Concomitant WT1 mutations predict poor prognosis in acute myeloid leukemia patients with double mutant CEBPA

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Supplementary Table 1. Clinical characteristics of AML patients according to the status of *CEBPA* mutations

<table>
<thead>
<tr>
<th>Variables</th>
<th>CEBP wt N=654</th>
<th>CEBP sm N=33</th>
<th>CEBP dm N=69</th>
<th>P value&lt;sup&gt;c&lt;/sup&gt;</th>
<th>P value&lt;sup&gt;d&lt;/sup&gt;</th>
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<tr>
<td>Sex</td>
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<td></td>
<td></td>
<td>0.685</td>
<td>0.909</td>
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<tr>
<td>Male</td>
<td>372</td>
<td>20</td>
<td>41</td>
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<tr>
<td>Female</td>
<td>282</td>
<td>13</td>
<td>28</td>
<td></td>
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<tr>
<td>Age, years&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49 (23-82)</td>
<td>52 (18-85)</td>
<td>40 (17-90)</td>
<td>&lt;0.0001</td>
<td>0.038</td>
</tr>
<tr>
<td>Lab&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>WBC (k/μL)</td>
<td>6.39 (0.3-212.7)</td>
<td>37.3 (0.98-271.5)</td>
<td>44.2 (2.41-387.4)</td>
<td>&lt;0.0001</td>
<td>0.739</td>
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<tr>
<td>Hb (g/dL)</td>
<td>8.1 (4.2-13.9)</td>
<td>8.2 (4.5-10.9)</td>
<td>8.8 (3-13.6)</td>
<td>0.006</td>
<td>0.045</td>
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<tr>
<td>Platelet (k/μL)</td>
<td>28 (5-122)</td>
<td>28 (10-712)</td>
<td>41 (5-204)</td>
<td>0.077</td>
<td>0.707</td>
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<tr>
<td>PB Blast (k/μL)</td>
<td>0.77 (0-134.0)</td>
<td>20.9 (0.21-260.6)</td>
<td>29.2 (0.72-371.9)</td>
<td>0.006</td>
<td>0.634</td>
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<tr>
<td>FAB&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td></td>
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<tr>
<td>M0</td>
<td>18 (2.8)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.403</td>
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<tr>
<td>M1</td>
<td>114 (17.6)</td>
<td>11 (33.3)</td>
<td>39 (56.5)</td>
<td>&lt;0.0001</td>
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<tr>
<td>M2</td>
<td>213 (32.4)</td>
<td>17 (51.5)</td>
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<td>0.237</td>
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<tr>
<td>M3</td>
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<td>M4</td>
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<tr>
<td>M6</td>
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<td>1 (3.0)</td>
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<td>0.162</td>
<td>0.324</td>
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<tr>
<td>Undetermined</td>
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<td>0 (0)</td>
<td>0 (0)</td>
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<td>Cytogenetics&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Favorable</td>
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<tr>
<td>Intermediate</td>
<td>392 (61.9)</td>
<td>30 (96.8)</td>
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<tr>
<td>Unfavorable</td>
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<td>1 (3.2)</td>
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<td>Recurrent geneic abnormalities</td>
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<tr>
<td>inv(16)</td>
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<td>0</td>
<td>0</td>
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<td>APL</td>
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<td>0.009</td>
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<td>-</td>
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<tr>
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<td>&gt;0.999</td>
<td>-</td>
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<td>0</td>
<td>-</td>
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<td>&lt;0.0001</td>
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<td>0</td>
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<td>Abbreviation</td>
<td>Median (range)</td>
<td>Number of patients (%)</td>
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<td></td>
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<td>------------------------</td>
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<td><em>NPM1</em></td>
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<td>Myelodysplasia-related change</td>
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<td>24</td>
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<td>&lt;0.0001</td>
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</tbody>
</table>

2017 ELN classification

**Favorable**

- t(8;21) 57 0 0 0.011 < 0.0001
- inv(16) 27 0 0 0.099 -
- *NPM1*+/*FLT3-ITD- 78 4 0 0.002 0.01
- *CEBPA*^sm^ 0 0 69 < 0.0001 < 0.0001

**Intermediate**

- *NPM1*+/*FLT3-ITD+ 53 4 0 0.014 0.01
- t(9;11) 9 0 0 > 0.999 -
- Other cytogenetics 107 17 0 < 0.0001 < 0.0001

**Unfavorable**

- t(6;9) 3 0 0 > 0.999 -
- t(v;11q23) 11 1 0 0.612 0.324
- t(9;22) 1 0 0 > 0.999 -
- inv(3) 3 0 0 > 0.999 -
- -5/-7 19 0 0 0.384 -
- complex 72 1 0 0.004 0.324
- *NPM1*-//*FLT3-ITD+ 50 1 0 0.01 0.324
- *RUNX1* 60 0 0 0.009 -
- *ASXL1* 35 4 0 0.04 0.01
- *TP53* 10 1 0 0.610 0.324

Abbreviations: APL, acute promyelocytic leukemia; *CEBPA*^sm^, *CEBPA* single mutation; *CEBPA*^dm^, *CEBPA* double mutation; NOS, not otherwise specified; PB, peripheral blood;

*a* Median (range)

*b* Number of patients (%)

*c* *CEBPA*^sm^ patients vs *CEBPA* wild-type patients

*d* *CEBPA*^sm^ patients vs *CEBPA*^dm^ patients
**Supplementary Table 2. Characterization of the mutations in the CEBPA coding region**

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<tr>
<th>UPN</th>
<th>Age/Sex</th>
<th>Karyotype</th>
<th>N terminal DNA change</th>
<th>N terminal Protein change</th>
<th>C terminal DNA change</th>
<th>C terminal Protein change</th>
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<td>CN</td>
<td>c.324C&gt;G</td>
<td>p.Y108X</td>
<td>c.992T&gt;G</td>
<td>p.L331R</td>
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<td>CN</td>
<td>c.196_197insGT</td>
<td>p.A66GfsX95</td>
<td>c.925_926insTGG</td>
<td>p.V308_E309insV</td>
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<tr>
<td>3</td>
<td>32F</td>
<td>CN</td>
<td>c.264_303delins20</td>
<td>p.Q88HfsX75</td>
<td>c.920_921ins9</td>
<td>p.R306_N307insKQR</td>
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<td>c.243_244insGTG</td>
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<td>c.930_931insAGC</td>
<td>p.T310_Q311insT</td>
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<td>c.68_69insC</td>
<td>p.H24AfsX64</td>
<td>c.772_787del</td>
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<td>p.Q305_R306insQ</td>
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<td>p.I62AfsX95</td>
<td>c.925_942del</td>
<td>p.E309_V314del</td>
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<td>CN</td>
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<td>p.P45HfsX115</td>
<td>c.916_917insAGC</td>
<td>p.Q305_R306insQ</td>
</tr>
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<td>p.N321&gt;SY</td>
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Abbreviations: CN, cytogenetically normal; NM, no mitosis; UPN, unique patient number
Supplementary Table 3. Additional mutations in the CEBPA\textsuperscript{dm} patients

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Supplementary Table 4. Prognostic factors for OS and DFS in total AML patients

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<td><strong>Age</strong>&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>1.773 (1.368-2.296)</td>
<td>0.003</td>
<td>1.435 (1.131-1.820)</td>
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<tr>
<td><strong>WBC</strong>&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>1.932 (1.471-2.537)</td>
<td>&lt;0.0001</td>
<td>1.757 (1.371-2.252)</td>
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<tr>
<td><strong>Cytogenetics</strong>&lt;sup&gt;c&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>2.923 (2.014-4.242)</td>
<td>&lt;0.0001</td>
<td>2.506 (1.763-3.563)</td>
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<tr>
<td><strong>CEBPA</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.002</td>
<td>0.420 (0.246-0.718)</td>
<td>0.006</td>
<td>0.544 (0.351-0.842)</td>
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<tr>
<td><strong>NPM1+/FLT3-ITD</strong>&lt;sup&gt;d&lt;/sup&gt;</td>
<td>&lt;0.0001</td>
<td>0.389 (0.232-0.652)</td>
<td>0.002</td>
<td>0.476 (0.300-0.753)</td>
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<td><strong>ASXL1</strong></td>
<td>0.970</td>
<td>0.992 (0.653-1.507)</td>
<td>0.634</td>
<td>1.097 (0.749-1.608)</td>
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<td><strong>TP53</strong></td>
<td>0.016</td>
<td>1.929 (1.129-3.295)</td>
<td>0.260</td>
<td>1.364 (0.795-2.339)</td>
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<td><strong>RUNX1</strong></td>
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<td>1.129 (0.753-1.694)</td>
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<td>1.220 (0.846-1.758)</td>
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<td><strong>IDH2</strong></td>
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<td>0.577 (0.372-0.897)</td>
<td>0.058</td>
<td>0.691 (0.471-1.013)</td>
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<td><strong>DNMT3A</strong></td>
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<td>1.851 (1.307-2.621)</td>
<td>0.006</td>
<td>1.581 (1.144-2.185)</td>
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<td><strong>SF</strong></td>
<td>&lt;0.0001</td>
<td>2.127 (1.405-3.220)</td>
<td>&lt;0.0001</td>
<td>2.159 (1.473-3.165)</td>
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</table>

Abbreviations: CI, confidence interval; RR, relative risk; SF, splicing factors, including SF3B1, SRSF2, U2AF1; WBC, white blood cell.

<sup>a</sup>Age ≥50 relative to age <50 (the reference)

<sup>b</sup>WBC greater than 50,000/μl vs. less than 50,000/μl.

<sup>c</sup>Unfavorable cytogenetics vs. others.

<sup>d</sup>NPM1+/FLT3-ITD− vs. other subtypes.
Supplementary Table 5. Clinical characteristics of CEBPA\textsuperscript{dm} AML patients according to the status of WT1 mutations\textsuperscript{a}

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<tr>
<td>Age, years\textsuperscript{b}</td>
<td>49 (28-69)</td>
<td>38.5 (17-90)</td>
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<td>Lab\textsuperscript{b}</td>
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<tr>
<td>WBC (k/μL)</td>
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<td>36.83 (2.41-380.2)</td>
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<td>Hb (g/dL)</td>
<td>7.1 (3.0-13.6)</td>
<td>9.0 (5.7-12.7)</td>
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<td>Platelet (k/μL)</td>
<td>43.5 (10-54)</td>
<td>40.5 (5-204)</td>
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<td>PB Blast (k/μL)</td>
<td>74.0 (2.95-371.9)</td>
<td>17.3 (0.72-354.0)</td>
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<td>LDH (U/L)</td>
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<td>Complete Resonse</td>
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<td>Relapse</td>
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Abbreviation: PB, peripheral blood

\textsuperscript{a} WT1 mutation data was available in 68 of 69 CEBPA\textsuperscript{dm} patients.

\textsuperscript{b} Median (range)

\textsuperscript{c} Number of patients (%)

\textsuperscript{d} Only the 61 patients who received conventional intensive induction chemotherapy and then consolidation chemotherapy if CR was achieved, as mentioned in the text, were included in the analysis.
## Supplementary Table 6. Sequential studies of WT1 mutations in AML patients

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<td>Relapse</td>
<td>R471T</td>
<td>38.3</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>1995/6/16</td>
<td>Initial</td>
<td>-</td>
<td>-</td>
<td>NRAS, FLT3-ITD</td>
</tr>
<tr>
<td></td>
<td>1996/6/3</td>
<td>CR1</td>
<td>-</td>
<td>-</td>
<td>NRAS, FLT3-ITD</td>
</tr>
<tr>
<td></td>
<td>1996/11/15</td>
<td>Relapse</td>
<td>H465Y</td>
<td>41.6</td>
<td>NRAS, FLT3-ITD</td>
</tr>
</tbody>
</table>

*aOnly patients with WT1 mutation at diagnosis, relapse or both are shown and those without the mutation at both diagnosis and relapse are not shown.*
Supplementary Table 7. Prognostic impact of concomitant mutations in CEBPA\textsuperscript{dm} patients

<table>
<thead>
<tr>
<th></th>
<th>No. of patients</th>
<th>CR1 (%)</th>
<th>Relapse (%)</th>
<th>Relapse in patients with C/T alone (%)</th>
<th>CR2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total pts</td>
<td>62</td>
<td>56 (90.2)</td>
<td>21 (37.5)</td>
<td>21/46 (45.6)</td>
<td>14/21 (66.7)</td>
</tr>
<tr>
<td>GATA2</td>
<td>21</td>
<td>20 (95.2)</td>
<td>6 (30.0)</td>
<td>6/16 (37.5)</td>
<td>5/6 (83.3)</td>
</tr>
<tr>
<td>FLT3-ITD</td>
<td>8</td>
<td>6 (75.0)</td>
<td>1 (16.7)</td>
<td>1/3 (33.3)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>NRAS</td>
<td>9</td>
<td>8 (88.9)</td>
<td>2 (25.0)</td>
<td>2/3 (67.7)</td>
<td>2/2 (100)</td>
</tr>
<tr>
<td>TET2</td>
<td>7</td>
<td>6 (85.7)</td>
<td>1 (16.7)</td>
<td>1/6 (16.7)</td>
<td>1/1 (100)</td>
</tr>
<tr>
<td>WT1</td>
<td>7</td>
<td>5 (71.4)</td>
<td>4 (80.0)*</td>
<td>4/4 (100)*</td>
<td>1/4 (25)</td>
</tr>
</tbody>
</table>

Abbreviations: C/T, chemotherapy; CR, complete remission

*denotes statistically significance compared to those without the mutation (P<0.05)
Supplementary Figure 1.
Kaplan-Meier plots for (A) OS and (B) DFS in CEBPA\textsuperscript{dm} patients according to transplantation or not in first CR

(A)
Supplementary Figure 2.

Kaplan-Meier plots for (A) OS and (B) DFS according to the status of different concomitant mutations

(A)
Wild-type, n=54

FLT3-ITD, n=8

P=0.578

Wild-type, n=37

GATA2 mutation, n=21

P=0.078

Wild-type, n=53

NRAS mutation, n=9

P=0.293

Wild-type, n=54

TET2 mutation, n=7

P=0.257