Co., Piscataway, NJ). The primers used for BCL2 expression were 5′-CCCT GGTGGACAACATCG-3′ (forward) and 5′-CAGGGAGAA ATCAAACAGAGGC-3′ (reverse). Housekeeping gene ABL1 was detected by RT-qPCR using 2 × SYBR Green PCR Mix (Multisciences, Hangzhou, China) [14–17]. Relative BCL2 mRNA levels were calculated using 2^−ΔΔCT method.

**Bioinformatics analyses**

The comparison of BCL2 expression in AML from TCGA data and controls was performed by GEPIA (http://geopia.cancer-pku.cn/detail.php) [18]. Differential gene expression analysis for RNA/microRNA sequencing data was calculated using the raw read counts with the R/Bioconductor package “edgeR”, all analyses were controlled for the false discovery rate (FDR) by the Benjamini-Hochberg procedure. Functional and signaling pathway enrichment was conducted using online website of STRING (http://string-db.org). The microRNA which could target BCL2 was identified by TargetScan (http://www.targetscan.org/vert_72/), mirDIP (http://ophid.utoronto.ca/mirDIP/), miRWalk (http://mirwalk.umm.uni-heidelberg.de/), and miRDB (http://mirdb.org/miRDB/). All basic statistical analyses were performed using the base functions in R version 3.4 (https://www.r-project.org).

**Statistical analyses**

SPSS 22.0 and GraphPad Prism 5.0 were used for statistical analyses and figures creation. Mann-Whitney’s U test was used for the comparison of continuous variables, whereas Pearson Chi-square analysis or Fisher exact test was applied for the comparison of categorical variables. The prognostic effect of BCL2 expression on leukemia-free survival (LFS) and overall survival (OS) was analyzed though Kaplan-Meier analysis using Log-rank test. Univariate and multivariate proportional hazard regression analysis was performed using Cox regression.
The P value (two-tailed) equal or less than 0.05 in all statistical analyses was defined as statistically significant.

Results

BCL2 overexpression in AML

A cohort of 173 de novo adult AML patients with BCL2 expression data from public TCGA datasets was used for differential expression analysis. By using the GEPIA (http://gepia.cancer-pku.cn/detail.php), we found BCL2 expression in AML patients was significantly increased compared with GTEx normal BM samples (P < 0.001, Fig. 1a). In order to confirm the results, we further analyzed BCL2 expression in the second cohort of 154 AML patients from our hospital. Similarly, BCL2 expression was markedly up-regulated in newly diagnosed AML compared with controls and AML patients achieved CR (P < 0.001 and = 0.041, Fig. 1b). Moreover, BCL2 transcript level was significantly increased in AML at relapse time compared with those at CR time (P = 0.024, Fig. 1b).

BCL2 expression identified specific FAB subtypes of AML

In order to explore the clinical implication of BCL2 expression in AML, we further divided these cases into two groups (BCL2\textsuperscript{high} and BCL2\textsuperscript{low}) based on median level of BCL2 transcript. The comparison of clinical/laboratory characteristics of the AML patients between two groups were summarized in Table 1. There were no significant differences between BCL2\textsuperscript{high} and BCL2\textsuperscript{low} groups in sex, age, BM blasts, and the distributions of cytogenetics (P > 0.05). However, BCL2\textsuperscript{high} cases had significantly lower white blood cells (WBC) and higher peripheral blood (PB) blasts compared with BCL2\textsuperscript{low} cases (P = 0.041 and 0.033). Additionally, significant differences in the distributions of BCL2 classifications and cytogenetics were found between two groups (P = 0.000). BCL2 overexpression was markedly correlated with FAB-M0/M1 (P = 0.038 and 0.015), whereas BCL2 under-expression was associated with FAB-M5 (P = 0.001). Among gene mutations, no significant differences

| Table 1 Correlation of BCL2 expression with clinic-pathologic characteristics in AML among TCGA cohort |
|----------------------------------|-----------------|-----------------|--------|
| Patient's parameters             | BCL2 expression |                 | P   |
|                                 | Low (n = 87)    | High (n = 86)   |       |
| Sex, male/female                | 49/38           | 43/43           | 0.448 |
| Median age, years (range)       | 61 (22–82)      | 56 (18–88)      | 0.106 |
| Median WBC, ×10\textsuperscript{9}/L (range) | 17.9 (0.6–223.8) | 15.25 (0.4–297.4) | 0.041 |
| Median PB blasts, % (range)     | 24 (0–94)       | 46 (0–98)       | 0.033 |
| Median BM blasts, % (range)     | 73 (30–98)      | 72 (30–100)     | 0.893 |
| FAB classifications             |                 |                 | 0.000 |
| M0                               | 4               | 12              | 0.038 |
| M1                               | 15              | 29              | 0.015 |
| M2                               | 21              | 17              | NS    |
| M3                               | 5               | 11              | NS    |
| M4                               | 22              | 12              | NS    |
| M5                               | 16              | 2               | 0.001 |
| M6                               | 1               | 1               | NS    |
| M7                               | 1               | 2               | NS    |
| No data                          | 2               | 0               | NS    |
| Cytogenetics                     |                 |                 | 0.239 |
| Normal                           | 44              | 32              | NS    |
| t(15;17)                         | 5               | 10              | NS    |
| t(8;21)                          | 6               | 1               | NS    |
| inv(16)                          | 3               | 7               | NS    |
| +8                               | 3               | 5               | NS    |
| del(5)                           | 0               | 1               | NS    |
| -7/del(7)                        | 4               | 4               | NS    |
| 11q23                            | 2               | 1               | NS    |
| Others                           | 10              | 9               | NS    |
| Complex                          | 9               | 15              | NS    |
| No data                          | 1               | 1               | NS    |
| Gene mutation                    |                 |                 |       |
| FLT3 (+/-)                       | 23/64           | 26/60           | 0.616 |
| NPM1 (+/-)                       | 28/59           | 20/66           | 0.235 |
| DNMT3A (+/-)                     | 23/64           | 19/67           | 0.595 |
| IDH2 (+/-)                       | 9/78            | 8/78            | 1.000 |
| IDH1 (+/-)                       | 5/82            | 11/75           | 0.124 |
| TET2 (+/-)                       | 9/78            | 6/80            | 0.590 |
| RUNX1 (+/-)                      | 5/82            | 10/76           | 0.188 |
| TP53 (+/-)                       | 6/81            | 8/78            | 0.590 |
| NRAS (+/-)                       | 5/82            | 7/79            | 0.566 |
| CEBPA (+/-)                      | 7/80            | 6/80            | 1.000 |
| WT1 (+/-)                        | 2/85            | 8/78            | 0.057 |
| PTPN11 (+/-)                     | 3/84            | 5/81            | 0.496 |
| KIT (+/-)                        | 5/82            | 2/84            | 0.443 |
| U2AF1 (+/-)                      | 4/83            | 3/83            | 1.000 |

AML acute myeloid leukemia, WBC white blood cells, PB peripheral blood, BM bone marrow, FAB French-American-British classification, NS no significant

The P value (two-tailed) equal or less than 0.05 in all statistical analyses was defined as statistically significant.
were found, besides \(BCL2^{\text{high}}\) tended to be associated with \(WT1\) mutations \((P = 0.057)\).

**BCL2 expression did not affect prognosis in AML**

Among the tested AML patients, a total of 73 cases received auto/allo-HSCT for consolidation treatment (after induction chemotherapy), whereas the other 100 cases only received chemotherapy. In both chemotherapy and auto/allo-HSCT groups, \(BCL2^{\text{high}}\) patients showed similar OS (median 26.3 vs 15.8 months) and LFS (median 11.1 vs 9.3 months) time compared with \(BCL2^{\text{low}}\) patients (Fig. 2a and c). Among cytogenetically normal AML (CN-AML), there was also no significant difference in OS (median 24.6 vs 18.1 months) and LFS (median 9.6 vs 11.6 months) time between \(BCL2^{\text{high}}\) and \(BCL2^{\text{low}}\) groups (Fig. 2b and d). Moreover, no matter in either chemotherapy or auto/allo-HSCT groups, no significant differences were found in OS and LFS time between \(BCL2^{\text{low}}\) and \(BCL2^{\text{high}}\) groups among whole-cohort-AML (Chemotherapy group: OS median 8.1 vs 8.0 months and LFS median 8.0 vs 5.9 months; auto/allo-HSCT group: OS median 30.0 vs 56.3 months and LFS median 14.6 vs 13.8 months) and CN-AML (Chemotherapy group: OS median 15.5 vs 8.2 months and LFS median 12.0 vs 8.2 months; auto/allo-HSCT group: OS median 24.6 vs 56.3 months and LFS median 8.6 vs 13.8 months) (Fig. 2e-l). Moreover, Cox regression analysis also confirmed that \(BCL2\) did not independently affect the OS and LFS in whole-cohort-AML (Table 2).

**High expression of BCL2 in AML patients did not benefit from transplantation**

To investigate whether AML patients with high expression of \(BCL2\) could benefit from auto/allo-HSCT, survival in patients with auto/allo-HSCT were compared among both \(BCL2^{\text{high}}\) and \(BCL2^{\text{low}}\) groups. In the \(BCL2^{\text{low}}\) group, the patients undergoing auto/allo-HSCT had significantly better OS and LFS compared with patients only received chemotherapy among both total AML (OS median 56.3 vs 8.0 months and LFS median 13.8 vs 5.9 months) and CN-AML (OS median 56.3 vs 8.2 months and LFS median 13.8 vs 8.2 months) (Fig. 3a-d). In the \(BCL2^{\text{high}}\) group, no significant differences in OS and LFS were found between auto/allo-HSCT and chemotherapy groups among both total AML (OS median 30.0 vs 8.1 months and LFS median 14.6 vs 8.0 months) and CN-AML (OS median 24.6 vs 15.5 months and LFS median 12.0 vs 8.6 months) (Fig. 3e-h).

**Molecular signatures associated with BCL2 in AML**

To gain insights into the biological function of \(BCL2\), we first compared the transcriptomes of \(BCL2^{\text{high}}\) and \(BCL2^{\text{low}}\) groups. This comparison yielded 1533 differentially expressed genes \((\text{FDR} < 0.05, |\log2 \text{FC}| > 1; \text{Fig. 4a and b; Additional file 2})\), in which 569 genes were positively correlated with \(BCL2\) expression, and 964 were negatively correlated. Several genes such as \(PAX2\), \(HOXC6\), \(HOXC10\), \(HOXC9\), \(SOX11\), \(HOXD13\), \(HOXD8\), \(WT1\), \(SALL4\), \(HOXC11\), \(HOXC4\), \(HOXC12\), \(HOXC5\), and \(HOXD12\) reported with proto-leukemia effects were
identified within this signature positively correlated with BCL2 expression. Among the negatively associated genes, BCL2 expression related to the anti-leukemia-associated genes such as CDKN2B, LGALS3, CDH6, THBS1, ITGB2, ROBO1, DOK2, DKK2, DKK1, and LEP. Furthermore, the Gene Ontology analysis revealed that these genes involved in biologic processes, including system development, signaling, cell communication, and cell adhesion (Fig. 4c).

Next, we also derived microRNA expression signatures associated with BCL2 expression. A total of 19 microRNAs was significantly correlated including 11 positive and 8 negative (FDR < 0.05, |log2 FC| > 1; Fig. 4d; Additional file 3). Negatively correlated microRNAs included miR-195, miR-497, miR-135a, miR-196a, miR-193b, miR-455, miR-375, and miR-205, which have been found to have anti-leukemia effects in previous studies. Of these microRNAs, miR-195 and miR-497 was identified as predicted microRNAs that could direct target BCL2 (Fig. 4e, Additional file 4).

Discussion
In this study, we found and verified that BCL2 expression was significantly up-regulated in newly diagnosed AML especially in relapsed AML among two independent cohorts in consistent with previous studies [19–28]. Previously, BCL2 overexpression showed heterogenous expression in the range of 34 to 87% [19]. Although

Fig. 2 The impact of BCL2 expression on survival of AML patients from TCGA cohort. a–d Kaplan–Meier survival curves of OS and LFS in both chemotherapy and HSCT groups. e–h Kaplan–Meier survival curves of OS and LFS in chemotherapy group. i–l Kaplan–Meier survival curves of OS and LFS in HSCT groups.
| Variables          | OS          | Multivariate analysis | OS          | Multivariate analysis |
|--------------------|-------------|-----------------------|-------------|-----------------------|
|                    | HR (95% CI) | P                     | HR (95% CI) | P                     |
| BCL2 expression    | 1.000 (1.000–1.000) | 0.185                 | 1.000 (1.000–1.000) | 0.761                 |
| Age                | 1.040 (1.027–1.054) | 0.000                 | 1.027 (1.011–1.042) | 0.001                 |
| WBC                | 1.003 (0.999–1.006) | 0.119                 | 1.007 (1.003–1.012) | 0.001                 |
| Karyotype risk     | 1.854 (1.465–2.346) | 0.000                 | 2.208 (1.591–3.063) | 0.000                 |
| Treatment regimens | 0.551 (0.389–0.780) | 0.001                 | 0.441 (0.284–0.687) | 0.000                 |
| FLT3 mutations     | 1.269 (0.869–1.852) | 0.217                 | 1.254 (0.859–1.829) | 0.241                 |
| NPM1 mutations     | 1.220 (0.837–1.778) | 0.301                 | 1.268 (0.869–1.848) | 0.218                 |
| CEBPA mutations    | 0.913 (0.464–1.796) | 0.792                 | 1.053 (0.535–2.073) | 0.881                 |
| DNMT3A mutations   | 1.615 (1.104–2.362) | 0.014                 | 1.472 (0.951–2.279) | 0.083                 |
| IDH1 mutations     | 0.843 (0.466–1.527) | 0.574                 | 0.890 (0.492–1.611) | 0.700                 |
| IDH2 mutations     | 1.113 (0.649–1.910) | 0.697                 | 0.987 (0.576–1.691) | 0.963                 |
| TET2 mutations     | 0.953 (0.514–1.767) | 0.879                 | 0.945 (0.510–1.751) | 0.857                 |
| RUNX1 mutations    | 1.853 (1.077–3.186) | 0.026                 | 1.692 (1.137–2.518) | 0.009                 |
| TP53 mutations     | 3.687 (2.144–6.339) | 0.000                 | 2.379 (1.211–4.673) | 0.012                 |

OS overall survival, LFS leukemia-free survival, HR hazard ratio, CI confidence interval, WBC white blood cells. Variables in multivariate analysis including BCL2 expression, age, WBC, karyotype (favorable vs. intermediate vs. poor), treatment regimens (without/with HSCT) and gene mutations (mutant vs. wild-type).

**Table 2** Cox regression analyses of variables for OS and LFS in whole-cohort-AML among TCGA cohort

**Fig. 3** The effect of HSCT on survival of AML patients among different BCL2 expression groups from TCGA cohort. a–d: Kaplan–Meier survival curves of OS and LFS in low BCL2 expression group. e–h: Kaplan–Meier survival curves of OS and LFS in high BCL2 expression group.
BCL2 overexpression in AML cells correlates with CD34 and CD117 positivity by other investigators [19, 20], we did not find the association of BCL2 expression with BM blasts, despite the fact that BCL2-high patients showed higher percentage of PB blasts. Among FAB subtypes, BCL2 overexpression was significantly correlated with FAB-M0/M1, whereas BCL2 under-expression was associated with FAB-M5, which was in consistent with previous reports [19]. Interestingly, although previous studies revealed that BCL2 overexpression correlated with poor response to chemotherapy [19–22], we did not find the negative effect of BCL2 overexpression on FAB-M0/M1. The molecular signatures associated with BCL2 in AML from TCGA cohort are illustrated in Fig. 4. A: Expression heatmap of differentially expressed genes between BCL2-low and BCL2-high AML patients among TCGA datasets (FDR < 0.05, P < 0.05 and |log2 FC| > 1). B: Volcano plot of differentially expressed genes between BCL2-low and BCL2-high AML patients. C: Gene Ontology analysis of DEGs conducted using online website of STRING (http://string-db.org). D: Expression heatmap of differentially expressed microRNAs between BCL2-low and BCL2-high AML patients among TCGA datasets (FDR < 0.05, P < 0.05 and |log2 FC| > 1). E: Venn results of microRNAs which could target BCL2 predicted by TargetScan (http://www.targetscan.org/vert_72/), mirDIP (http://ophid.utoronto.ca/mirDIP/), miRWalk (http://mirwalk.umm.uni-heidelberg.de/), and miRDB (http://mirdb.org/miRDB/).
clinical outcome of AML. Similarly, several investigators also did not show the significant association of $BCL2$ overexpression with prognosis [23, 24]. In addition, increasing studies attempted to show the transcript ratio of $FLT3 + KIT/BCL2$, $FLT3/BCL2$, and $BAX/BCL2$ (or combined with $WT1$ or $MDR1$) may affect prognosis in AML [25–28]. Thus, we deduced that $BCL2$ expression was not a valuable single factor that affecting prognosis in AML.

Apoptosis plays crucial roles in the command of tissue homeostasis, and is important in the clearance of infected, unwanted, or otherwise damaged cells [29]. Meanwhile, deregulation of apoptosis may give rise to neoplastic transformation [9]. It has been well demonstrated that $BCL2$ acted as a negative regulator on cellular apoptosis and is a druggable target [9, 30–32]. In hematologic malignancies, the impairment of apoptosis process is often caused by $BCL2$ overexpression [32]. Taking these into account, targeting $BCL2$ proteins to cause apoptosis is considered as a potential therapeutic approach in hematological malignancies [33–36]. Early efforts in $BCL2$ inhibitor including ABT-737 and ABT-263/navitoclax were encountered with disappointment in clinic because of dose-dependent thrombocytopenia [31]. In 2013, Souers et al. recently reported the re-engineering of ABT-263/navitoclax to create ABT-199/venetoclax, which was a highly potent and selective inhibitor of $BCL2$ [11]. By clinical studies, venetoclax presented high rate of treatment response as a single drugs in refractory/relapsed CLL [37]. Of note, ABT-199/venetoclax has been authorized by FDA for relapsed or refractory CLL with $17p$ deletion in 2016. In addition to CLL, ABT-199 also powerfully kills a various array of non-Hodgkin lymphoma and AML cell lines [12], suggesting that the drug has the potential to be efficacious in multiple hematologic malignancies. From our study, we observed that AML patients with $BCL2$ underexpression could benefit from auto/allo-HSCT, whereas patients with $BCL2$ overexpression did not benefit from auto/allo-HSCT.

Herein, we further determined the molecular signatures associated with $BCL2$ in AML to further get better understanding of AML biology. We found that $BCL2$ dysregulation was significantly associated with $HOX$ gene family, which was reported highly correlated with hematopoiesis and leukemogenesis [38, 39]. Moreover, for microRNAs, we found $BCL2$ expression was negatively correlated with several microRNAs such as $miR-195$, $miR-497$, $miR-135a$, $miR-196a$, $miR-193b$, $miR-455$, $miR-375$, and $miR-205$, which were found to be associated with AML pathogenesis or patients prognosis by previous studies [40–44]. Of these microRNAs, $miR-195$ and $miR-497$ was identified as predicted microRNAs that could direct target $BCL2$. Obviously, further studies are needed to confirm the direct connections of $BCL2$ with microRNAs by luciferase assay.

**Conclusion**

$BCL2$ overexpression identified specific FAB subtypes of AML, but it did not affect prognosis. Patients with $BCL2$ overexpression did not benefit from auto/allo-HSCT among whole-cohort-AML and CN-AML.

**Additional files**

- **Additional file 1:** Clinic-pathologic characteristics in AML from our cohort. (DOCX 19 kb)
- **Additional file 2:** Different expressed genes of microRNA for $BCL2^+$ and $BCL2^−$. (XLSX 50 kb)
- **Additional file 3:** Different expressed genes of RNA for $BCL2^+$ and $BCL2^−$. (XLSX 1682 kb)
- **Additional file 4:** Venn results of microRNAs targeting $BCL2$. (TXT 37 kb)

**Abbreviations**

AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; BM: Bone marrow; BMNc: BM mononuclear cell; CLL: Chronic lymphoid leukemia; CN-AML: Cyto-genetically normal AML; CR: Complete remission; FDA: Food and Drug Administration; HSCT: Hematopoietic stem cell transplantation; LFS: Leukemia-free survival; OS: Overall survival; RT-qPCR: Real-time quantitative PCR; TCGA: The Cancer Genome Atlas

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**Authors’ contributions**

J-dZ and JQ conceived and designed the experiments; J-dZ and T-jZ performed the experiments; J-dZ and J-cM analyzed the data; WZ and LY collected the clinical data; JL, J-cM, HG, X-mW, X-hL offered technique support; J-dZ wrote the paper. All authors read and approved the final manuscript.

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**Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

The present study approved by the Ethics Committee and Institutional Review Board of the Affiliated People’s Hospital of Jiangsu University.

**Consent for publication**

Written informed consents were obtained from all enrolled individuals prior to their participation.

**Competing interests**

The authors declare that they have no competing interests.
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