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

Frank G. Rücker[1],[^] | Andrea Corbacioglu[1],[^] | Julia Krzykalla[2] |
Sibylle Cocciardi[1] | Claudia Lengerke[3] | Ulrich Germing[4] | Gerald Wulf[5] |
Maisun A. Samra[6] | Lino L. Teichmann[7] | Michael Lübbert[8] |
Michael W. M. Kühn[9] | Martin Bentz[10] | Jörg Westermann[11] | Lars Bullinger[11] |
Verena I. Gaidzik[1] | Annika Meid[1] | Sophia Aicher[1] | Frank Stegelmann[1] |
Daniela Weber[1] | Anika Schrade[1] | Felicitas Thol[12] | Michael Heuser[12] |
Arnold Ganser[12] | Axel Benner[2] | Hartmut Döhner[1] | Konstanze Döhner[1] |
for the German-Austrian Acute Myeloid Leukemia Study Group (AMLSG)

Correspondence: Konstanze Döhner (konstanze.doehner@uniklinik-ulm.de)

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* (*CEBPA*^mut) 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 N-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 *CEBPA*^mut AML exhibit biallelic mutations (*CEBPA*^bi), typically consisting of one TAD and one bZIP mutation on separate alleles.[3,4] Based on specific genetic features and its prognostic impact, *CEBPA*^bi 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 *CEBPA*^bZIP

[1]Department of Internal Medicine III, University Hospital of Ulm, Ulm, Germany
[2]Division of Biostatistics, German Cancer Research Center, Heidelberg, Germany
[3]Medical Clinic, Department of Hematology, Oncology, Clinical Immunology and Rheumatology, University Hospital Tübingen, Tübingen, Germany
[4]Department of Hematology, Oncology and Clinical Immunology, Heinrich Heine University, Düsseldorf, Germany
[5]Department of Hematology and Medical Oncology, University Medical Center Göttingen, Göttingen, Germany
[6]Department of Internal Medicine IV, University Hospital of Gießen, Gießen, Germany
[7]Department of Medicine and Polyclinic III, Bonn University Hospital, Bonn, Germany

[8]Department of Hematology, Oncology and Stem Cell Transplantation, University of Freiburg Medical Center, Freiburg, Germany
[9]Department of Hematology and Oncology, University Medical Center Mainz, Mainz, Germany
[10]Department of Internal Medicine III, Hospital of Karlsruhe, Karlsruhe, Germany
[11]Department of Hematology, Oncology and Tumor Immunology, Charité University Medicine, Berlin, Germany
[12]Department of Hematology, Hemostasis, Oncology and Stem Cell Transplantation, Hannover Medical School, Hannover, Germany
[^]Frank G. Rücker and Andrea Corbacioglu contributed equally to this work.

mutations, and in particular, in-frame mutations (*CEBPA*[bZIP_inf]), to be associated with a unique gene-expression profile and favorable outcome, regardless of the mono- or biallelic status.[8–10] Based on these data, the former entity of AML with *CEBPA*[bi] was expanded by single mutations in bZIP (smbZIP-*CEBPA*) in the current 2022 WHO classification and replaced by AML with *CEBPA*[bZIP_inf] (irrespective of the allelic status) within the 2022 International Consensus Classification (ICC) of myeloid neoplasm and acute leukemias.[11,12] Furthermore, *CEBPA*[bZIP_inf] (irrespective of the allelic status) is now categorized as favorable in the 2022 ELN risk stratification.[13]
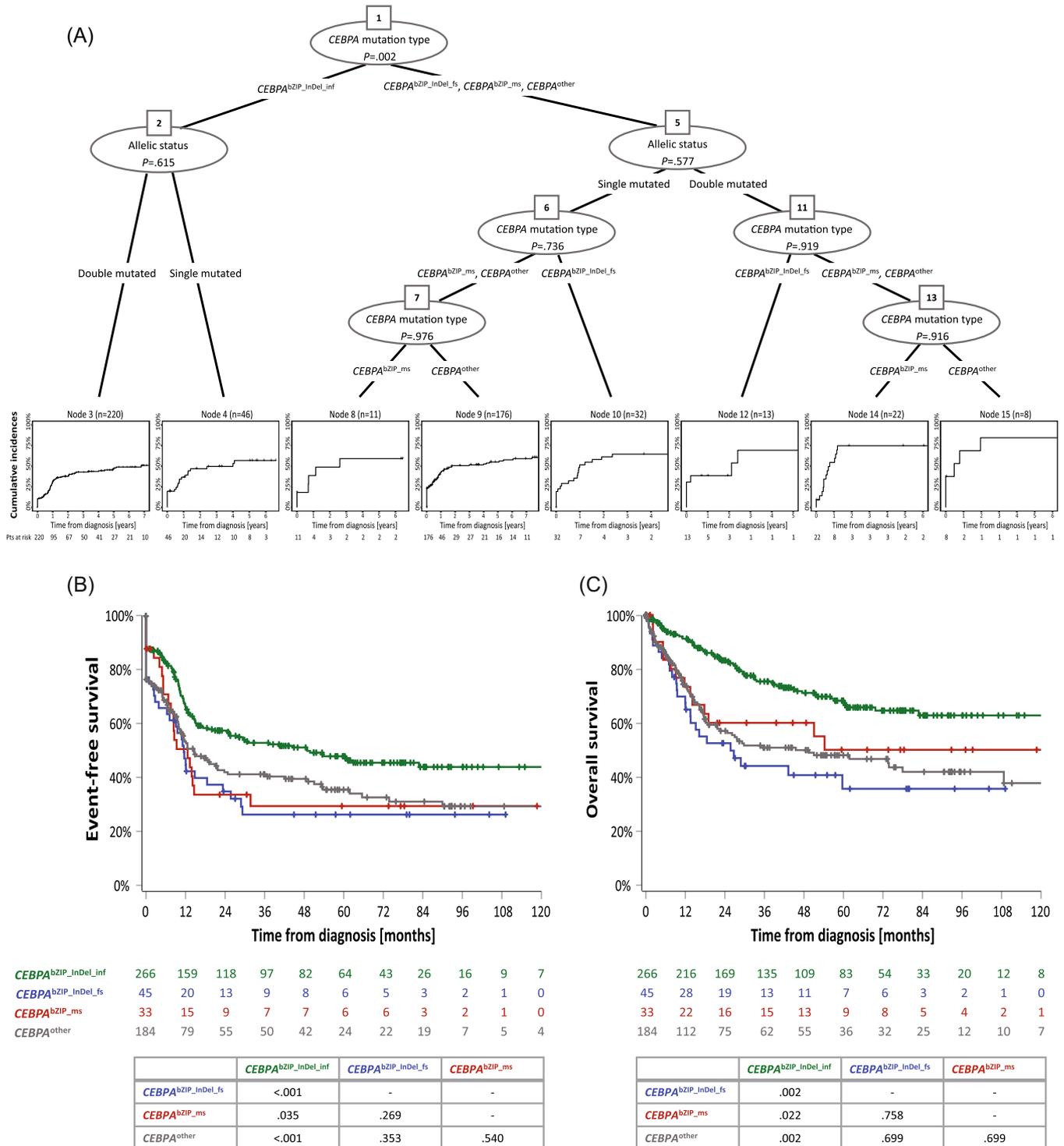
FIGURE 1 Outcome of the 528 patients with *CEBPA*-mutated AML according to *CEBPA* genotypes. Conditional inference tree-structured event-free survival model for *CEBPA* mutation types with estimates of cumulative incidence of events (refractory disease, relapse, and/or death) in the terminal nodes. 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 $CEBPA^{bZIP}$, in particular to further characterize $CEBPA^{bZIP\_inf}$ mutations, we retrospectively analyzed 528 intensively treated adult $CEBPA^{mut}$ 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 of mutations (TAD1/2 and bZIP), and allelic status. Two hundred forty-three (46%) patients were enrolled in one of 12 AMLSG treatment trials (Supporting Information S1: Appendix) and 285 (54%) received intensive treatment according to the standard of care. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent for treatment and genetic testing was obtained from all patients.

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: 14.4–30.6) 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 ($dmCEBPA^{bZIP\_InDel\_inf}$, n = 220), (2) frame-shift insertion/deletion or nonsense mutation in bZIP ($dmCEBPA^{bZIP\_InDel\_fs}$, n = 13), (3) missense mutation in bZIP ($dmCEBPA^{bZIP\_ms}$, n = 22), (4) other ($dmCEBPA^{other}$, n = 8), (5) $smCEBPA^{bZIP\_InDel\_inf}$ (n = 46), (6) $smCEBPA^{bZIP\_InDel\_fs}$ (n = 32), (7) $smCEBPA^{bZIP\_ms}$ (n = 11), or (8) $smCEBPA^{other}$ (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, $dmCEBPA^{bZIP\_InDel\_inf}$ and $smCEBPA^{bZIP\_InDel\_inf}$ 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 $CEBPA^{bZIP\_InDel\_inf}$ 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).

Based on these findings, patients were subsequently categorized as $CEBPA^{bZIP\_InDel\_inf}$ (n = 266), $CEBPA^{bZIP\_InDel\_fs}$ (n = 45), $CEBPA^{bZIP\_ms}$ (n = 33), or $CEBPA^{other}$ (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, $CEBPA^{bZIP\_InDel\_inf}$ patients were younger (median age in years: 49 vs. 66 vs 60 vs 61; p < .001) and achieved a higher CR1 rate (91.4% vs. 81.8% vs. 83.3% vs. 76.2%; p < .001) (Supporting Information S1: Table 2). Irrespective of the allelic status, $CEBPA^{bZIP\_InDel\_inf}$ patients had a significantly improved EFS (median [95% CI] 49.8 months [16.9–82.7] vs. 11.5 [8.3–14.6] for $CEBPA^{bZIP\_InDel\_fs}$ vs. 12.6 [6.2–19.1] for $CEBPA^{bZIP\_ms}$ vs 14.6 [7.7–21.5] for $CEBPA^{other}$; p < .001) and OS (median [95% CI] NA for patients with $CEBPA^{bZIP\_InDel\_inf}$ [NA–NA] vs. 25.7 months [10.2–41.3] for $CEBPA^{bZIP\_InDel\_fs}$ vs. 54.3 [14.6–NA] for $CEBPA^{bZIP\_ms}$ vs. 45.5 [13.1–77.9] for $CEBPA^{other}$; p < .001) (Figure 1B,C). 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 1 Multivariable analyses for outcome and response determining the prognostic significance of CEBPA mutation types.

	EFS		OS		CR	
	HR (95% CI)	p	HR (95% CI)	p	OR (95% CI)	p
Age (10-year increase)	1.18 (1.08–1.30)	<.001	1.48 (1.29–1.65)	<.001	0.84 (0.69–1.02)	.070
Female	1.11 (0.87–1.40)	.407	1.23 (0.92–1.64)	.171	1.53 (0.89–2.60)	.121
WBC (log10)	1.48 (1.18–1.84)	<.001	1.49 (1.15–1.95)	.003	0.79 (0.50–1.25)	.311
BM blasts	1.00 (0.99–1.01)	.926	1.00 (0.99–1.01)	.516	1.00 (0.99–1.01)	.887
De novo AML	1		1		1	
sAML	1.57 (1.00–2.46)	.052	1.31 (0.77–2.24)	.326	0.97 (0.37–2.53)	.943
tAML	0.61 (0.24–1.52)	.287	0.48 (0.17–1.34)	.163	2.36 (0.27–20.23)	.435
FLT3-ITD positive	1.22 (0.86–1.72)	.268	1.24 (0.83–1.87)	.292	0.75 (0.36–1.53)	.422
NPM1 mutation	0.61 (0.43–0.87)	.006	0.49 (0.33–0.75)	<.001	2.83 (1.33–6.03)	.007
$CEBPA^{bZIP\_InDel\_inf}$	1		1		1	
$CEBPA^{bZIP\_InDel\_fs}$	1.65 (1.08–2.54)	.022	2.29 (1.39–3.78)	.001	0.44 (0.17–1.12)	.084
$CEBPA^{bZIP\_ms}$	1.67 (1.04–2.69)	.033	1.49 (0.83–2.66)	.185	0.55 (0.19–1.64)	.285
$CEBPA^{other}$	1.66 (1.19–2.31)	.003	2.29 (1.55–3.39)	<.001	0.24 (0.12–0.47)	<.001
HCT in CR1	0.38 (0.25–0.59)	<.001	0.50 (0.32–0.80)	.003	-	-

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.

(log10 transformed), bone marrow blasts, and age including HCT in CR1 as a time-dependent variable (Supporting Information S1: Appendix) revealed increasing age and higher WBC as unfavorable factors, whereas $CEBPA^{bZIP\_InDel\_inf}$, $NPM1^{mut}$, and HCT in CR1 were favorable (Table 1).

A recent meta-analysis of 1010 adult $CEBPA^{mut}$ AML from six different study groups characterized *CEBPA* mutational subgroups in more detail by evaluating their clinical and genetic features as well as their prognostic impact.[14] In line with our data, the authors showed that $CEBPA^{bZIP\_InDel\_inf}$ mutations represent a subset of AML with distinct disease biology and clinical outcomes. Despite certain limitations, in particular, the retrospective nature spanning almost three decades with evolving different treatment approaches, both studies independently confirm the less favorable impact of in-frame bZIP missense mutations (currently subsumed in the category AML with $CEBPA^{bZIP\_inf}$ in the ICC and ELN risk classification). The less favorable impact of bZIP missense mutations might be explained by the finding that bZIP missense mutations localize differently and therefore have diverse functional consequences. $CEBPA^{bZIP\_InDel\_inf}$ predominantly affects the fork region of bZIP, while $CEBPA^{bZIP\_ms}$ cluster in the basic region (Supporting Information S1: Figure 4). These different mutation patterns imply that $CEBPA^{bZIP\_ms}$ impair DNA binding, while $CEBPA^{bZIP\_InDel\_inf}$ affects dimerization. Murine data have shown that missense mutations in the basic region of bZIP lead to a myeloproliferative disease transforming into overt AML,[7] while AML arises upon transplantation of transgenic cells carrying the most common in-frame insertion in bZIP (K313dup, K-allele) alone or in combination with an N-terminal mutation (L-allele), with the K/L combination driving the most aggressive AML.[15]

In this retrospective, exploratory analysis of 528 adult patients with newly diagnosed intensively treated patients with $CEBPA^{mut}$ AML, we further refined the prognostic impact of different *CEBPA* mutation types, in particular, *CEBPA* mutations that are located in the bZIP domain. Our study shows that the beneficial effect is restricted to *CEBPA* bZIP InDel in-frame mutations, irrespective of the allelic status, whereas *CEBPA* bZIP missense mutations are associated with inferior outcomes. Our data as well as the data from Georgi et al. provide novel and clinically relevant results contributing to a further refinement of $CEBPA^{mut}$ AML in the current ICC and WHO classifications as well as for risk stratification as recommended by the ELN.

AUTHOR CONTRIBUTIONS
Frank G. Rücker, Andrea Corbacioglu, Hartmut Döhner, and Konstanze Döhner designed the study. Frank G. Rücker, Andrea Corbacioglu, Sibylle Cocciardi, Verena I. Gaidzik, Annika Meid, and Sophia Aicher performed experiments and validated data. Frank G. Rücker, Julia Krzykalla, Daniela Weber, and Axel Benner performed statistical analyses. Frank G. Rücker, Andrea Corbacioglu, Julia Krzykalla, Daniela Weber, Axel Benner, Hartmut Döhner, and Konstanze Döhner analyzed the results. Claudia Lengerke, Ulrich Germing, Gerald Wulf, Maisun A. Samra, Lino L. Teichmann, Michael Lübbert, Michael W. M. Kühn, Martin Bentz, Jörg Westermann, Lars Bullinger, Frank Stegelmann, Anika Schrade, Felicitas Thol, Michael Heuser, and Arnold Ganser collected and provided patient samples and clinical information. Frank G. Rücker, Andrea Corbacioglu, Hartmut Döhner, and Konstanze Döhner wrote the first draft of the manuscript; and all authors undertook manuscript writing, editing, and approval, revised the manuscript, and reviewed and approved the final version.

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.

ORCID
Frank G. Rücker http://orcid.org/0009-0003-1941-967X
Lino L. Teichmann http://orcid.org/0000-0001-9489-7282

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

REFERENCES
1. Zhang DE, Zhang P, Wang N, Hetherington CJ, Darlington GJ, Tenen DG. Absence of granulocyte colony-stimulating factor signaling and neutrophil development in CCAAT enhancer binding protein α-deficient mice. *Proc Natl Acad Sci*. 1997;94(2):569-574. doi:10.1073/pnas.94.2.569

2. Pabst T, Mueller BU, Zhang P, et al. Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-α (C/EBPα), in acute myeloid leukemia. *Nat Genet*. 2001;27(3):263-270. doi:10.1038/85820

3. Nerlov C. C/EBPα mutations in acute myeloid leukaemias. *Nat Rev Cancer*. 2004;4(5):394-400. doi:10.1038/nrc1363

4. 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. doi:10.1056/NEJMoa1516192

5. Jahn E, Saadati M, Fenaux P, et al. Clinical impact of the genomic landscape and leukemogenic trajectories in non-intensively treated elderly acute myeloid leukemia patients. *Leukemia*. 2023;37(11):2187-2196. doi:10.1038/s41375-023-01999-6

6. Kirstetter P, Schuster MB, Bereshchenko O, et al. Modeling of C/EBPα mutant acute myeloid leukemia reveals a common expression signature of committed myeloid leukemia-initiating cells. *Cancer Cell*. 2008;13(4):299-310. doi:10.1016/j.ccr.2008.02.008

7. Porse BT, Bryder D, Theilgaard-Mönch K, et al. Loss of C/EBPα cell cycle control increases myeloid progenitor proliferation and transforms the neutrophil granulocyte lineage. *J Exp Med*. 2005;202(1):85-96. doi:10.1084/jem.20050067

8. Tarlock K, Lamble AJ, Wang YC, et al. CEBPA-bZip mutations are associated with favorable prognosis in de novo AML: a report from the Children's Oncology Group. *Blood*. 2021;138(13):1137-1147. doi:10.1182/blood.2020009652

9. Taube F, Georgi JA, Kramer M, et al. CEBPAmutations in 4708 patients with acute myeloid leukemia: differential impact of bZIP and TAD mutations on outcome. *Blood*. 2022;139(1):87-103. doi:10.1182/blood.2020009680

10. Wakita S, Sakaguchi M, Oh I, et al. Prognostic impact of CEBPA bZIP domain mutation in acute myeloid leukemia. *Blood Adv*. 2022;6(1):238-247. doi:10.1182/bloodadvances.2021004292

11. Khoury JD, Solary E, Abla O, et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: myeloid and histiocytic/dendritic neoplasms. *Leukemia*. 2022;36(7):1703-1719. doi:10.1038/s41375-022-01613-1

12. Arber DA, Orazi A, Hasserjian RP, et al. International consensus classification of myeloid neoplasms and acute leukemias: integrating morphologic, clinical, and genomic data. *Blood*. 2022;140(11):1200-1228. doi:10.1182/blood.2022015850

13. Döhner H, Wei AH, Appelbaum FR, et al. Diagnosis and management of AML in adults: 2022 recommendations from an international expert panel on behalf of the ELN. *Blood*. 2022;140(12):1345-1377. doi:10.1182/blood.2022016867

14. Georgi JA, Stasik S, Kramer M, et al. Prognostic Impact of CEBPA-mutational subgroups in adult AML. *Leukemia*. 2024;38(2):281-290. doi:10.1038/s41375-024-02140-x

15. Bereshchenko O, Mancini E, Moore S, et al. Hematopoietic stem cell expansion precedes the generation of committed myeloid leukemia-initiating cells in C/EBPα mutant AML. *Cancer Cell*. 2009;16(5):390-400. doi:10.1016/j.ccr.2009.09.036