Frequency and Clinical Impact of WT1 Mutations in the Context of CEBPA-Mutated Acute Myeloid Leukemia

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Research Article

Keywords: Acute myeloid leukemia, WT1, CEBPA, mutation

Posted Date: May 27th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-526446/v1>

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Abstract

Introduction: Several studies have confirmed that mutations in the Wilms tumor 1 (WT1) gene occur in adult acute myeloid leukemia (AML). However, few data are available regarding the incidence of WT1 mutations in CEBPA^{mut} AML and their impact.

Methods: We retrospectively analyzed the frequency and clinical impact of WT1 mutations in 220 newly diagnosed AML patients with CEBPA mutations. Chromosome karyotype analysis was performed by R or G banding method and further confirmed either by fluorescence in situ hybridization (FISH) and/or by multiple reverse transcription polymerase chain reaction (multiple RT-PCR). Mutations were detected with a panel of 112 mutational genes using next-generation sequencing (NGS). FLT3-ITD, NPM1 and CEBPA mutation were detected by PCR Sanger sequencing.

Results: Overall, 30 WT1 mutations were detected in 29 of the 220 patients (13.18%) screened. These mutations clustered overwhelmingly in exon 7 (16 mutations in 15 patients), but they were also detected in exon 8 (n=6) and exon 9 (n=8). WT1-mutated (WT1^{mut}) patients presented with lower platelet counts ($P=0.0481$), higher WT1 expression ($P=0.0371$), and a negative trend with the M5 subtype ($P=0.07$) compared to WT wild-type (WT^{wt}) patients. WT1 mutations were found to be significantly more frequent in AML patients with double-mutated CEBPA (CEBPA^{dm}) than in AML patients with single-mutated CEBPA ($P=0.043$). Of note, the concomitant mutations in epigenetic regulators were inversely correlated with WT1 mutations ($P=0.003$). Patients with newly diagnosed WT1^{mut} AML had inferior relapse-free survival, event-free survival and overall survival compared with patients with WT1^{wt} AML ($P=0.002$, 0.004, and 0.010, respectively).

Conclusion: Our data showed that WT1 mutations are frequently identified in CEBPA^{mut} AML, especially in CEBPA^{dm} AML. Patients with WT1 mutations show distinct clinical features, a spectrum of comutations, and poor prognosis compared to WT1 wild-type patients.

Introduction

CCAAT/enhancer binding protein a (CEBPA) plays a crucial role in the repression of self-renewal, cell cycle arrest, and myeloid differentiation of mature neutrophils during normal hematopoiesis[1, 2]. Mutations in the CEBPA gene can occur across the whole coding region and have been described in approximately 6–24% of all acute myeloid leukemia (AML) patients[3–5]. CEBPA-mutated (CEBPA^{mut}) patients can be subdivided into two different subgroups: (1) AML carrying one mutation on one allele (single-mutated CEBPA, CEBPAsm) and (2) AML with two CEBPA mutations (double-mutated CEBPA, CEBPA^{dm}), typically showing an N-terminal mutation and a basic leucine zipper gene mutation. However, only patients who harbor CEBPA double mutations are associated with favorable outcomes[6, 7]. In the 2016 World Health Organization (WHO) classification of leukemia, AML with CEBPA^{dm} was included as a distinctive disease entity due to its unique biological and clinical profiles[8].

With the application of next-generation sequencing (NGS), > 85% of CEBPA^{mut} AML patients were observed to have other known concurrent mutations, such as WT1, CSF3R, and GATA2[9, 10]. However, studies have shown conflicting data on the impact of CEBPA mutations on prognosis when identified with other concomitant mutations[9–11]. These findings suggest that it is necessary to further explore co-mutations in the context of CEBPA mutations in AML.

Recently, mutations in the Wilms tumor 1 (WT1) gene have been observed in adult and childhood leukemia, commonly in AML[12, 13]. In several studies investigating more than 1000 AML samples total, mutations in WT1 were found in 2%-8.3% of the patients[12–15]. However, in AML patients with CEBPA^{dm}, the frequency of WT1 mutations increased to 13.6%-18.52%[10, 14]. To the best of our knowledge, few data are available regarding the impact of WT1 mutations on CEBPA^{mut} AML. We report here the frequency and types of WT1 mutations and their association with clinical features and outcomes in a cohort of 220 CEBPA^{mut} AML patients.

Patients And Methods

Patients

A total of 220 selected *de novo* AML patients with CEBPA mutations from five medical institutions of hematology (Affiliated Changzhou Second Hospital of Nanjing Medical University, Affiliated Hospital of Jiangnan University, Wuxi No. 2 People's Hospital, The Third Affiliated Hospital of Soochow University, and The First Affiliated Hospital of Soochow University) between August 2014 and November 2020 were analyzed. Of the patients, 114 were male and 106 were female. The median age was 39 years (range: 18-88 years), with 190 patients being <60 years and 30 patients being ≥60 years. According to the French–American–British (FAB) classification, 14, 96, 33, 62, 5, and 10 patients were diagnosed with M1, M2, M4, M5, M6 and undetermined types, respectively. All patients gave their written informed consent for genetic analysis and for the use of the laboratory results for scientific studies. The study was approved by the research ethics board of each participating hospital and adhered to the tenets of the Declaration of Helsinki.

Cytomorphology, cytogenetics and immunophenotyping

Cytomorphologic assessment was performed by May-Grünwald-Giemsa staining, myeloperoxidase (MPO) reaction, and nonspecific esterase (NSE) staining using alpha-naphthyl-acetate following the FAB and WHO classifications. Karyotyping by the G or R-banding method was performed following standard methods on bone marrow (BM) cells after short culture. When possible, at least 20 metaphases were analyzed for each case. Immunophenotyping was performed as previously described[16].

Mutation screening

BM smear or peripheral blood (PB) samples at the time of initial diagnosis were collected. A sensitive next-generation amplicon deep-sequencing assay was used with an Illumina next-generation sequencer. A high depth of coverage (1000×) was obtained for 112 genes, including the whole coding regions that are known to be frequently mutated in hematologic malignancies. Altered DNA sequences were deemed mutations or variants in IGV software analysis and were identified by the COSMIC database and needed to exclude dbSNPs. Mutations in NPM1, FLT3, and CEBPA were identified by polymerase chain reaction (PCR) followed by direct Sanger sequencing, as previously described[17-19]. The mutations of the 112 genes analyzed in this study are listed in supplemental table 1 (Table S1).

Fusion gene and WT1 mRNA expression

Forty-one fusion genes were detected by multiple reverse transcription PCR amplification, which was performed as 8 parallel multiplex reactions on a 7500 Real-time PCR System (Applied Biosystems). We evaluated WT1 mRNA expression in BM or PB using a WT1 mRNA assay kit (Otsuka Pharmaceutical, Tokyo, Japan), as previously reported[20].

Treatment and outcome

Of the 220 AML patients with CEBPA mutations, 161 patients received a standard chemotherapy regimen (DA/IA, daunorubicin/idarubicin 60/10 mg/m² d1-3, cytarabine 100 mg/m² d1-7) for induction therapy, 31 patients were prescribed a reduced intensity DA/IA (daunorubicin/idarubicin 45/6-8 mg/m² d1-3, cytarabine 100 mg/m² d1-7) regimen, 13 patients received an HAD regimen (homoharringtonine 2 mg/m² d1-7, cytarabine 100 mg/m² d1-7, daunorubicin 40 mg/m² d1-3), 14 patients received decitabine-based induction due to older age or underlying comorbidity, and 1 patient discontinued chemotherapy because of severe infection and respiratory failure.

Complete remission (CR) was observed in patients after one course of induction chemotherapy. Relapse-free survival (RFS) was defined as the time from diagnosis to relapse or the last follow-up in CR. Event-free survival (EFS) was calculated as the time interval from the date of diagnosis to the date of first evidence of an event, which included relapse, progression, recurrence, change, and death. Overall survival (OS) was defined as the time from diagnosis to death or the last follow-up.

Statistical analysis

Student's t-test was used to analyze continuous factors with a normal distribution, and the Mann–Whitney U test was used for comparisons of data that failed the normality test between different groups. Analysis of categorical variables between different cohorts was performed by the chi-square test and Fisher's exact test. Survival curves were calculated for RFS, OS and EFS based on the Kaplan–Meier method and compared using the two-sided log rank test. Patients who had undergone allogeneic bone

marrow transplantation were censored at the time of transplantation. SPSS software version 20.0 (SPSS version 20.0; SPSS Institute, Chicago, IL, USA) was used for statistical analysis, and the results were considered significant at $P < 0.05$.

Results

Patients' characteristics

A total of 99 patients (45.0%) harboring single CEBPA mutations and 121 patients (55.0%) with biallelic double mutations were identified. Compared with CEBPAsm AML patients, CEBPA^{dm} AML patients had lower platelet counts (median: $29 \times 10^9/L$ vs. $43 \times 10^9/L$, $P=0.002$), higher hemoglobin levels (median: 93 g/L vs. 82 g/L, $P=0.0162$), and lower WT1 expression levels (median: 12.385% vs. 42.35%, $P=0.000$). A total of 52.07% (63/121) of CEBPA^{dm} AML patients presented with the FAB-M2 subtype, which occurred at a distinctively higher rate than that in CEBPAsm patients (52.07% vs. 33.33%, $P=0.0053$). AML patients with CEBPAsm were more likely to show the M5 subtype than those with CEBPA^{dm} (41.4% vs. 17.35%, $P=0.0001$). No significant differences were identified regarding sex, age, white blood cell (WBC) count or cytogenetic karyotype between the CEBPAsm and CEBPA^{dm} AML groups. Table 1 lists different categories of clinical information of the enrolled patients.

Frequency and types of WT1 mutations

Overall, 30 WT1 mutations were detected in 29 of the 220 patients (13.18%) screened. WT1 mutations occurred significantly more frequently in CEBPA^{dm} AML (21/121, 17.36%) than in CEBPAsm (8/99, 8.08%) AML patients ($P=0.043$). Mutations clustered overwhelmingly in exon 7 (16 mutations in 15 patients), but they were also detected in exon 8 (n=6) and exon 9 (n=8). One patient presented with two kinds of mutations (no. 29). The amino acid changes and mutation information of the WT1 gene are shown in Fig. 1 and Table 2.

Association of WT1 mutations with clinical characteristics

WT1-mutated (WT1^{mut}) patients had lower platelet counts (median: $25 \times 10^9/L$ vs. $38 \times 10^9/L$, $P=0.0481$), higher WT1 expression levels (median: 29.72% vs. 16.81%, $P=0.0371$), and a negative trend for the M5 subtype (median: 17.24% vs. 29.84%, $P=0.07$) compared to WT wild-type (WT^{wt}) patients. No significant differences were identified regarding sex, age, WBC count, hemoglobin, or cytogenetic karyotype between the WT1^{mut} and WT1^{wt} groups. The clinical characteristics are shown in table 1.

Companion gene mutations

Based on NGS, we detected 59 mutated genes by screening the 112-gene panel. In addition to WT1, 33 of the 59 genes could be categorized as signaling pathways, epigenetic regulators, transcription factors, spliceosomes, tumor suppressors, chromatin modifiers, and cohesin. A total of 94.54% (208/220) of CEBPA mutations were accompanied by additional mutations and frequently co-occurred with FLT3-ITD (n=39, 17.73%), NRAS (n=34, 15.45%), GATA2 (n=34, 15.45%), RUNX1 (n=30, 13.64%), WT1 (n=29, 13.18%), FAT1 (n=29, 13.18%), TET2 (n=23, 10.45%), CSF3R (n=20, 9.09%), KIT (n=21, 9.54%) and NOTCH2 (n=18, 8.18%). Other genes had a mutation prevalence of <8%. Functionally, mutations involved in signaling pathways (n=123, 55.91%) and transcription factors (n=47, 21.36%) were found frequently in CEBPA^{mut} AML patients.

Among WT1-mutated patients, the most common co-mutation was FLT3-ITD (n=7, 24.14%), followed by NRAS (n=5, 17.24%), CSF3R (n=4, 13.79%), GATA2 (n=4, 13.79%), KIT (n=4, 13.79%), and FAT1 (n=3, 10.34%). Fewer common co-mutations (2%–10.25%) were found in NPM1, SF3B1, NOTCH1/2, PTPN11, JAK2 and others. The most frequent functional pathway was signaling pathways in as many as 62.07% of cases. Notably, no mutations in TET2, IDH1/2, or DNMT3A were identified in WT^{mut} patients. The concomitant mutations in epigenetic regulators were inversely correlated with WT1 mutations ($P=0.003$). There were no differences in the incidences of FLT3-ITD, NRAS, CSF3R, GATA2, PTPN11, SF3B1 and NOTCH1/2 between WT^{mut} and WT^{wt} patients. The difference in the mutational spectrum between WT-mutated and WT1 wild-type AML is shown in table 3. The frequencies of the detected gene mutations and gene functional groups are shown in Figure 2.

Prognostic impact of WT1 mutations in the context of CEBPA double mutations

Of 121 patients with CEBPA double mutations, 111 patients were evaluable for complete remission (CR) after the first induction therapy. No differences were observed in the CR rate between patients with or without WT1 mutations in CEBPA^{dm} AML (89.5% vs. 91.3%, $P=1.000$). The median follow up was 30.5 (range 0.5-60.6) months. We next investigated the impact of WT1 mutations on survival. WT1-mutated patients had significantly inferior RFS, EFS, and OS than WT1 wild-type patients (3-year RFS rate, 40% vs. 70%, median, 8.9 months vs. NR, $P=0.002$; 3-year EFS rate, 36% vs. 65%, median, 9.9 months vs. NR, $P=0.004$; 3-year OS rate, 48% vs. 72%, 20.4 months vs. NR, $P=0.010$) (Fig. 3a/b/c and Table 4).

Discussion

The WT1 gene, located on chromosome 11p13, encodes a zinc-finger protein that exists in multiple isoforms and functions as a transcription factor involved in cellular growth, proliferation and differentiation and may therefore act as an oncogene[21–23]. The WT1 gene was initially identified as a tumor suppressor gene linked with nephroblastoma[24, 25]. Over the past years, numerous studies have highlighted that WT1 is aberrantly expressed or mutated in hematopoietic malignancies, including acute leukemia (AL), myelodysplastic syndrome (MDS) and chronic myelogenous leukemia (CML)[26–30]. However, few data are available regarding the frequency of WT1 mutations and their impact on CEBPA^{mut} AML.

Recently, WT1 zinc-finger mutations (exons 7–10) were detected in 8.3% of pediatric AML patients[12], 10.6% of AML patients with a normal karyotype (NK-AML) [32], and 13.6% of biallelic CEBPA^{mut} AML patients[14]. We revealed that WT1 mutations occurred in 17.36% of CEBPA^{dm} AML patients in exons 7, 8 and 9. This percentage of cases is slightly higher than that reported in CEBPA^{dm} AML by Krauth MT et al. (13.16%) [14]. The difference could be explained by the fact that in the present study, we analyzed WT1 mutations in all exons, while in the study by Krauth MT et al., only mutations in exons 7 and 9 were analyzed. Similar to the findings of Fasan A et al.[3], we also found that WT1 was significantly more frequently mutated in CEBPA^{dm} patients than in CEBPAsm patients, suggesting that the implication of the WT1 mutation may differ in CEBPA^{dm} and CEBPAsm AML.

Zidan et al.[31] observed that WT1 mutations were significantly associated with younger age, and no associations were detected between the presence of WT1 mutations and sex, hemoglobin concentration, platelet count, and FAB subtype at diagnosis in NK-AML. Haider I and colleagues[13] showed that there were also no statistically significant differences in clinical parameters, such as hemoglobin level, WBC count and platelet count, between WT1^{mut} and WT1^{wt} patients. Krauth MT et al.[14] found that WT1 mutations were more frequent in females than in males and in patients < 60 years than in patients ≥ 60 years (95/1,449; 6.6% vs. 80/1,708; 4.7%; $P=0.014$). The median age of patients with WT1 mutations was lower (55.4 years compared to 67.5 years for WT1 wild-type) in AML; when the study population was limited to the subgroup with CEBPA^{dm} AML, 16.1% of WT1^{mut} patients were < 60 years old and 11.1% of WT1^{mut} patients were ≥ 60 years old ($P=0.317$). In the present study, there was no significant difference between WT1^{mut} and WT1^{wt} AML patients with regard to age, sex, WBC count, and Hb. However, WT1^{mut} patients showed a significantly lower platelet count and a trend toward a negative association with FAB-M5 compared to WT1^{wt} patients. The reason for these differences with the above studies might be due to the age distribution of the cohort, the patient enrollment criteria, the small sample size, the regions, or the research background.

Becker H et al. found that in WT1-mutated patients, FLT3-ITD more frequently occurred in older patients with primary NK-AML[33]. Krauth MT and colleagues found that WT1 mutations were rarely concomitant with DNMT3A (4.4%, $P=0.014$), ASXL1 (1.7%, $P=0.001$), IDH2R140 (1.7%, $P=0.001$) and IDH1R132 (0.9%, $P=0.001$) mutations[14]. In this study, concomitant mutations in epigenetic regulators were inversely correlated with WT1 mutations ($P=0.003$). This observation suggests that WT1 mutations are anticorrelated with epigenetic regulator mutations in CEBPA^{mut} AML. Rampal R et al. observed that WT1-mutant AML patients had reduced 5-hydroxymethylcytosine (5hmC) levels, similar to TET2/IDH1/IDH2 mutant AML patients[34]. Wang Yiping et al. showed that WT1 physically interacts with and recruits TET2 to its target genes to activate their expression. The interaction between WT1 and TET2 is disrupted by multiple AML-derived TET2 mutations[35]. These findings, including our results, suggest that mutations in WT1 and TET2 may have interactive effects. Further studies are needed to reveal the interaction mechanism between WT1 and epigenetic regulators in the context of CEBPA mutations.

WT1 overexpression remains a valuable molecular marker in AML for predicting the survival of patients without any leukemia-specific mutation, especially if NGS is not routinely available[26, 27]. Nagasaki J et al. showed that OS and progression-free

survival (PFS) were significantly worse in elderly MDS patients with higher levels of WT1 at diagnosis[36]. Lyu Xiaodong et al. showed that WT1 overexpression by itself is an independent and negative indicator for predicting the CR rate, DFS and OS of NK-AML patients[26]. Our data revealed that WT1^{mut} patients showed significantly higher levels of WT1 expression than WT1^{wt} patients, but further investigation is needed to explore the effect of WT1 expression on prognosis in the background of WT^{mut}/CEBPA^{mut} AML.

There are still some controversies regarding the prognostic significance of WT1 mutations in patients with AML. Studies from Hou HA and Virappane P demonstrated that the WT1 mutation was associated with poor prognosis in NK-AML and nonselective AML patients[37, 38]. Tien Feng-Ming and colleagues found that WT1^{mut} patients with CEBPA^{dm} AML tended to have a lower CR rate, a higher relapse rate, and significantly shorter OS and disease-free survival (DFS) than those with wild-type WT1[39]. Krauth MT et al. indicated that WT1^{mut} was associated with shorter EFS in NK-AML patients, but it had no impact on prognosis in subgroups with high WT1^{mut} incidences (CEBPA^{dm}, PML-RARA)[14]. An analysis based on the TCGA database showed that the WT1-mutated group had lower rates of CR but higher rates of minimal residual disease (MRD) after one and two courses of induction chemotherapy. In NK-AML pediatric patients, those with WT1 mutations had significantly worse OS and EFS in both univariate and multivariate survival analyses[40]. In our study, WT1^{mut} patients had significantly inferior 3-year RFS, EFS and OS rates than WT1^{wt} patients in the context of CEBPA double mutations. This result is consistent with that reported by Tien Feng-Ming et al. but different from that reported by Krauth MT et al. This inconsistency may be attributed to differences in the characteristics of the populations investigated, such as sex, karyotype, age, combination with other mutations such as FLT3-ITD, or different ethnicities.

In conclusion, this study confirms that WT1 mutations are frequently identified in CEBPA^{mut} AML patients, especially in CEBPA^{dm} AML patients. Patients with WT1 mutations show distinct clinical features, a spectrum of co-mutations, and poor prognosis compared to WT1 wild-type patients.

Declarations

Ethics approval and consent to participate

All patients gave their written informed consent for genetic analysis and for the use of the laboratory results for scientific studies. The study was performed in accordance with the World Medical Association Declaration of Helsinki and Ethics Committee approval of Changzhou No.2 People's Hospital was obtained ([2015]KY004-1).

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

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

Competing interests

The authors declare no competing financial interests.

Funding

This work was partly supported by grants from Young Scientists Foundation of Changzhou No. 2 People's Hospital (NO.2019K002), Science and Technology Project of Changzhou Health Committee (NO.QN202035), Science and Technology Development Fund project of Nanjing Medical University(No. NMUB201), Major Science Project of Changzhou Health Commission(ZD202018).

Authors' contributions

TW, HYH, BW, WQ, PW and HYC had contributions in collecting clinical information of selected patients. ZW, BW, HYC and PW completed the entire experimental processes. TW and HYC completed all statistical analyses and constructed figures. TW wrote

the manuscript. HYC and XZL were the main contributors in revising the manuscript. Other authors provided some advice on the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors thank all the physicians who provided leukemia samples and clinical data.

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Tables

Table 1. Pretreatment clinical characteristics according to CEBPA and WT1 mutation status

Variable	Total (n=220)	CEBPA sm (n=99)	CEBPA ^{dm} (n=121)	<i>P1</i>	WT ^{mut} (n=29)	WT1 ^{wt} (n=191)	<i>P2</i>
Gender							
Male, n (%)	114 (51.8%)	46 (46.5%)	68 (56.2%)	0.1506	12 (41.38%)	102 (53.4%)	0.2273
Female, n (%)	106 (48.18%)	53 (53.5%)	53 (43.8%)		17 (58.62%)	89 (46.6%)	
Age (year)							
Median (range)	39 (18-88)	38 (18-76)	40 (18-88)	0.2371	43 (18-66)	39 (18-78)	0.7165
WBC (×10 ⁹ /L)							
Median (range)	16.21 (0.61-287.6)	12 (0.61-195.7)	18.67 (1.7-287.6)	0.3663	18.67 (0.8-195.7)	15.7 (0.61-287.6)	0.6242
Hb (g/L)							
Median (range)	87.5 (35-158)	82 (35-151)	93 (55-158)	0.0162	91 (35-158)	87 (49-147)	0.5934
PLT (×10 ⁹ /L)							
Median (range)	36.5 (5-389)	43 (11-389)	29 (5-322)	0.002	25 (5-129)	38 (7-389)	0.0481
Cytogenetic karyotype							
normal, n (%)	167 (76.6%)	73 (74.5%)	94 (78.3%)	0.5049	22 (75.9%)	145 (76.72%)	0.9192
abnormal, n (%)	51 (23.4%)	25 (25.5%)	26 (21.7%)		7 (24.1%)	44 (23.28%)	
NA	2	1	1		0	2	
WT1 expression (%)							
Median (Range)	18.74 (0.01-317)	42.35(0.01-213.07)	12.385 (0.21-317)	0.000	29.72 (8.44-294)	16.81 (0.01-317)	0.0371
NA	59				8	51	
FAB classification							
M1, n(%)	14 (6.36%)	6 (6.06%)	8 (6.61%)	0.8768	1 (3.44%)	13 (6.8%)	0.3
M2, n(%)	96 (43.63%)	33 (33.33%)	63 (52.07%)	0.0053	16 (55.17%)	80 (41.88%)	0.1788
M4, n(%)	33 (15.0%)	11 (11.11%)	22 (18.2%)	0.1440	5 (17.24%)	28 (14.66%)	0.2
M5, n(%)	62 (28.18%)	41 (41.4%)	21(17.35%)	0.0001	5 (17.24%)	57 (29.84%)	0.07
M6, n(%)	5 (2.27%)	3 (3.03%)	2(1.65%)	0.28	0	5 (2.62%)	1
Undetermined,n(%)	10 (4.54%)	5 (5.05%)	5 (4.13%)	0.24	2 (6.89%)	8 (4.19%)	0.26

Abbreviations: *P*, *p*-value, *P*1, CEBPAsm AML group vs.CEBPA^{dm} AML group; *P*2, WT1^{mut} AML group vs. WT1^{wt} AML group; n, number of patients; AML,acute myeloid leukemia; F, female; M, male; WBC, white blood cell count, HB, hemoglobin; PLT, platelet; NA, not available.

Table 2 WT1 mutations information in 29 patients with CEBPA^{mut} AML

Case No. /Sex/Age(y)	WBC (×10 ⁹ /L)	HB (g/L)	PLT (×10 ⁹ /L)	FAB subtype	Karyotype	WT1 ^{mut} a.a. change	Multiple RT-PCR	WT1 expression(%)
1/Male/54	10.27	87	25	M2	47,XY, +22	S435X	(-)	76.57
2/Male/18	37	66	50	M2	47,XY, +8, del(9) (q13q22)	A382fs	(-)	NA
3/Female/49	62.14	74	66	M4	46,XX, der(7)	R440fs	(-)	28.15
4/Male/52	0.8	62	38	M5	45,XY, -7	R462P	(-)	294
5/Female/23	18.9	102	44	M2	45,XX, -8	A382fs	(-)	29.72
6/Female/43	37	91	18	Undetermined	46,XX, del(9) (q13q22)	R370fs	(-)	78.03
7/Female/66	17.4	63	5	M2	46,XX, del(9) (q13q22)	A382fs	(-)	184.66
8/Female/34	195.7	57	23	M4	46,XX	R462L	(-)	NA
9/Female/40	15.0	93	57	M4	46,XX	S381X	(-)	10.66
10/Male/54	46.9	98	9	M4	46,XY	S381X	(-)	12.82
11/Male/20	4.0	61	27	M1	46,XY	S381X	(-)	NA
12/Female/18	86.0	104	20	M2	46,XX	R462P	(-)	NA
13/Female/46	11.9	79	29	M5	46,XX	K414fs	(-)	8.44
14/Female/62	4.11	65	25	Undetermined	46,XX	H465Y	(-)	NA
15/Male/60	10.39	132	26	M5	46,XY	A382fs	(-)	NA
16/Male/57	8.15	106	17	M2	46,XY	R462P	(-)	67.22
17/Male/66	27.88	119	16	M2	46,XY	S435X	(-)	47.44
18/Female/30	90.13	105	24	M5	46,XX	R434H	(-)	27.89
19/Male/62	8.96	103	27	M2	46,XY	S381X	(-)	16.7
20/Female/19	50.31	63	23	M2	46,XX	R380G	(-)	22.51
21/Female/50	7.07	88	16	M2	46,XX	A382fs	(-)	204.18
22/Female/45	35	128	23	M4	46,XX	V379fs	(-)	160.35
23/Male/22	50.24	35	21	M2	46,XY	H465G	(-)	18.84
24/Male/29	4.44	158	129	M2	46,XY	H445fs	(-)	21.27
25/Female/20	18.67	102	23	M2	46,XX	R462L	(-)	82.69
26/Male/19	76.79	91	38	M5	46,XY	R380fs	(-)	42.02
27/Male/54	9.28	93	38	M2	46,XY	C423Y	(-)	17.03
28/Female/30	2.09	85	54	M2	46,XX	R462P	(-)	NA
29/Female/30	79.74	63	53	M2	46,XX	R380G, S381fs	(-)	NA

Abbreviations: AML, acute myeloid leukemia (AML) ; F, female; M, male; WBC, white blood cell count, HB, hemoglobin; PLT, platelet; a.a., amino acid;

NA, not available; (+), positive; (-), negative; RT-PCR, reverse transcription polymerase chain reaction

Table 3. Concomitant gene abnormalities according to WT1 mutation status

Mutational genes	Total (n=220)	WT1 ^{mut} AML (n=29)	WT1 ^{wt} AML (n=191)	<i>P</i>
Signaling pathways, n (%)	123 (123/220, 55.91%)	17 (18/29, 62.07%)	106 (106/191, 55.59%)	0.506
c-KIT, n (%)	21 (21/220, 9.54%)	4 (4/29, 13.79%)	17 (17/191, 8.9%)	0.493
NRAS, n (%)	34 (34/220, 15.45%)	5 (5/29, 17.24%)	29 (29/191, 15.18%)	0.784
KRAS, n (%)	7 (7/220, 3.18%)	0	7 (7/191, 3.66%)	0.598
FLT3-ITD, n (%)	39 (39/220, 17.73%)	7 (7/29, 24.14%)	32 (32/191, 16.75%)	0.332
CSF3R, n (%)	20 (20/220, 9.09%)	4 (4/29, 13.79%)	16 (16/191, 8.38%)	0.311
RELN, n (%)	13 (13/220, 5.91%)	2 (2/29, 6.89%)	11 (11/191, 5.75%)	0.268
NOTCH1, n (%)	11 (11/220, 5.0%)	1 (1/29, 3.45%)	10 (10/191, 5.23%)	1
NOTCH2, n (%)	18 (18/220, 8.18%)	2 (2/29, 6.89%)	16 (16/191, 8.38%)	1
JAK2, n (%)	4 (4/220, 1.82%)	1 (1/29, 3.45%)	3 (3/191, 1.57%)	0.434
SH2B3, n (%)	8 (8/220, 3.64%)	1 (1/29, 3.45%)	7 (7/191, 3.66%)	1
PTPN11, n (%)	11 (11/220, 5.0%)	2 (2/29, 6.89%)	9 (9/191, 4.71%)	1
Epigenetic regulators, n (%)	41 (41/220, 18.64%)	0	41 (41/191, 21.46%)	0.003
TET2, n (%)	23 (23/220, 10.45%)	0	23 (23/191, 12.04%)	0.05
IDH1, n (%)	9 (9/220, 4.09%)	0	9 (9/191, 4.71%)	0.610
IDH2, n (%)	3 (3/220, 1.36%)	0	3 (3/191, 1.57%)	1
DNMT3A, n (%)	14 (14/220, 6.36%)	0	14 (14/191, 7.33%)	0.225
Transcription factors, n (%)	47*(47/220, 21.36%)	4*(4/29, 13.79%)	43*(43/191, 22.5%)	0.286
ETV6, n (%)	1 (1/220, 0.45%)	0	1 (1/191, 0.52%)	1
RUNX1, n (%)	30 (30/220, 13.64%)	0	6 (6/191, 3.14%)	1
GATA2, n (%)	34 (34/220, 15.45%)	4 (4/29, 13.79%)	30 (30/191, 15.71%)	1
SETBP1, n (%)	9 (9/220, 4.09%)	0	9 (9/191, 4.71%)	0.607
CEBPA ^{dm} , n (%)	121 (121/220, 55%)	21 (21/29, 72.4%)	100 (100/191, 52.36%)	0.043
CEBPA Sm , n (%)	99 (99/220, 45.0%)	8 (8/29, 27.58%)	91 (91/191, 47.64%)	
Spliceosomes, n (%)	9 (9/220, 4.09%)	1 (1/29, 3.45%)	8 (8/191, 4.19%)	1
SRSF2, n (%)	1 (1/220, 0.45%)	0	1 (1/191, 0.52%)	1
SF3B1, n (%)	6 (6/220, 2.73%)	1 (1/29, 3.45%)	5 (5/191, 2.62%)	0.576
ZRSR2, n (%)	2 (2/220, 0.90%)	0	2 (2/191, 1.05%)	1
Tumour suppressors, n (%)	38 (38/220, 17.27%)	3 (3/29, 10.34%)	35 (35/191, 18.32%)	0.429
TP53, n (%)	9 (9/220, 4.09%)	0	9 (9/191, 4.71%)	0.610
FAT1, n (%)	29 (29/220, 13.18%)	3 (3/29, 10.34%)	26 (26/191, 13.61%)	0.775
Cohesin, n (%)	(7/191, 3.66%)	0	7 (7/191, 3.66%)	0.598
RAD21, n (%)	5 (5/220, 2.27%)	0	5 (5/191, 2.62%)	1
SMC1A, n (%)	1 (1/220, 0.45%)	0	1 (1/191, 0.52%)	1
SMC3, n (%)	1 (1/220, 0.45%)	0	1 (1/191, 0.52%)	1

Chromatin modifiers, n (%)	24 (24/220, 10.91%)	1 (1/29, 3.45%)	23 (23/191, 12.04%)	0.215
ASXL1, n (%)	10 (10/220, 4.55%)	0	10 (10/191, 5.23%)	0.366
EZH2, n (%)	8 (8/220, 3.64%)	1 (1/29, 3.45%)	8 (8/191, 4.19%)	1
BCOR, n (%)	5 (5/220, 2.27%)	0	1 (1/191, 0.52%)	1
BCORL1, n (%)	4 (4/220, 1.82%)	0	4 (4/191, 2.09%)	1

Abbreviations: *P*, *p*-value, WT1^{mut} AML group vs. WT1^{wt} AML group; n, number of patients; AML, acute myeloid leukemia; *The WT1 mutation was excluded.

Table 4. Univariable outcome analyses according to WT1 mutation status

Clinical endpoint	Total (n=121)	WT1 ^{mut} /CEBPA ^{dm} (n=21)	WT1 ^{wt} /CEBPA ^{dm} (n=100)	<i>P</i>
CR rate, %	101/111, 91.0%	17/19, 89.5%	84/92, 91.3%	1.000
missing, n	10	2*	8	
RFS				0.002
Median, mo (95% CI)	NR	8.9 (0-21.7)	NR	
3-year DFS, % (SE)	66 (5)	40 (14)	70 (6)	
missing, n	19	4	15	
EFS		2	7	0.004
Median, mo (95% CI)	NR	9.9 (8.4-11.4)	NR	
3-year EFS, % (SE)	61 (5)	36 (12)	65 (5)	
missing, n	9	2	7	
OS				0.010
Median, mo (95% CI)	NR	20.4 (NA-NA)	NR	
3-year OS, % (SE)	68 (5)	48 (13)	72 (6)	
missing, n	9	2	7	

Abbreviations: CR, complete remission; n, numbers; RFS, relapse-free survival; EFS, event-free survival; OS, overall survival; mo, months; NA: not available; *P*, *p*-value; NR, not reached; SE, standard error. *P*-value is from Fisher's exact test for CR rate and from the log-rank test for survival end-points; Statistically significant results are highlighted in bold font. *, including an early death case during induction.

Figures

Figure 1

Schematic showing the locations of WT1 mutations found in CEBPA mutated AML. One patient had both the R380G and S381fs mutations.

Figure 2

Spectrum of acquired co-mutations in 29 WT1 mutated patients with CEBPA mutated AML divided into different mutational categories. Each bar represents a distinct driver gene.

Figure 3

Influence of mutations in WT1 on survival. a. Kaplan–Meier estimates of Relapse-free survival (RFS) , b. event-free survival (EFS) and c. overall survival (OS) in newly diagnosed patients with CEBPA double mutated AML, according to the presence or absence of mutations in WT1.

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