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Genetic heterogeneity of cytogenetically normal AML with mutations of *CEBPA*

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Key Points

- bi*CEBPA*-mutated AML is a heterogeneous subentity that consists of at least 2 subgroups with different genetic and clinical features.

Biallelic mutations of the CCAAT/enhancer binding protein α (*CEBPA*) gene define a distinct genetic entity of acute myeloid leukemia (AML) with favorable prognosis. The presence of *GATA2* and *CSF3R* mutations that are specifically associated with this subgroup but not mutated in all samples suggests a genetic heterogeneity of bi*CEBPA*-mutated AML. We characterized the mutational landscape of *CEBPA*-mutated cytogenetically normal AML by targeted amplicon resequencing. We analyzed 48 biallelically mutated *CEBPA* (bi*CEBPA*), 32 monoallelically mutated *CEBPA* (mo*CEBPA*), and 287 wild-type *CEBPA* (wt*CEBPA*) patient samples from German AML Cooperative Group studies or registry. Targeted sequencing of 42 genes revealed that mo*CEBPA* patients had significantly more additional mutations and additional mutated genes than bi*CEBPA* patients. Within the group of bi*CEBPA* patients, we identified 2 genetic subgroups defined by the presence or absence of mutations in chromatin/DNA modifiers (C), cohesin complex (C), and splicing (S) genes: bi*CEBPA*$^{\mathrm{CCSpos}}$ (25/48 [52%]) and bi*CEBPA*$^{\mathrm{CCSneg}}$ (23/48 [48%]). Equivalent subgroups were identified in 51 bi*CEBPA* patients from the Cancer Genome Project. Patients in the bi*CEBPA*$^{\mathrm{CCSpos}}$ group were significantly older and had poorer overall survival and lower complete remission rates following intensive chemotherapy regimens compared with patients in the bi*CEBPA*$^{\mathrm{CCSneg}}$ group. Patients with available remission samples from the bi*CEBPA*$^{\mathrm{CCSpos}}$ group cleared the bi*CEBPA* mutations, but most had persisting CCS mutations in complete remission, suggesting the presence of a preleukemic clone. In conclusion, CCS mutations define a distinct biological subgroup of bi*CEBPA* AML that might refine prognostic classification of AML. This trial was registered at www.clinicaltrials.gov as #NCT00266136 and NCT01382147.

Introduction

Mutations in the CCAAT/enhancer binding protein α (*CEBPA*) gene are detected in ∼10% of patients with cytogenetically normal (CN) acute myeloid leukemia (AML). *CEBPA* mutations can be either biallelic or monoallelic. Most patients with 2 *CEBPA* mutations carry 1 frameshift mutation in the N-terminal part of the

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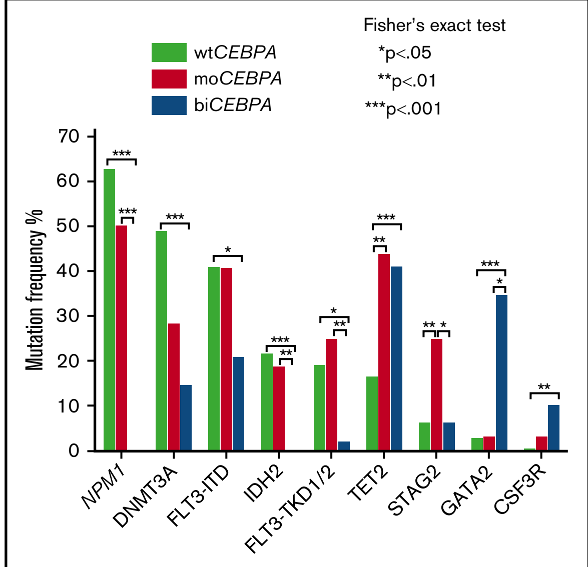

Figure 1. Mutation spectrum of mo*CEBPA*, bi*CEBPA*, and wt*CEBPA*. Evaluation of the mutation spectrum of mo*CEBPA* (n = 32) and bi*CEBPA* (n = 48) patients in comparison with wt*CEBPA* samples (n = 287). Eight of 20 genes with a mutation frequency of ≥5% were significantly associated with ≥1 groups.

protein and the other one in the bZIP domain, which is located at the C terminus.[1,2] A single *CEBPA* mutation is most often found at the N terminus. N-terminal frameshift mutations specifically abolish the translation of the full-length (42-kDa) protein of *CEBPA*, leading to the overexpression of a shorter, dominant-negative 30-kDa isoform of *CEBPA*.[3] C-terminal in-frame mutations disrupt the homo- and heterodimerization domains and therefore impair the DNA-binding activity of the *CEBPA* protein.[4,5] Only patients with biallelically mutated *CEBPA* (bi*CEBPA*) have favorable outcomes when compared with other CN-AML patients.[2,6-12] Because of its unique characteristics, AML with bi*CEBPA* mutations are classified as a distinct entity in the 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia.[10] bi*CEBPA* mutations are rarely associated with other prognostic mutations such like internal tandem duplications (ITD) of the fms-like tyrosine kinase 3 (*FLT3*) gene, mutations in the tyrosine kinase domain (TKD) of *FLT3*, mutations in nucleophosmin 1 gene (*NPM1*), or partial tandem duplications of the lysine methyltransferase 2A (*KMT2A*) gene. We previously identified a specific association of bi*CEBPA* mutations with mutations in the transcription factor *GATA2* in 39% of cases.[13] *CSF3R* mutations are described to be also frequently mutated (29%) in bi*CEBPA* patients.[14] In the present study, we aimed to characterize the mutational spectrum of mo*CEBPA* and bi*CEBPA* CN-AML patients. We also analyzed outcome in patients with bi*CEBPA* mutations based on these additional mutations.

Methods

Targeted amplicon resequencing (Agilent Haloplex; target region, ~62 kbp) was used to analyze 42 genes and hotspots in 80 *CEBPA* mutated AML patients (32 monoallelically mutated *CEBPA* [mo*CEBPA*] and 48 bi*CEBPA*); 65 of these AML patients (40 bi*CEBPA* and 25 mo*CEBPA*) were enrolled in the German AML Cooperative Group (AMLCG) 1999 or 2008 multicenter

Table 1. Characteristics of mo*CEBPA* vs bi*CEBPA* patients

Characteristic	mo*CEBPA*	bi*CEBPA*	*P*
Age, y			.69
Median	62	57	
Range	16-78	20-84	
Sex			
Female	23 (72)	23 (48)	**.04**
WBC count, ×10⁹/L	31/32	47/48	.47
Median	30	28	
Range	0.4-188	1.3-408.6	
Hemoglobin, g/dL	29/32	44/48	.13
Median	9.1	10	
Range	2.7-13.2	6.4-13.8	
Platelet count, ×10⁹/L	28/32	47/48	.09
Median	52	32	
Range	3-291	3-151	
Bone marrow blasts, %	19/32	40/48	.65
Median	85	71	
Range	30-95	20-100	
LDH, U/dL	20/32	36/48	.67
Median	392	479	
Range	152-2666	205-2510	

Data represent number or n (%) of patients, unless otherwise indicated. Bold indicates significant *P* values.
LDH, lactate dehydrogenase; WBC, white blood cell.

randomized phase 3 trials or in the AMLCG registry. All other patients were treated according to standard intensive induction/postremission protocols. Long-term follow-up data were available for all mo*CEBPA* patients and 45 of 48 bi*CEBPA* patients.

Treatment protocols of the AMLCG 1999 (registered at www.clinicaltrials.gov as #NCT00266136) or AMLCG 2008 (#NCT01382147) trials have been reported previously.[15,16] Study protocols were approved by the ethics committees of the participating centers. From all patients, written informed consent to the scientific use of surplus samples was obtained in accordance with the Declaration of Helsinki.

Genomic DNA was extracted from bone marrow or peripheral blood samples using QIAcube technology (QIAGEN, Hilden, Germany). 200 ng double-stranded genomic DNA, as quantified by a Qubit Fluorometer 2.0 (Life Technologies, Carlsbad, CA), was used for the target capture reaction from each sample. A custom-design HaloPlex Target Enrichment kit (1-500 kb; Agilent, Boeblingen, Germany) was employed to capture the target regions according to the HaloPlex Target Enrichment System-Fast Protocol Version B. Paired-end sequencing (2 × 250 bp) was performed on an Illumina MiSeq instrument (Illumina, San Diego, CA). Sequence alignment and variant detection was performed as described previously.[17] A variant allele frequency threshold of 2% was set for mutation detection. *CEBPA* mutations in diagnosis and if available in remission samples were identified using fragment-length analysis with subsequent Sanger sequencing.[1] *FLT3*-ITD status was analyzed via fragment length analysis.[18] *CSF3R* (NM_156039) exons 14-17 were analyzed by Sanger sequencing.[19] Sequence traces were analyzed with Sequencher software (Gene Codes, Ann Arbor, MI). Target regions

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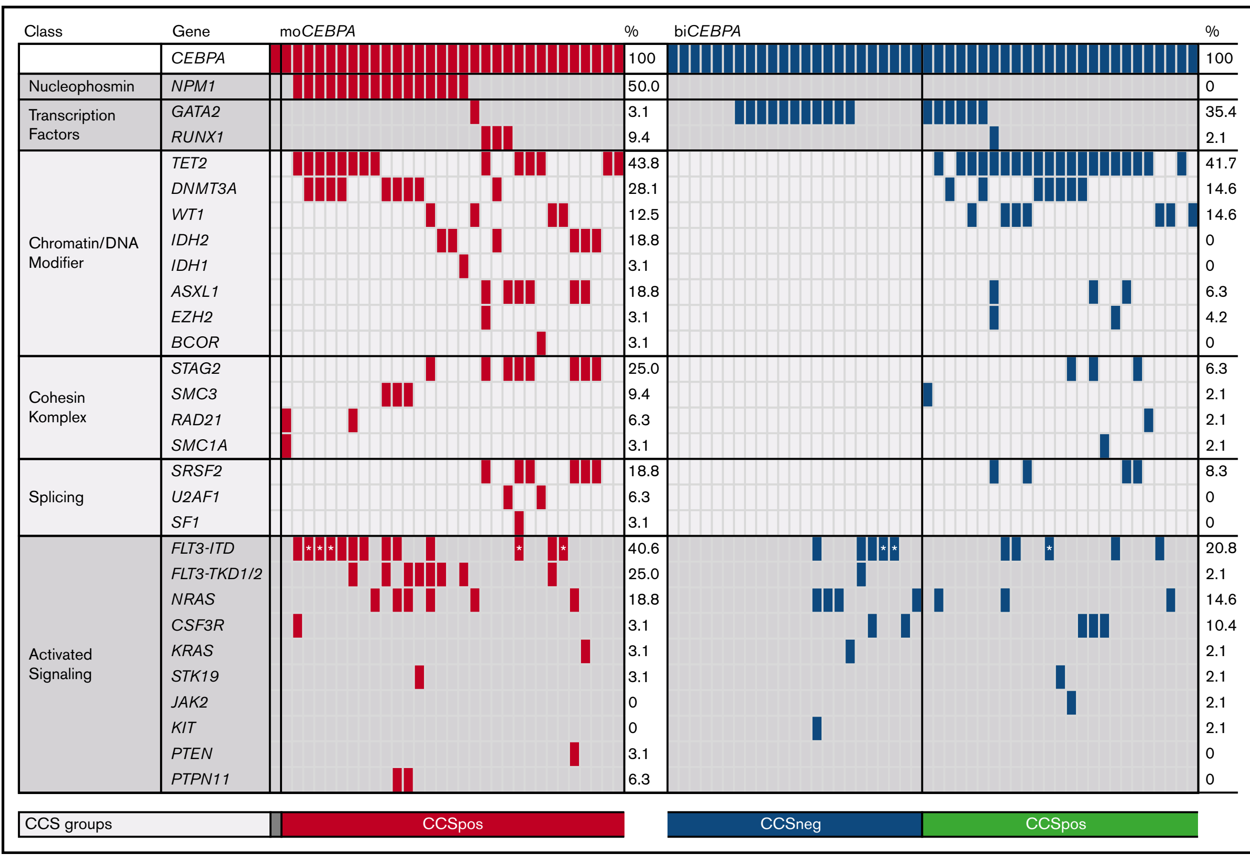

Figure 2. Frequency of genetic alterations organized by categories of related genes and genetic groups. The heatmap includes all genes that were mutated in either the mo*CEBPA* (red) or bi*CEBPA* (dark blue) subgroup. bi*CEBPA* patient samples were further separated in 2 biological groups: CCSneg (blue) and CCSpos (green). Patients with a signal ratio of *FLT3*-ITD ≥0.5 are marked with an asterisk.

of remission samples were analyzed by sequencing analysis on the Ion PGM system (coverage ~1000-fold).

Differences in patient characteristics were calculated using the Fisher's exact test for categorical variables and Mann-Whitney *U* test for continuous variables. The Fisher's exact test was used to compare pairwise mutation frequencies among the 3 *CEBPA* groups (mo*CEBPA* or bi*CEBPA* and wild-type *CEBPA* [wt*CEBPA*]) for genes with a mutation frequency ≥5% in at least 1 of the *CEBPA* groups. Adjustment for multiple hypothesis testing was performed using the method described by Benjamini and Hochberg.[20] To evaluate differences in additional mutated genes or number of additional gene mutations between mo*CEBPA* and bi*CEBPA* patients, we used the Mann-Whitney *U* test. We further characterized the mutation profile of bi*CEBPA*-mutated patients. There is evidence that AML patients with mutations in chromatin modifier and splicing genes have poor outcomes.[21] We therefore analyzed outcomes depending on presence and absence of chromatin modifiers and splicing genes and added cohesion genes to this group upon its interaction with chromatin. All clinical end points were defined according to generally accepted criteria.[22] Overall survival (OS) was calculated from the date of randomization for patients in clinical trials or at the date of first diagnosis for patients in registry until death from any cause. Relapse-free survival (RFS) was determined for responders from the first day of complete remission (CR) until relapse or death from any cause. Estimated probabilities of OS and RFS were calculated using the Kaplan-Meier method. The log-rank test evaluated statistical differences (*P* value) between survival distributions. Patients who had undergone allogeneic bone marrow transplantation were censored at the time of transplantation. For all tests, $P \leq .05$ was considered significant. Statistical computations were performed using SPSS software version 20.0 (SPSS, Chicago, IL) and the R software package version 3.3.1 (R Foundation for Statistical Computing; www.r-project.org).

Results

Using targeted resequencing, we analyzed a cohort of 80 *CEBPA*-mutated CN-AML patients (n = 48 bi*CEBPA*, n = 32 mo*CEBPA*) for mutations in 42 genes (supplemental Tables 1 and 2). *CEBPA* and *FLT3*-ITD mutational status was analyzed using conventional techniques (supplemental Tables 3 and 4).

We evaluated the mutational spectrum of our *CEBPA* cohort in comparison with a cohort of 287 wt*CEBPA* patients.[17] Among the 20 genes with a mutation frequency of ≥5%, 8 were significantly associated with *CEBPA* mutation status (Figure 1).

We found significantly more *STAG2* (25%; $P = .04$) and *FLT3*-TKD1/2 (25%; $P = .01$) mutations in the mo*CEBPA* subgroup than in the bi*CEBPA* group. Most of the TKD mutations in the mo*CEBPA* cohort were subclonal. *GATA2* (35%) and *CSF3R* (10%) mutations were most frequently found in bi*CEBPA*-mutated

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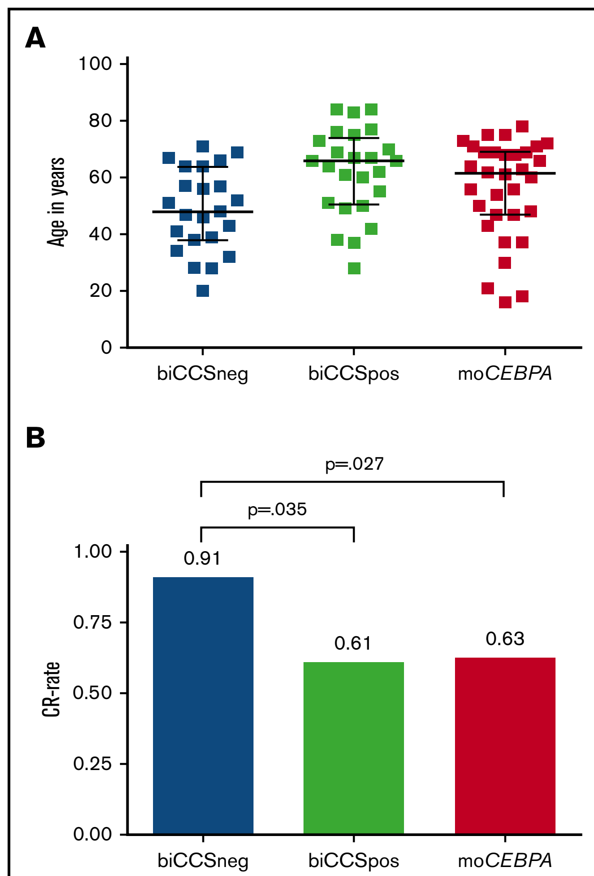

Figure 3. Age and CR rate of bi*CEBPA*^{CCSpos}, bi*CEBPA*^{CCSneg}, and mo*CEBPA* patients. (A) Age of bi*CEBPA*^{CCSneg} (median = 48), bi*CEBPA*^{CCSpos} (median = 66), and mo*CEBPA* (median = 62) patients. Scatter dot plot, median with interquartile range. (B) The CR rate was significantly higher in bi*CEBPA*^{CCSneg} patients (91% [20/22]) than bi*CEBPA*^{CCSpos} (61% [14/23]; P = .035) and mo*CEBPA* patients (P = .027).

patient samples. In our cohort, *IDH2* and *NPM1* mutations were mutually exclusive of bi*CEBPA* mutations.

DNMT3A was most frequently detected in wt*CEBPA* (49%) patients. The frequency of *DNMT3A* mutations in wt*CEBPA* was significantly different from those in bi*CEBPA* (15%; $P < .001$), but not mo*CEBPA* (28%; $P = .07$).

We found a higher frequency of *FLT3*-ITD mutations in mo*CEBPA* (41%; $P = .13$) and wt*CEBPA* (41%; $P = .02$) patients than bi*CEBPA* patients (21%).

TET2 mutations were significantly more frequent in mo*CEBPA* (44%, $P = .003$) and bi*CEBPA* (42%, $P = .001$) patients than wt*CEBPA* patients. In our data set, the frequency of *TET2* mutations is much higher than that of other *CEBPA*-mutated AML cohorts.[23] The frequency in our *CEBPA* cohort is also higher when compared with other AML cohorts.[24,25]

TET2 mutations were located within the conserved region of the gene and are thus likely pathogenic.[26,27] These data show that *TET2* mutations define a subgroup of *CEBPA*-mutated samples regardless of the *CEBPA* allelic mutation status.

Table 2. Characteristics of bi*CEBPA*^{CCSneg} vs bi*CEBPA*^{CCSpos} patients

Characteristic	bi*CEBPA*^{CCSneg}	bi*CEBPA*^{CCSpos}	*P*
Age, y			**.005**
Median	48	66	
Range	20-71	28-84	
Sex			.77
Female	12 (52)	11 (44)	
WBC count, ×10⁹/L		24/25	.39
Median	11.8	30.8	
Range	1.3-408.6	1.7-284.4	
Hemoglobin, g/dL	21/23	23/25	.12
Median	10.1	9.7	
Range	7.9-13.8	6.4-12.1	
Platelet count, ×10⁹/L		24/25	.89
Median	28	35	
Range	3-151	10-120	
Bone marrow blasts, %	19/23	21/25	.58
Median	66	75	
Range	20-97	31-100	
LDH, U/dL	19/23	17/25	.14
Median	427	561	
Range	205-2510	229-1660	

Data represent number or n (%) of patients, unless otherwise indicated. Bold indicates significant *P* values.

Next, we compared both *CEBPA* subgroups. With the exception of sex, there were no significant differences in baseline characteristics between mo*CEBPA* and bi*CEBPA* patient samples (Table 1).

Targeted sequencing analysis of these 42 genes revealed that mo*CEBPA* patients had significantly more additional mutations than bi*CEBPA* patients (mean: 4.0 ± 1.7 vs 2.2 ± 1.5; $P < .001$). The number of additional mutated genes was also significantly higher in mo*CEBPA* patients than bi*CEBPA* patients (mean: 3.7 ± 1.6 vs 2.0 ± 1.3; $P < .001$) (supplemental Figure 1). The 2 groups differed significantly with regard to mutations in the following genes: *NPM1*, *FLT3*-TKD1/2, *IDH2*, *STAG2*, and *GATA2* (supplemental Figure 2). Patients without any additional mutations were more frequently found in the bi*CEBPA* group than in the mo*CEBPA* cohort (6/48, 13% vs 1/32, 3%; $P = .23$; Figure 2).

Moreover, we identified 2 distinct genetic subgroups in bi*CEBPA* patients based on comutated genes (Figure 2). bi*CEBPA* patients with mutations in chromatin/DNA modifiers (C), cohesin complex (C), and splicing (S) genes were defined as bi*CEBPA*^{CCSpos} (n = 25). The bi*CEBPA*^{CCSpos} group is mainly defined by *TET2* (20/25), *DNMT3A* (7/25), and *WT1* (7/25) mutations. The other group, bi*CEBPA*^{CCSneg}, includes bi*CEBPA* patients with mutations in signaling factors only and/or *GATA2* and patients with no additional mutations in the 42 genes analyzed. In a cohort of 51 bi*CEBPA* patients without recurrent chromosomal abnormalities and genomic rearrangements (analyzed in a large AML cohort of 1540 patients reported by the Cancer Genome Project), we also identified a bi*CEBPA*^{CCSneg} subgroup; however, this group is smaller (31%) than ours (48%) (supplemental Figure 3). The most frequently

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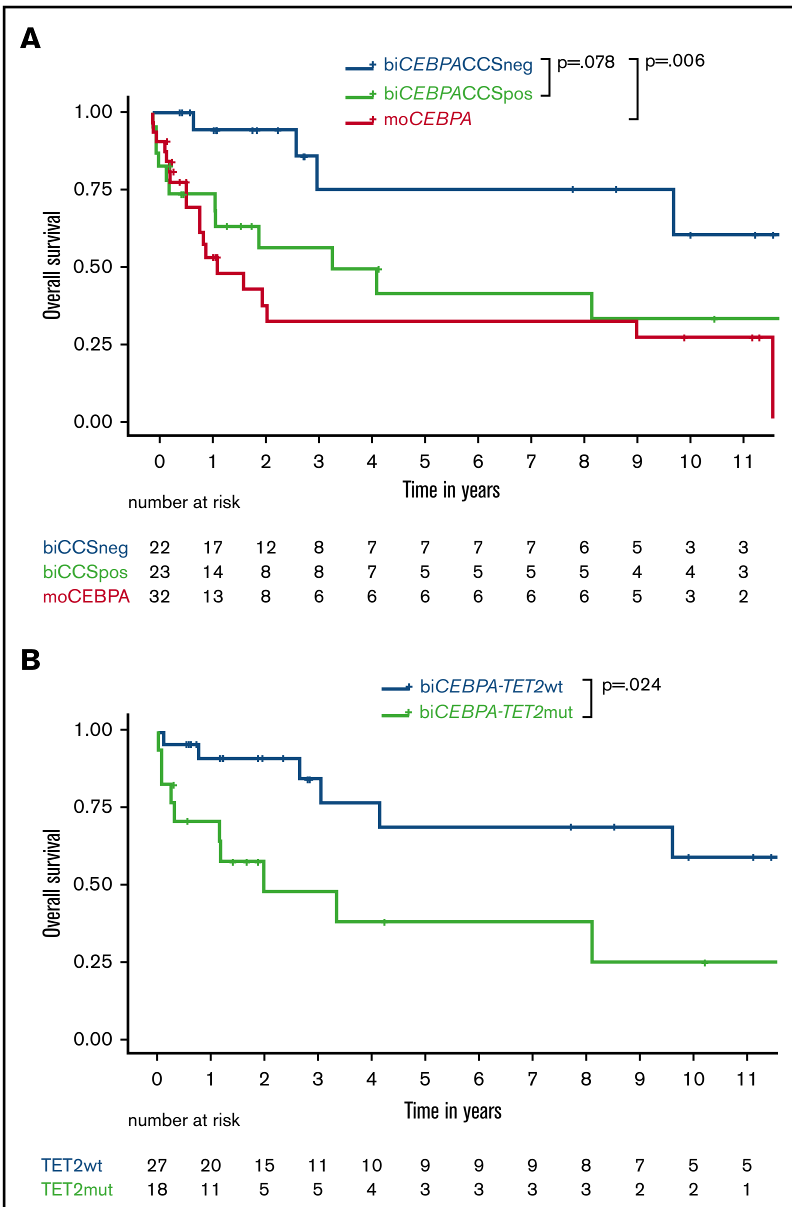

Figure 4. Survival data depending on biogroup and *TET2* status. (A) OS of bi*CEBPA*^{CCSneg}, bi*CEBPA*^{CCSpos}, and mo*CEBPA* patients. (B) OS of bi*CEBPA* patients depending on *TET2* mutational status.

mutated gene in the bi*CEBPA*^{CCSpos} group of the Cancer Genome Project was *WT1* (20%).[21]

bi*CEBPA*^{CCSpos} patients were significantly older than bi*CEBPA*^{CCSneg} patients (66 vs 48 years, $P = .005$; Figure 3A; Table 2). Patients in CCSpos subgroup also had a lower CR rate (Figure 3B) and a higher early death rate (supplemental Table 6).

bi*CEBPA*^{CCSpos} patients had a trend to poorer outcome in terms of OS than bi*CEBPA*^{CCSneg} patients (hazard ratio [HR], 2.6; 95% confidence interval [CI], 0.9-7.4; $P = .078$; Figure 4A). RFS and cumulative incidence of relapse were not statistically significant (supplemental Figure 4)

Multivariable Cox regression including CCS status and age suggested that age does not fully explain the potential differences in OS (adjusted CCS HR, 2.0; 95% CI, 0.7-6.0; $P = .22$; supplemental Table 5). In a multivariable logistic regression model to assess the association between CR and CCS after adjusting for age, the score was prognostic for patients in CR (adjusted CCS OR, 0.17; 95% CI, 0.03-1.01; $P = .05$), but not patients in CR or CR with incomplete hematologic recovery (adjusted CCS OR, 0.58; 95% CI, 0.08-3.97; $P = .58$; supplemental Table 7). For the end point early death at day 60, the model proved to be unsuitable, because there was no early-death event in the bi*CEBPA*^{CCSneg} cohort and only 4 in the bi*CEBPA*^{CCSpos} cohort (supplemental Table 6). Accounting for a reduced statistical power due to small numbers, CCS status might have an independent influence on survival. Survival of bi*CEBPA*^{CCSpos} and mo*CEBPA* patients was comparable (Figure 4A).[28]

bi*CEBPA*^{CCSpos} and bi*CEBPA*^{CCSneg} patients did not significantly differ in treatment regimens, which consisted of high-dose cytarabine and mitoxantrone vs 6-thioguanine, standard-dose cytarabine and daunorubicin (TAD9) (supplemental Table 8).

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Figure 5. Mutational status of bi*CEBPA*CCSpos and bi*CEBPA*CCSneg remission samples (age is given in brackets). (A) Four out of 5 bi*CEBPA*CCSpos patients had persisting clones in CR, indicating a clonal hematopoiesis. (B) None of the 5 bi*CEBPA*CCSneg samples had a detectable mutation load at the time of remission. Dx, diagnosis.

A

	biCEBPA CCSpos									
	UPN21 (60)		UPN43 (67)		UPN25 (75)		UPN69 (84)		UPN71 (76)	
	Dx	CR	Dx	CR	Dx	CR	Dx	CR	Dx	CR
CEBPA	+	-	+	-	+	-	+	-	+	-
TET2	+	+	+	+	+	+	+	+	+	-
DNMT3A	+	+	+	+	+	-				
WT1									+	-
SRSF2									+	-

B

	biCEBPA CCSneg									
	UPN24 (43)		UPN64 (46)		UPN65 (57)		UPN63 (41)		UPN18 (38)	
	Dx	CR	Dx	CR	Dx	CR	Dx	CR	Dx	CR
CEBPA	+	-	+	-	+	-	+	-	+	-
GATA2	+	-	+	-	+	-	+	-		
KRAS							+	-		
CSF3R									+	-

As most of the patients in bi*CEBPA*CCSpos group (20/25, 80%) were defined by *TET2* mutations, we further analyzed the prognostic effect of *TET2*. OS (HR, 3.1; 95% CI, 1.2-8.1; $P = .024$) was significantly worse in bi*CEBPA* patients with a *TET2* mutation than those without a *TET2* mutation (Figure 4B). In bi*CEBPA* patients, there was a trend for an effect of *TET2* on RFS (supplemental Figure 5).

We also analyzed OS and RFS in mo*CEBPA* patients (supplemental Figure 6A-B). In 32 (n = 14 *TET2* mutated) and 21 (n = 8 *TET2* mutated) mo*CEBPA* patients, there was no significant difference in OS (HR, 2.0; 95% CI, 0.8-5.2; $P = .15$) and RFS (HR, 0.9; 95% CI, 0.3-2.9; $P = .99$), respectively.

TET2 and *DNTM3A* mutations, which largely define our bi*CEBPA*CCSpos subgroup, are frequently present during complete morphologic remission as a result of clonal hematopoiesis.[29,30] This finding leads us to the hypothesis that in some bi*CEBPA*CCSpos patients, a preleukemic clone might have been present (supplemental Figure 7). In 4 out of 5 patients for whom material from complete morphologic remission was available, we could identify persisting *TET2* or *DNMT3A* mutations (Figure 5A; supplemental Table 9). It is very likely that in these patients, the *TET2* and *DNMT3A* mutations might have preceded the *CEBPA* mutations. In contrast, we did not find persisting mutations in available remission samples (n = 5) from the bi*CEBPA*CCSneg subgroup (Figure 5B).

In 6% to 10% of bi*CEBPA*-mutated AML patients, 1 of the *CEBPA* mutations is of germline origin.[31,32] Because of younger age and fewer or no concomitant mutations, a germline *CEBPA* variant would be more likely to occur in bi*CEBPA*CCSneg patients. Remission material was available from 8 out of 23 bi*CEBPA*CCSneg samples, and these patients had no persisting *CEBPA* mutation. In 7 out of 25 bi*CEBPA*CCSpos samples, we also did not detect a *CEBPA* germline mutation.

Discussion

Our study provides novel key findings with genetic relevance in *CEBPA*-mutated AML. We studied a cohort of 80 *CEBPA*-mutated CN-AML patients, including 48 patients with biallelic mutations. Samples from these patients were genetically well characterized by deep amplicon sequencing, with a special focus on mutation patterns between mo*CEBPA* and bi*CEBPA* patient samples and within the bi*CEBPA* subgroup.

In this dataset, we identified novel associations of mo*CEBPA* with *STAG2* and *FLT3*- TKD1/2 mutations.[2,23] Previously, Lavallee et al identified recurrent mutations (T618I) in the *CSF3R* (29%) gene in a small cohort of 14 patients with double-mutated *CEBPA*.[14] *CSF3R* is the receptor for colony-stimulating factor 3 and, like *CEBPA*, is crucial for normal granulopoiesis.[33] In this cohort of bi*CEBPA* patients, the mutation frequency of the T618I *CSF3R* was 10%. Because of the larger size of our bi*CEBPA* cohort and the fact that we only included normal-karyotype-AML the mutation frequency of *CSF3R* in our cohort might be more representative of bi*CEBPA* CN-AML patients.

We found a higher frequency of *TET2* mutations in mo*CEBPA* (44%) and bi*CEBPA* patients (42%) than in other AML or *CEBPA*-mutated AML cohorts (8% to 24%).[23-25] When we compared these data with our wt*CEBPA* cohort data set, we found that *TET2*

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mutations define a distinct subgroup of *CEBPA*-mutated samples regardless of allelic status. Most mutations result in a frame shift or premature stop codon, underscoring their functional relevance. The presence of a *TET2* mutation in bi*CEBPA* patients has a negative impact on OS, which is in concordance with the findings of Grossmann et al.[31]

We also could show that patients in the bi*CEBPA* subgroup had a significantly lower frequency of cooccurring mutations. Although we did not perform genome-wide analysis, these data suggest that the second hit in the *CEBPA* gene may drive leukemogenesis, and fewer cooperating mutations in other genes might therefore be required.

We identified 2 genetic subgroups in bi*CEBPA*-mutated AML and validated this finding in a bi*CEBPA* patient cohort from Papaemmanuil et al.[21] Patients in the bi*CEBPA*^{CCSpos} group were significantly older and had a poorer prognosis, and most patients with remission samples available had persisting mutations in *TET2* and/or *DNMT3A*, suggesting preleukemic clonal hematopoiesis even before the onset of leukemia. These data suggest that CCS mutations are the initiating events in this subgroup, followed by the acquisition of bi*CEBPA* mutations. In contrast, bi*CEBPA*^{CCSneg} patients were younger, had a unique pattern of co-occurring mutations, cleared all mutations in remission, and had a favorable prognosis.

Two patients with mo*CEBPA* mutation also had persisting *DNMT3A* mutations. Due to a lack of samples, we could not perform any further analysis in this cohort.

The poorer prognosis, higher early death rate, and lower CR rate in bi*CBEPA*^{CCSpos} patients could only partly be explained by older age. Clonal hematopoiesis might also be a risk factor for early death, although our study lacks the statistical power to test this hypothesis. Recent data indicate that healthy individuals with clonal hematopoiesis of indeterminate potential have a higher risk of cardiovascular disease.[34] In a logistic regression model, the CCS score for bi*CEBPA*-mutated patient samples was prognostic for patients in CR.

In conclusion, our results suggest that bi*CEBPA* mutations can be further subdivided by CCS mutation status in 2 groups with distinct genetic characteristics. This subclassification provides insight in the pathogenesis of the disease and might help to further refine prognostic classification of AML.

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Authorship

Contribution: N.P.K., A.D., and K.S. designed the study; N.P.K., K.H.M., and M.R.-T. analyzed sequencing data; N.P.K., E.H., F.P., and T. Herold performed statistical analysis; N.P.K., S.K.B., S.S., B.K., S.T., and A.D. were involved in the laboratory characterization of patient samples; F.P. and M.C.S. provided clinical follow-up data. T. Hinrichsen and H.-G.K. performed sequencing on the Ion PGM system and analyzed the data; J.B., W.E.B., B.J.W., M.C.S., and W.H. coordinated the AMLCG clinical trial; K.S., J.B., W.H., S.K.B., and K.H.M. were involved in patient care; N.P.K., K.S., F.P., and T. Herold wrote the manuscript with help from all authors; K.S. supervised the project; and all authors had access to primary clinical trial data.

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