Refinement of the prognostic impact of somatic CEBPA bZIP domain mutations in acute myeloid leukemia: Results of the AML Study Group (AMLSG)

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The transcription factor CCAAT/enhancer binding protein alpha (CEBPA) is a key regulator of myelopoiesis and granulocyte differentiation.1,2 The intronless CEBPA gene on chromosome 19q13.1 encodes two DNA-binding protein isoforms: a full-length 42-kDa protein (p42) and a shorter 30-kDa isoform (p30), initiated from two distinct start sites.2 The p42 isoform contains two N-terminal transactivation domains (TAD1, TAD2), whereas the p30 isoform lacks TAD1. Both isoforms contain the highly conserved C-terminal basic DNA-binding domain and the leucine zipper (bZIP) involved in DNA binding and protein dimerization. In younger adult patients, mutations of CEBPA (CEBPAmut) are present in 5%–10% of newly diagnosed acute myeloid leukemia (AML); the frequency in older patients is considerably lower.2,5 There are two mutational patterns: the first one clusters at the C-terminus involving the two TADs, typically frame-shift mutations; the second one at the C-terminus affecting bZIP, typically in-frame mutations. Out-of-frame TAD mutations result in the truncated p30 isoform that has been shown to act as a dominant negative of the p42 isoform and to be associated with increased proliferation and minimal differentiation of myeloid progenitors.2,6 Depending on the position, in-frame bZIP mutations cause a p42 isoform defective either in DNA binding or homo- and heterodimerization.3,7

Approximately half of the CEBPAmut AML exhibit biallelic mutations (CEBPABi), typically consisting of one TAD and one bZIP mutation on separate alleles.3,4 Based on specific genetic features and its prognostic impact, CEBPABi was defined as a distinct entity within the 2016 WHO classification and was categorized as favorable in the risk stratification of the 2017 European LeukemiaNet (ELN) recommendations. Recent studies in pediatric and adult AML have demonstrated CEBPABi...
mutations, and in particular, in-frame mutations (CEBPA<sub>bZIP<sub>inf</sub></sub>), to be associated with a unique gene-expression profile and favorable outcome, regardless of the mono- or biallelic status. Based on these data, the former entity of AML with CEBPA<sup>bi</sup> was expanded by single mutations in bZIP (smbZIP-CEBPA) in the current 2022 WHO classification and replaced by AML with CEBPA<sub>bZIP<sub>inf</sub></sub> (irrespective of the allelic status) within the 2022 International Consensus Classification (ICC) of myeloid neoplasm and acute leukemias. Furthermore, CEBPA<sub>bZIP<sub>inf</sub></sub> (irrespective of the allelic status) is now categorized as favorable in the 2022 ELN risk stratification.

**FIGURE 1** Outcome of the 528 patients with CEBPA-mutated AML according to CEBPA genotypes. Conditional inference tree-structured event-free survival model for CEBPA mutation types with estimates of cumulative incidence of events (refractory disease, relapse, and/or death) in the terminal nodes. Hematopoietic-cell transplantation in first complete remission (HCT in CR1) was considered a competing event (A). Kaplan–Meier curves for event-free survival (B) and overall survival (C) according to CEBPA mutation types, irrespective of the allelic status. HCT in CR1 was not considered a competing event. Results of pairwise comparisons are provided below the x-axis.
To evaluate the prognostic impact of CEBPA\textsuperscript{bZIP}, in particular to further characterize CEBPA\textsuperscript{bZIP,inf} mutations, we retrospectively analyzed 528 intensively treated adult CEBPA\textsuperscript{mut} AML patients (median age: 54 years; range: 18–82; ≤60 years: n = 340, >60 years: n = 188) enrolled in treatment trials of the German-Austrian AML Study Group (AMLSG) and/or entered into the AMLSG Bio Registry study (NCT01252485). CEBPA mutation status was evaluated centrally in two reference laboratories of the AMLSG; assays were harmonized and cross-validated between the two laboratories. Sequences were analyzed for type of CEBPA mutations (insertions/deletions either in-frame or frameshift, missense, and nonsense mutations), the precise localization for type of cross-reference laboratories of the AMLSG; assays were harmonized and cross-validated between the two laboratories. Studies were performed for type of CEBPA mutations (insertions/deletions either in-frame or frameshift, missense, and nonsense mutations), the precise localization for type of cross-reference laboratories of the AMLSG; assays were harmonized and cross-validated between the two laboratories.

Complete remission (CR), including CR with incomplete hematologic recovery during induction cycles 1 and 2, was achieved in 425/501 (85%) evaluable patients. Allogeneic hematopoietic-cell transplantation (HCT) in the first CR (CR1) was performed in 109 (21%) patients. The median follow-up time was 55.5 months (95% confidence interval [95% CI], 51.2–59.5). Median event-free survival (EFS) and median overall survival (OS) were 20.0 (95% CI: 14.4–26.5) and 172.4 months (95% CI: 72.5–NA), respectively. The 5-year EFS and OS rates were 42% and 55%, respectively. One hundred forty-one (26.7%) patients relapsed, and 195 (36.9%) died.

Of the 528 patients, 263 (49.8%) exhibited ≥2 CEBPA mutations (dmCEBPA), and 265 had monoallelic CEBPA mutations (smCEBPA). To further refine CEBPA mutation types and to address their prognostic impact, patients were categorized into eight groups based on allelic status (dmCEBPA vs. smCEBPA) and mutation type: (1) dmCEBPA with in-frame insertion/deletion in bZIP (dmCEBPA\textsuperscript{bZIP,InDel,inf}, n = 220), (2) frame-shift insertion/deletion or nonsense mutation in bZIP (dmCEBPA\textsuperscript{bZIP,InDel,fs}, n = 13), (3) missense mutation in bZIP (dmCEBPA\textsuperscript{bZIP,ms}, n = 22), (4) other (dmCEBPA\textsuperscript{other}, n = 8), (5) smCEBPA\textsuperscript{bZIP,InDel,inf} (n = 46), (6) smCEBPA\textsuperscript{bZIP,InDel,fs} (n = 32), (7) smCEBPA\textsuperscript{bZIP,ms} (n = 11), and (8) smCEBPA\textsuperscript{other} (n = 176). These eight groups differed significantly with regard to several clinical and concurrent genetic features as well as achievement of CR1 and outcome (Supporting Information S1: Table 1).

To evaluate the prognostic impact of the eight CEBPA mutation types, we performed conditional inference tree models for EFS and OS. Of the eight equally relevant groups, dmCEBPA\textsuperscript{bZIP,InDel,inf} and smCEBPA\textsuperscript{bZIP,InDel,inf} patients separated from the other groups in the first tree based on significantly lower cumulative incidences of events (refractory disease, relapse, and/or death). No further partitioning was observed (Supporting Information S1: Figure 1). Assuming a prognostic impact primarily for mutation type rather than allelic status, subsequent conditional inference tree models were performed by splitting up CEBPA mutation types and allelic status for EFS and OS. In both the models, the CEBPA\textsuperscript{bZIP,InDel,inf} group again separated significantly from the other three groups in the first tree without further separation, confirming the significantly favorable prognosis, regardless of the allelic status (Figure 1A and Supporting Information S1: Figure 2).

As these findings, patients were subsequently categorized as CEBPA\textsuperscript{bZIP,InDel,inf} (n = 266), CEBPA\textsuperscript{bZIP,InDel,fs} (n = 45), CEBPA\textsuperscript{ms} (n = 33), or CEBPA\textsuperscript{other} (n = 184), irrespective of the allelic status. These four groups differed significantly with regard to several clinical and concurrent genetic features as well as achievement of CR1. Most obviously, CEBPA\textsuperscript{bZIP,InDel,inf} patients were younger (median age in years: 49 vs. 66 vs. 60 vs 61; p < 0.001) and achieved a higher CR1 rate (91.4% vs. 81.8% vs. 83.3% vs. 76.2%; p < 0.001) (Supporting Information S1: Table 2). Irrespective of the allelic status, CEBPA\textsuperscript{bZIP,InDel,inf} patients had a significantly improved EFS (median [95% CI] 49.8 months [16.9–82.7] vs. 11.5 [8.3–14.6] for CEBPA\textsuperscript{bZIP,InDel,fs} vs. 12.6 [6.2–19.1] for CEBPA\textsuperscript{ms vs. 14.6 (7.7–21.5) for CEBPA\textsuperscript{other}; p < 0.001) and OS (median [95% CI] NA for patients with CEBPA\textsuperscript{bZIP,InDel,inf} [NA–NA] vs. 25.7 months [10.2–41.3] for CEBPA\textsuperscript{bZIP,InDel,fs} vs. 54.3 [14.6–NA] for CEBPA\textsuperscript{ms} vs. 45.5 [13.1–77.9] for CEBPA\textsuperscript{other}; p < 0.001) (Figure 1B). Of note, a sensitivity analysis in which survival times were censored at the date of HCT in CR1 revealed almost identical results (Supporting Information S1: Figure 3).

Multivariate Cox models for EFS and OS adjusted for sex, type of AML, FLT3-ITD, NPM1 mutation status, white blood cell (WBC) count

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Multivariable analyses for outcome and response determining the prognostic significance of CEBPA mutation types.</th>
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<tbody>
<tr>
<td></td>
<td>EFS HR (95% CI)</td>
</tr>
<tr>
<td>Age (10-year increase)</td>
<td>1.18 (1.08–1.30)</td>
</tr>
<tr>
<td>Female</td>
<td>1.11 (0.87–1.40)</td>
</tr>
<tr>
<td>WBC (log10)</td>
<td>1.48 (1.18–1.84)</td>
</tr>
<tr>
<td>BM blasts</td>
<td>1.00 (0.99–1.01)</td>
</tr>
<tr>
<td>De novo AML</td>
<td>1</td>
</tr>
<tr>
<td>sAML</td>
<td>1.57 (1.00–2.46)</td>
</tr>
<tr>
<td>tAML</td>
<td>0.61 (0.24–1.52)</td>
</tr>
<tr>
<td>FLT3–ITD positive</td>
<td>1.22 (0.86–1.72)</td>
</tr>
<tr>
<td>NPM1 mutation</td>
<td>0.61 (0.43–0.87)</td>
</tr>
<tr>
<td>CEBPA\textsuperscript{bZIP,InDel,inf}</td>
<td>1</td>
</tr>
<tr>
<td>CEBPA\textsuperscript{bZIP,InDel,fs}</td>
<td>1.65 (1.08–2.54)</td>
</tr>
<tr>
<td>CEBPA\textsuperscript{ms}</td>
<td>1.67 (1.04–2.69)</td>
</tr>
<tr>
<td>CEBPA\textsuperscript{other}</td>
<td>1.66 (1.19–2.31)</td>
</tr>
<tr>
<td>HCT in CR1</td>
<td>0.38 (0.25–0.59)</td>
</tr>
</tbody>
</table>

Abbreviations: 95% CI, 95% confidence interval; BM, bone marrow; CR, complete remission; EFS, event-free survival; ITD, internal tandem duplication; HCT in CR1, allogeneic hematopoietic cell transplantation in first CR; HR, hazard ratio; OS, overall survival; p, p value; sAML, secondary acute myeloid leukemia following myelodysplastic syndrome; tAML, therapy-related AML; WBC, white blood cell count.
CONFLICT OF INTEREST STATEMENT
Frank G. Rücker reports honoraria from and consultancy for Jazz Pharmaceuticals, Novartis, and BMS/Celgene; travel support from Jazz Pharmaceuticals. Michael Lübbert reports an advisory role for Abbvie, Astex Pharmaceuticals, Imago BioSciences, Janssen, Otsuka, and Syros; research support from Janssen and Cheplapharm. Michael W. M. Kühn reports honoraria from and consultancy for Pfizer, Kura Oncology, Jazz Pharmaceuticals, BMS/Celgene, and Abbvie; speakers bureau of Gilead. Lars Bullinger reports honoraria from Abbvie, Amgen, Astellas, BMS/Celgene, Daiichi Sankyo, Gilead, Janssen, Jazz Pharmaceuticals, Menarini, Novartis, Pfizer, Roche, and Sanofi; research support from Bayer and Jazz Pharmaceuticals. Verena I. Gaidzik reports an advisory role for Jazz Pharmaceuticals, Abbvie, and Boehringer-Ingelheim; speakers bureau of Pfizer, Janssen, and Abbvie; and travel support from Abbvie. Frank Stegelmann reports honoraria from and consultancy for AOP Pharma, MorphoSys, BMS/Celgene, Incyte, Novartis, and Pfizer. Felicitas Thol reports an advisory role for Novartis, BMS, Abbvie, Menarini, and Rigel. Michael Heuser reports honoraria from Cerrara, Jazz Pharmaceuticals, Janssen, Novartis, and Sobi; paid consultancy for Abbvie, Agios, Astellas, BMS/Celgene, Glycostem, LabDelbert, Pfizer, PinotBio, and Servier; and research funding to his institution from Abbvie, Agios, Astellas, BMS/Celgene, Glycostem, Jazz Pharmaceuticals, Karyopharm, Luxo Oncology, and PinotBio. Hartmut Döhner declares being in an advisory role for Abbvie, Agios, Amgen, Astellas, AstraZeneca, Berlin Chemie, BMS/Celgene, Daiichi Sankyo, GEMoAb, Gilead, Janssen, Jazz Pharmaceuticals, Novartis, Servier, Stemline, and Syndax; research funding from Abbvie, Agios, Amgen, Astellas, BMS/Celgene, Jazz Pharmaceuticals, Kronos Bio, Novartis, and Pfizer. Konstanze Döhner reports an advisory role for Amgen, BMS/Celgene, Daiichi Sankyo, Janssen, Jazz Pharmaceuticals, Novartis, and Roche; research funding from Agios, Astex, Astellas, BMS/Celgene, and Novartis. All other authors declare no competing interest. The remaining authors declared no conflicts of interest.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions. For original data, please contact Konstanze Döhner (konstanze.doehner@uniklinik-ulm.de). Individual participant data will not be shared.

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SUPPORTING INFORMATION
Additional supporting information can be found in the online version of this article.

REFERENCES


