FCO • 15(1) • 2024 • 29–35
DOI: 10.2478/fco-2023-0037

sciendo

Forum of Clinical **Oncology**

CEBPA mutation in acute myeloid leukemia: prognostic impact of bZIP domain mutation

Research Article

Raj Roy*, Prithibi Das

North Bengal Medical College, Siliguri, India

Received 4 February 2024; Accepted 24 June 2024

Abstract: One of the most important prognostic genes for acute myeloid leukemia (AML) is *CEPBA*, and its mutation (CEBPAmu) is present in nearly 10%–15% of *de novo* AML cases. CEBPAdm is associated with a favorable prognosis; however, CEBPAsm does not appear to have a better prognosis than CEBPAdm. We reviewed CEBPAmu-bZIP in 8694 cases across studies for prognostic impact in patients with *de novo* AML. It was observed that CEBPAmu in the basic leucine zipper domain (bZIP) was strongly associated with a favorable prognosis, but CEBPAmu out of the bZIP domain was not. CEBPA-bZIP mutations were discovered in 562 (6.46%) of 8694 cases, with 366 (65.1%) harboring a second *CEBPA* mutation (CEBPA-double-mutated [CEBPAdm]) and 196 (34.9%) having a single CEBPA-bZIP only mutation. Multivariate analysis of three studies consisting of 1028, 4708, and 2958 patients showed that CEBPAmu in bZIP was the most potent predictor of overall survival (OS) with overall survivability of 53%, 62%, and 89%, respectively, independently. However, complete remission of disease with bZIP mutation in the same studies was found to be 80%, 62%, and 78.6%, respectively. These findings indicate that CEBPAmu in bZIP is a potent marker for AML prognosis.

Keywords: *AML • mutation • prognosis*

1. Introduction

The *CCAAT*/enhancer-binding protein alpha (CEBPA) is a transcription factor essential for the differentiation of granulocytes. The expression of transcription factor CEBPA initiates at the time of dedication of stem cells to myeloid lineage, but it is specifically upregulated in granulocytes and is downregulated in mature peripheral blood monocytes[1]. It is well demonstrated that in the presence of defective CEPBA, only myelocytic lineages are affected while other hematopoietic lineages remain as usual.

Leukemia develops from the serial possession of somatic mutations in hematopoietic stem and ancestor cells with the capacity to self-replicate and propagate the neoplastic clone.[2,3] Although some mutations, such as those in *DNMT3A*, *TET2*, and *ASXL1*, are more common in clonal haematopoiesis and appear to be fairly early events in leukemogenesis, others tend to be acquired later in the course of leukemia development, including mutations in *FLT3*, *NRAS*, and *RUNX1*. The combinations of mutations that eventually drive leukemogenesis are influenced by biological cooperativity and mutual exclusivity between mutated genes. Due to their overriding impact on disease phenotype and disease outcome, genetic aberrations are given priority in defining acute myeloid leukemia (AML) disease classification. The classification system of myeloid malignancies that recently appeared is hereby named the International Consensus Classification (ICC) [table 1] and the fifth edition of the World Health Organization (WHO) classification (WHO-HAEM5) [table 2].[4,5] Since 2017, new data has surfaced that illuminates the need to acclimate the risk classification. In addition to baseline genetic characterization, the significance of response to initial therapy and assessment of early MRD in individual risk assignment is stressed.[3,6] For further backing, an ELN AML risk classification has been developed based on data from intensively treated patients [table 3].[7]

Genetic abnormalities are the most potent prognostic markers for AML. In recent times, mutated genes in AML have been assessed along with traditional chromosomal analysis, which helps in the invention of more advanced targeted therapies. One of the most important prognostic genes for AML is *CEPBA*, and its mutation (CEBPAmu)

* *E-mail: rajroy2632@gmail.com*

Table 1: International Consensus Classification (ICC) 2022 of AML.

ICC 2022
AML with recurrent genetic abnormalities (requiring equal or greater than 10% blasts, except *)
AML with t(8;21)(q22;q22.1)/*RUNX1::RUNX1T1*
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB::MYH11*
Acute promyelocytic leukemia (APL) with t(15;17)(q24.1;q21.2)/*PML::RARA*; APL with other *RARA* rearrangements
AML with t(9;11)(p21.3;q23.3)/*MLLT3::KMT2A*; AML with other *KMT2A* rearrangements
AML with t(6;9)(p22.3;q34.1)/*DEK::NUP214*
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2::MECOM*(*EVI1*); AML with other *MECOM* rearrangements
AML with *BCR::ABL1* fusion*
AML with other rare recurring translocations
AML with mutated *NPM1*
AML with in-frame bZIP CEBPA mutations
*AML requiring equal or greater than 20% blasts***
AML with mutated *TP53* (VAF >10%)
AML with myelodysplasia-related gene mutations
AML with myelodysplasia-related cytogenetic abnormalities
Therapy-related myeloid neoplasms
NA (BECOMING DIAGNOSTIC QUALIFIER)
AML not otherwise specified; subtyping optional
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia***
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Myeloid sarcoma

Table 2: WHO classification (WHO-HAEM5) of AML.

WHO 2022
AML with defining genetic abnormalities (no blast % cut-off, except *)
AML with *RUNX1::RUNX1T1* fusion
AML with *CBFB::MYH11* fusion
Acute promyelocytic leukemia with *PML::RARA* fusion
AML with *KMT2A* rearrangement
AML with *DEK::NUP214* fusion
AML with *MECOM* rearrangement
AML with *RBM15::MRTFA* fusion
AML with *BCR::ABL1* fusion*
AML with *NUP98 rearrangement*
AML with other (rare) defined genetic alterations*
AML with *NPM1* mutation
AML with CEBPA mutation#*
AML requiring equal or greater than 20% blasts
AML, myelodysplasia-related
Therapy-related myeloid neoplasms
NA (BECOMING NEW ENTITY OF SECONDARY MYELOID NEOPLAMS)
AML, defined by differentiation
AML with minimal differentiation
AML without maturation
AML with maturation
Acute myelomonocytic leukemia
Acute monoblastic/monocytic leukemia
Pure erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Myeloid sarcoma

is present in almost 10%–15% of *de novo* AML cases. One-third of CEBPAmu is single-mutated *CEPBA* (CEBPAsm), a heterozygous monoallelic mutation, and two thirds are double-mutated *CEBPA* (CEBPAdm), mostly biallelic N- and C-terminal mutations. However, CEBPAsm has a poorer prognosis than CEBPAdm; thus, the utility of CEBPAsm as a prognostic factor has not been clarified.[8-12] Though it is not certified, CEBPAsm has a poorer prognosis than CEBPAdm.[13]

CEPBA messenger RNA has two translation initiation sites and two isoforms: p42 (42 kDa) and p30 (30 kDa). The basic leucine zipper (bZIP) domain located on the C-terminal is common to both isoforms and it helps in DNA binding to the major groove of the DNA molecule by protein dimerization. One-third of CEBPAsm is in mutation in the bZIP domain, whereas the remaining are out of the bZIP domain. In contrast, mutations in CEBPAdm can involve both bZIP domains, one of them, or none.

This study mainly focuses on the effect of the bZIP domain mutation of CEBPAmu on the prognosis of AML.

2. Materials and methods

2.1. Sources of data and inclusion criteria

We searched PubMed, Google Scholar, and EMBASE for studies related to our study that were published after September 30, 2021. Synonyms used for the search process were acute myeloid leukemia, *CEBPA*, mutation, prognosis. The search was confined to human studies without language limitations. We did a manual search of reference lists in applicable studies to find out the applicability of the information to our study. We considered the study if it met the following criteria: (i) published after September 30, 2021; (ii) studies considering patients diagnosed with AML; (iii) studies pointing to the *CEBPA* mutation in bZIP domain; and (iv) studies having survival information of the patients.

2.2. Exclusion criteria

Studies did not consider any particular treatment protocol and did not give information on prognosis.

Table 3: ELN AML risk classification.

Risk category†	Genetic abnormality
Favorable	• t(8;21)(q22;q22.1)/*RUNX1*::*RUNX1T1*†,‡ • inv(16)(p13.1q22) or t(16;16)(p13.1;q22)/*CBFB*::*MYH11*†,‡ • Mutated *NPM1*†,§ without *FLT3*-ITD • bZIP in-frame mutated *CEBPA*‖
Intermediate	• Mutated *NPM1*†,§ with *FLT3*-ITD • Wild-type *NPM1* with *FLT3*-ITD (without adverse-risk genetic lesions) • t(9;11)(p21.3;q23.3)/*MLLT3*::*KMT2A*†,¶ • Cytogenetic and/or molecular abnormalities not classified as favorable or adverse
Adverse	• t(6;9)(p23.3;q34.1)/*DEK*::*NUP214* • t(v;11q23.3)/*KMT2A*-rearranged# • t(9;22)(q34.1;q11.2)/*BCR*::*ABL1* • t(8;16)(p11.2;p13.3)/*KAT6A*::*CREBBP* • inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)/*GATA2, MECOM(EVI1)* • t(3q26.2;v)/*MECOM*(*EVI1*)-rearranged • −5 or del(5q); −7; −17/abn(17p) • Complex karyotype,** monosomal karyotype†† • Mutated *ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, and/or ZRSR2*‡‡ • Mutated *TP53*[a]

*Frequencies, response rates, and outcome measures should be reported by risk category, and, if sufficient numbers are available, by specific genetic lesions indicated.
†Mainly based on results observed in intensively treated patients. Initial risk assignment may change during the treatment course based on the results from analyses of measurable residual disease.
‡Concurrent *KIT* and/or *FLT3* gene mutation does not alter risk categorization.
§AML with *NPM1* mutation and adverse risk cytogenetic abnormalities are categorized as adverse-risk.
‖Only in-frame mutations affecting the basic leucine zipper (bZIP) region of *CEBPA*, irrespective of whether they occur as monoallelic or biallelic mutations, have been associated with favorable outcome.
¶The presence of t(9;11)(p21.3;q23.3) takes precedence over rare, concurrent adverse risk gene mutations.
#Excluding *KMT2A* partial tandem duplication (PTD).
**Complex karyotype: ≥3 unrelated chromosome abnormalities in the absence of other class-defining recurring genetic abnormalities; excludes hyperdiploid karyotypes with three or more trisomies (or polysomies) without structural abnormalities.
††Monosomal karyotype: presence of two or more distinct monosomies (excluding loss of X or Y), or one single autosomal monosomy in combination with at least one structural chromosome abnormality (excluding core-binding factor AML).
‡‡For the time being, these markers should not be used as an adverse prognostic marker if they co-occur with favorable-risk AML subtypes.
[a]*TP53* mutation at a variant allele fraction of at least 10%, irrespective of the *TP53* allelic status (mono- or biallelic mutation); *TP53* mutations are significantly associated with AML with complex and monosomal karyotype

2.3. Data analysis

The review team discussed the inclusion criteria for studies and extracted data independently from articles. Data were double checked before they were put into statistical analysis to count out any discrepancies. Data excluded from papers were name of first author, time of publication, number of subjects, age group, prevalence of *CEBPA* mutations, therapeutic strategy, and complete remission (CR).

2.4. Statistical analysis

The Kaplan–Meier method was used to estimate overall survival (OS), disease-free survival (DFS), and relapse rate (RR) in those concerned studies. The authors of this study did not perform any additional Kaplan–Meier method. OS was defined as the time from study entry to death or date of last follow-up in surviving patients; DFS was defined as the time from end of course 1 for patients in CR until relapse or death or date of last follow-up for those without an event.[14] These above-mentioned definitions of OS and DFS were stated by original studies. In addition, definition of end point was uniform across three studies. For the rest of the analysis, authors used Statistical Package for the Social Sciences (SPSS) 2023. To indicate statistical significance, a 5% level of probability was used.

3. Results

Three studies comprising 8694 patients were included in our study[15-17]. Studies included in our study originated from Japan, Germany, and Seatle (USA). We considered every age group for our study.

3.1. *CEBPA* mutations and their frequency

The frequency of all CEBPAmu is shown in Figure 1. There were 59 patients with CEBPAsm and 103 patients with CEBPAdm among the total 1028 patients in the study conducted by Wakita.[15] Of the patients with CEBPAdm, 91.3% (94 of 103) had a combination of mutations in the bZIP domain (CEBPAmu in bZIP) and mutations out of the bZIP domain (i.e., CEBPAmu out of bZIP), 2.9% (3 of 103) of the patients had two CEBPAmu in bZIP, and 5.8% (6 of 103) of the patients had two CEBPAmu out of bZIP. Furthermore, 32.2% (19 of 59) of the patients with CEBPAsm had mutations in the bZIP domain (CEBPAsm in bZIP) and 67.8% (40 of 59) of patients had mutations out of the bZIP domain (CEBPAsm out of bZIP).

A total of 240 of the 4708 patients, which corresponds to 5.1% of the total patients, were positive for the *CEBPA* mutation in the study conducted by Taube.[16] In addition, 131 of 240 patients (54.6%) showed two *CEBPA* mutations mostly associated with combinations of TAD and bZIP mutations, mentioned as CEBPA[bi] and

CEBPAsm mutations, respectively, found in 109 out of 240 patients (45.4%), most of which were associated with independent mutations of TAD and bZIP.

Mutations in the bZIP domain were identified in 160 (5.4%) of 2958 patients. Among patients with bZIP mutations, 132 (82.5%) harbored a cooperating TAD domain mutation on the other allele and the remaining 28 patients (17.5%) lacked a second *CEBPA* mutation. [17]

Figure 2 shows the frequency of CEBPAmu mutations in the three studies considered in our study.

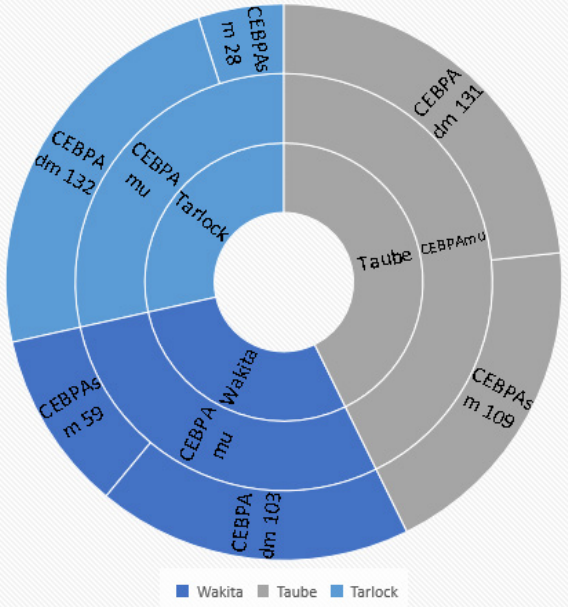

Figure 1: Frequency distribution of mutations reported in studies separated on the basis of author's name.

3.2. Clinical correlation of AML with CEBPAmu in bZIP

Most of the patients with AML with CEBPAmu on bZIP who were studied in the studies mentioned above belonged to immediate-risk chromosomal classification. Compared to patients bearing *CEBPA* WT mutation, those with *CEBPA* biallelic mutation were significantly younger (median age, 46 years) at diagnosis, similar to those with CEBPAsm on bZIP (median age, 50 years), whereas patients with CEBPAsm on TAD were significantly older (median age, 63 years) and were more comparable to the *CEBPA* WT mutation group (median, 57 years).

3.3. Comparison between CEBPAsm in bZIP and CEBPAsm out of bZIP

FLT3–ITD mutations were detected in 32.5% (13 of 40) of patients in the CEBPAsm out-of-bZIP group, which was higher than the 5.3% (1 of 19) of patients in the CEBPAsm in-bZIP group, and *NPM1* mutations were

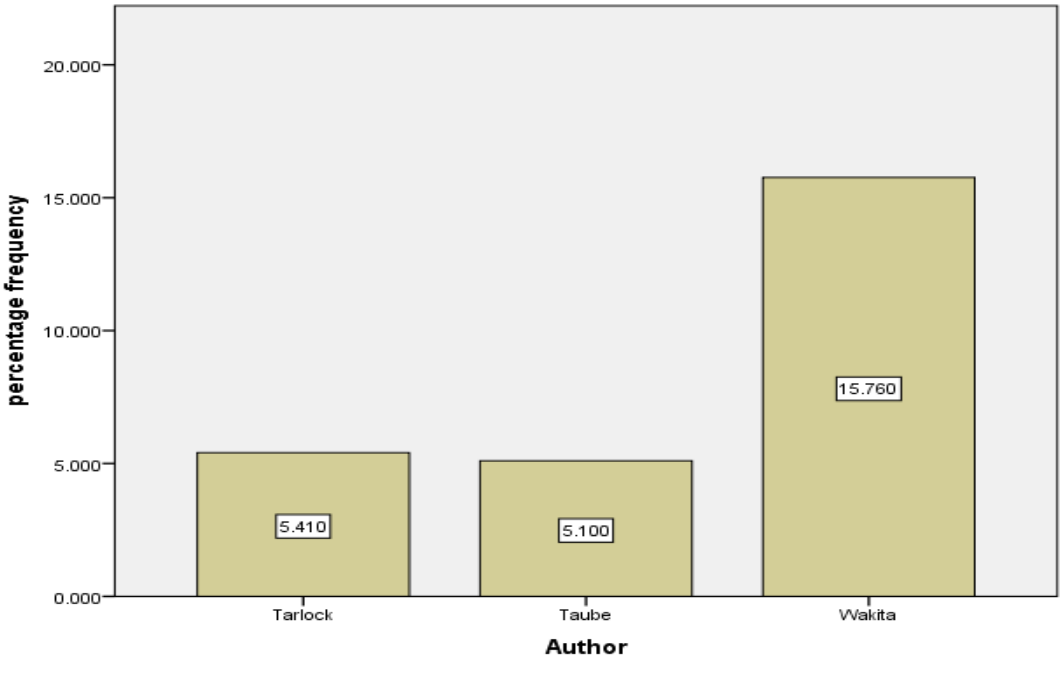

Figure 2: Comparison of percentage frequency of CEBPAmu found out of total study population.

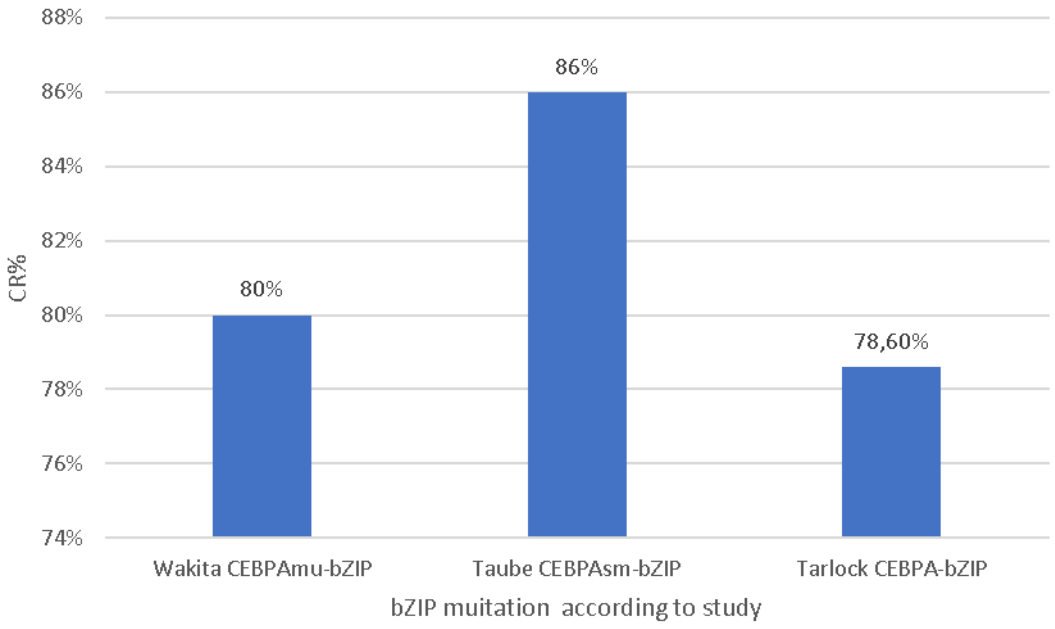

Figure 3: Complete remission (CR) rate percentage in the three studies.

detected in 35.0% (14 of 40) of patients in the CEBPAsm out-of-bZIP group and 26.3% (5 of 19) of patients in the CEBPAsm in-bZIP group according to the study conducted by Wakita et al.[15]

Based on the targeted NGS approach, additional mutations were identified in 208 of 240 patients with a *CEBPA* mutation (86.7%). The most frequently mutated genes were *TET2* (70 of 240; 29.2%), *GATA2* (68 of 240; 28.3%), *DNMT3A* (45 of 240; 18.8%), *FLT3–ITD* (39 of 240; 16.3%), *NPM1* and *NRAS* (31 of 240; 12.9% each), and *WT1* (30 of 240; 12.5%), according to the study by Taube et al.[16]

3.4. *CEBPA* mutations and outcome

Multivariate analyses for OS and cumulative incidence of relapse (CIR) were conducted on patients in a study conducted by Wakita et al., and they subsequently found OS (hazard ratio of 0.3287) and multivariate analysis for CIR in 525 patients who achieved CR showed that CEBPAmu in bZIP was an independent favorable prognostic factor of the CIR hazard ratio of 0.6157. A study conducted by Taube found that the *CEBPA* biallelic mutation had a significantly higher rate of CR as well as a higher median OS (CEBPA biallelic, 103.2 months; CEBPAsm, 21.9 months). Patients carrying CEBPAsm on bZIP showed significantly higher CR, with a remission rate close to 86% (Figure 3). Another study done by Tarlock presented the OS of the CEBPA bZIP mutation as 89% (Figure 4) and the OS of CEBPAdm as 81%.[17]

4. Discussion

We analyzed 8694 patients in a combination of three studies by Wakita, Taube, and Tarlock and double checked all the data extracted from those studies.

In the study conducted by Wakita et al., CEBPAdm was found to be a favorable prognostic marker. In addition, it was seen that if a single mutation is seen in *CEBPA* in the bZIP domain or in the bZIP domain, a mutation in the single or double position in the case of CEBPAdm is markedly favorable for diagnosis. CEBPAdm, which has been recommended in many previous guidelines, was found to be a confounding factor in CEBPAmu in the bZIP group and was not obtained as an independent prognostic factor. They also speculated that altered bZIP function acquired by the *CEBPA* bZIP mutant determines the sensitivity of the disease to chemotherapy. This surely explains why CEBPAdm is a better prognostic factor than CEBPAsm if linked with the bZIP domain, though it still needs further discussion. They also found CEBPAmu-bZIP to have an overall survivability of 53% (Figure 4) and CR of 80%.

In the study done by Tarlock et al. in a large cohort of 2948 children and young adults with newly diagnosed AML, they demonstrated that patients with *CEBPA*

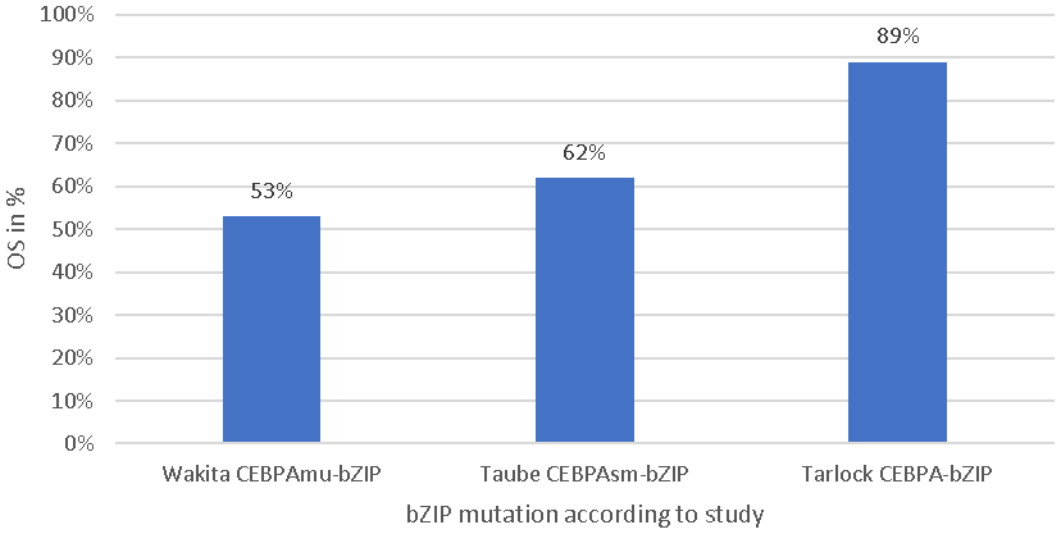

Figure 4: Overall survival (OS) rate in percentage in the three studies, showing favorable prognosis.

mutations that had single bZIP domain mutations experienced outcomes nearly identical to those of patients with biallelic *CEBPA* mutations. Georgi et al. reported on a cohort of 4578 adult patients with AML and showed analogous outcomes for patients with CEBPA-dm and CEBPA-bZIP.[18] In this study, CR was noted in 22 of 28 patients with CEBPA-bZIP, which corresponds to 78.6%. It was also found that 64% of patients with the CEBPA bZIP mutation experienced 5-year event-free survival.

Taube et al. found that only 90% of patients with biallelic *CEBPA* mutations containing typical bZIP mutations show a better outcome, which indicates that only those should be assigned to the favorable risk group. A typical bZIP mutation was also found in 90% of biallelic *CEBPA* mutations.

Acknowledgement

I thank all authors for their expertise in the concerned field.

References

[1] Radomska HS, Huettner CS, Zhang P, Cheng T, Scadden DT, Tenen DG. CCAAT/enhancer binding protein alpha is a regulatory switch sufficient for induction of granulocytic development from bipotential myeloid progenitors. Mol Cell Biol 1998; 18: 4301–4314.

[2] Ley TJ, Miller C, Ding L, Raphael BJ, Mungall AJ, Robertson A, et al; Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. N Engl J Med. 2013;368(22):2059-2074.

[3] Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209-2221.

[4] Arber DA, Orazi A, Hasserjian RP, Borowitz MJ, Calvo KR, Kvasnicka HM, et al. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. Blood. 2022 Sep 15;140(11):1200-1228. doi: 10.1182/blood.2022015850. PMID: 35767897; PMCID: PMC9479031.

[5] Khoury JD, Solary E, Abla O, Akkari Y, Alaggio R, Apperley JF et al. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. Leukemia. 2022 Jul;36(7):1703-1719. doi: 10.1038/s41375-022-01613-1. Epub 2022 Jun 22. PMID: 35732831; PMCID: PMC9252913.

[6] Jaiswal S, Ebert BL. Clonal hematopoiesis in human aging and disease. Science. 2019;366(6465):eaan4673.

[7] Hartmut Döhner, Andrew H. Wei, Frederick R. Appelbaum, Charles Craddock, Courtney D. DiNardo, Hervé Dombret, 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: https://doi.org/10.1182/blood.2022016867.

[8] Wouters BJ, Lowenberg B, Erpelinck-Verschueren CA, van Putten WL, Valk PJ, Delwel R. Double CEBPA mutations, but not single CEBPA mutations, € define a subgroup of acute myeloid leukemia with a distinctive gene expression profile that is uniquely associated with a favorable outcome. Blood. 2009;113(13):3088-3091.

[9] Pabst T, Eyholzer M, Fos J, Mueller BU. Heterogeneity within AML with CEBPA mutations; only CEBPA double mutations, but not single CEBPA mutations are associated with favourable prognosis. Br J Cancer. 2009;100(8):1343-1346.

[10] Green CL, Koo KK, Hills RK, Burnett AK, Linch DC, Gale RE. Prognostic significance of CEBPA mutations in a large cohort of younger adult patients with acute myeloid leukemia: impact of double CEBPA mutations and the interaction with FLT3 and NPM1 mutations. J Clin Oncol. 2010;28(16):2739-2747.

[11] Dufour A, Schneider F, Metzeler KH, Hoster E, Schneider S, Zellmeier E, et al. Acute myeloid leukemia with biallelic CEBPA gene mutations and normal karyotype represents a distinct genetic entity associated with a favorable clinical outcome. J Clin Oncol. 2010 Feb 1;28(4):570-7. doi: 10.1200/JCO.2008.21.6010. Epub 2009 Dec 28. PMID: 20038735.

[12] Taskesen E, Bullinger L, Corbacioglu A, Sanders MA, Erpelinck CA, Wouters BJ, et al. Prognostic impact, concurrent genetic mutations, and gene expression features of AML with CEBPA mutations in a cohort of 1182 cytogenetically normal AML patients: further evidence for CEBPA double mutant AML as a distinctive disease entity. Blood. 2011;117(8):2469-2475.

[13] Schlenk RF, Taskesen E, van Norden Y, Krauter J, Ganser A, Bullinger L, et al. The value of allogeneic and autologous hematopoietic stem cell transplantation in prognostically favorable acute myeloid leukemia with double mutant CEBPA. Blood. 2013;122(9):1576-1582.

[14] Kalbfleisch JD, Prentice RL. The Statistical Analysis of Failure Time Data. Hoboken, NJ: John Wiley & Sons, Inc; 2002.

[15] Wakita S, Sakaguchi M, Oh I, Kako S, Toya T, Najima Y, et al. Prognostic impact of CEBPA bZIP domain mutation in acute myeloid leukemia. Blood Adv. 2022 Jan 11;6(1):238-247. doi: 10.1182/bloodadvances.2021004292. PMID: 34448807; PMCID: PMC8753195.

[16] Taube F, Georgi JA, Kramer M, Stasik S, Middeke JM, Röllig C, et al. CEBPA mutations in 4708 patients with acute myeloid leukemia: differential impact of bZIP and TAD mutations on outcome. Blood. 2022 Jan 6;139(1):87-103. doi: 10.1182/blood.2020009680. PMID: 34320176.

[17] Tarlock K, Lamble AJ, Wang YC, Gerbing RB, Ries RE, Loken MR, et al. CEBPA-bZip mutations are associated with favorable prognosis in de novo AML: a report from the Children's Oncology Group. Blood. 2021 Sep 30;138(13):1137-1147. doi: 10.1182/blood.2020009652. Erratum in: Blood. 2022 Mar 10;139(10):1601. PMID: 33951732; PMCID: PMC8570058.

[18] Georgi J-A, Taube F, Kramer M, Herold S, Schaefer-Eckart K, Schmitz N, et al. Differences between CEBPA bZIP and TAD mutations and their effect on outcome-an analysis in 4578 patients with acute myeloid leukemia [abstract]. Blood. 2016;128(22). Abstract 283.