LETTER TO THE EDITOR

Core-binding factor acute myeloid leukemia with inv(16): Older age and high white blood cell count are risk factors for treatment failure

Dear Editors:

The core-binding factor (CBF) and acute myeloid leukemias (AML) are considered a distinct subgroup of AML due to similar molecular pathogenesis and clinical features.[1] The two forms of CBF-AML have rearrangements t(8;21)(q22;q22.1) [t(8;21)] and inv(16) (p13.1q22) or t(16;16)(p13.1;q22) [inv(16)] which result in the fusion genes *RUNX1/RUNXT1* and *CBFB/MYH11*, respectively. Although survival of CBF-AML is superior than other subtypes of AML, relapse occurs in approximately 30%-40% of patients.[1-4] Finding risk factors for treatment failure (eg, relapse or death) may be useful to manage patients individually. However, the relative rarity of CBF-AML (approximately 15%-20% of AML cases) and its relatively good prognosis has confounded these efforts.[2,3] Moreover, recent studies clearly indicate each CBF-AML is a distinct disease regarding patient and disease characteristics,[2-5] and should be evaluated separately. We previously published a scoring system (I-CBFit) for treatment failure for t(8;21),[6] now here we present our analysis for inv(16).

Eleven US and Europe centers collected data on 550 patients with CBF-AML; 290 patients had inv(16). Inclusion criteria were as follows: (a) AML patients with inv(16), t(16;16), or *CBFB/MYH11* by fluorescence in situ hybridization confirmed by the reporting institutions; (b) cases diagnosed between July 1996 and January 2017, (c) diagnostic or at least postinduction bone marrow sample was available for review in the participating institution's pathology department. Data were uniformly collected using a predesigned data spreadsheet. The data forms included the following: patient characteristics (age, sex, and race); disease characteristics [date of diagnosis, white blood cell count (WBC) at diagnosis ($\times 10^9$/L), cytogenetics, *KIT* D816V mutational status, primary or secondary AML]; hematopoietic cell transplantation (HCT) (autologous HCT or allogeneic HCT (alloHCT), donor type, remission status at HCT); and events (relapse, death, or alive at last contact). Deidentified patient data were transferred to the University of Minnesota where the main database was created and managed. This study was approved by the Institutional Review Board: Human Subjects Committee at the University of Minnesota.

Secondary AML was assigned if a patient had a history of chemotherapy/radiation therapy for a malignancy and/or had a history of preleukemic neoplastic bone marrow disease. The presence of 46 chromosomes in one clone or each clone was defined

TABLE 1 Characteristics of patients with inv(16) achieving CR

Variable	Total
n	264
Age (median [range])	49 [5-78]
Sex, n (%)	
Female	129 (48.9)
Male	117 (44.3)
Missing	18 (6.8)
WBC at Diagnosis (10^9/L) (median [range])	21.30 [1-373]
Missing n (%)	5 (1.9)
AML type, n (%)	
Primary	219 (83.0)
Secondary	32 (12.1)
Missing	13 (4.9)
Chromosome 4 abnormalities, n (%)	
No	243 (92.0)
Yes	5 (1.9)
Missing	16 (6.1)
Chromosome 5 or 7 abnormalities, n (%)	
No	233 (88.3)
Yes	15 (5.7)
Missing	16 (6.1)
+8, n (%)	
No	212 (80.3)
Yes	36 (13.6)
Missing	16 (6.1)
Chromosome 9 abnormalities, n (%)	
No	243 (92.0)
Yes	5 (1.9)
Missing	16 (6.1)
+ 22, n (%)	
No	208 (78.8)
Yes	40 (15.2)
Missing	16 (6.1)

(Continues)

TABLE 1 (Continued)

Variable	Total
-X,n (%)	
No	245 (92.8)
Yes	3 (1.1)
Missing	16 (6.1)
-Y,n (%)	
No	239 (90.5)
Yes	9 (3.4)
Missing	16 (6.1)
Number of chromosomes, n (%)	
<46	6 (2.3)
=46	180 (68.2)
>46	62 (23.5)
Missing	16 (61)
KIT D816V mutation, n (%)	
Negative	135 (51.1)
Positive	29 (11.0)
Missing	100 (37.9)
AlloHCT, n (%)	92 (34.8)
Disease status at alloHCT, n (%)	
No CR	7 (7.7)
CR1	28 (30.8)
CR2	54 (59.3)
>CR2	1 (1)
Missing	2 (2)
Relapsed within 2 y (%) in CR1	91 (34.5)
Nonrelapse mortality within 2 y in CR1, n (%)	15 (5.7)
EFS duration, months (median)	25.5
DFS duration, months (median)	29.5
OS duration, months (median)	Not reached

Abbreviations: AlloHCT, allogeneic hematopoietic cell transplantation; CR, complete remission; DFS, disease-free survival; EFS, event-free survival; OS, overall survival; WBC, white blood cell count.

as pseudodiploidy (due to inversion or t(16;16) it was not named diploidy), and if the chromosome number was higher or lower than 46 chromosomes in any clone (in patients with more than one clone) it was defined as hyperdiploidy and hypodiploidy, respectively. Event-free survival (EFS) was defined as the time from diagnosis to relapse, death from any cause, or failure to achieve complete remission. Disease-free survival (DFS) was defined as the time from complete remission to relapse or death from any cause. Overall survival (OS) was defined as the time from diagnosis to last follow-up alive or date of death of any cause. We described patients' characteristics using the median and range for continuous variables, and frequency and percentage for categorical variables. All patients were included EFS and OS analyses, but subjects who did not achieve complete remission (CR) were excluded from DFS analysis. Subjects that were censored before 2 years of follow-up were considered to be missing for the primary outcome. Covariates considered for inclusion were sex, age, race, primary or secondary AML, WBC at diagnosis, + 22, +8, and diploid status. Other chromosome abnormalities (4, 5, 7, 9, X, Y) were not considered for the prognostic model due to their rarity in our sample. *KIT* mutations were not included due to too many with missing data. Missing data were imputed with multivariate Imputation by Chained Equations in R. Twenty-five imputed datasets were generated. Stepwise model selection based on Akaike Information Criterion was performed on each imputed dataset. Covariates that were selected in at least half of the imputed datasets were included in the final model. Parameter estimates and standard errors were computed according to Rubin's rules. A Cox proportional hazards model was initially used to investigate the effects of age, sex, WBC at diagnosis, +8, +22, and hyperdiploidy on EFS. An event was defined as either relapse, death, or treatment failure (defined as not achieving CR at 30 days after diagnosis). Patients were considered to be censored if they were lost to follow-up or received alloHCT in CR1. When it was found that age and WBC were the only significant predictors, cutoff values were determined to group patients into high- and low-risk categories. Models for DFS and OS were also
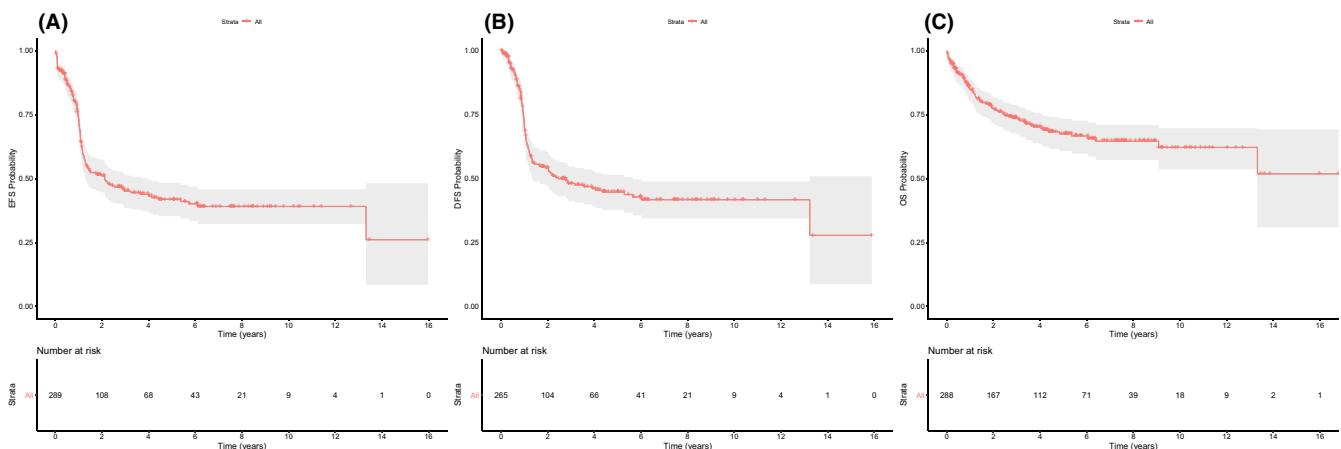

FIGURE 1 EFS (A), DFS (B), and OS (C) in patients with inv(16) [Colour figure can be viewed at wileyonlinelibrary.com]
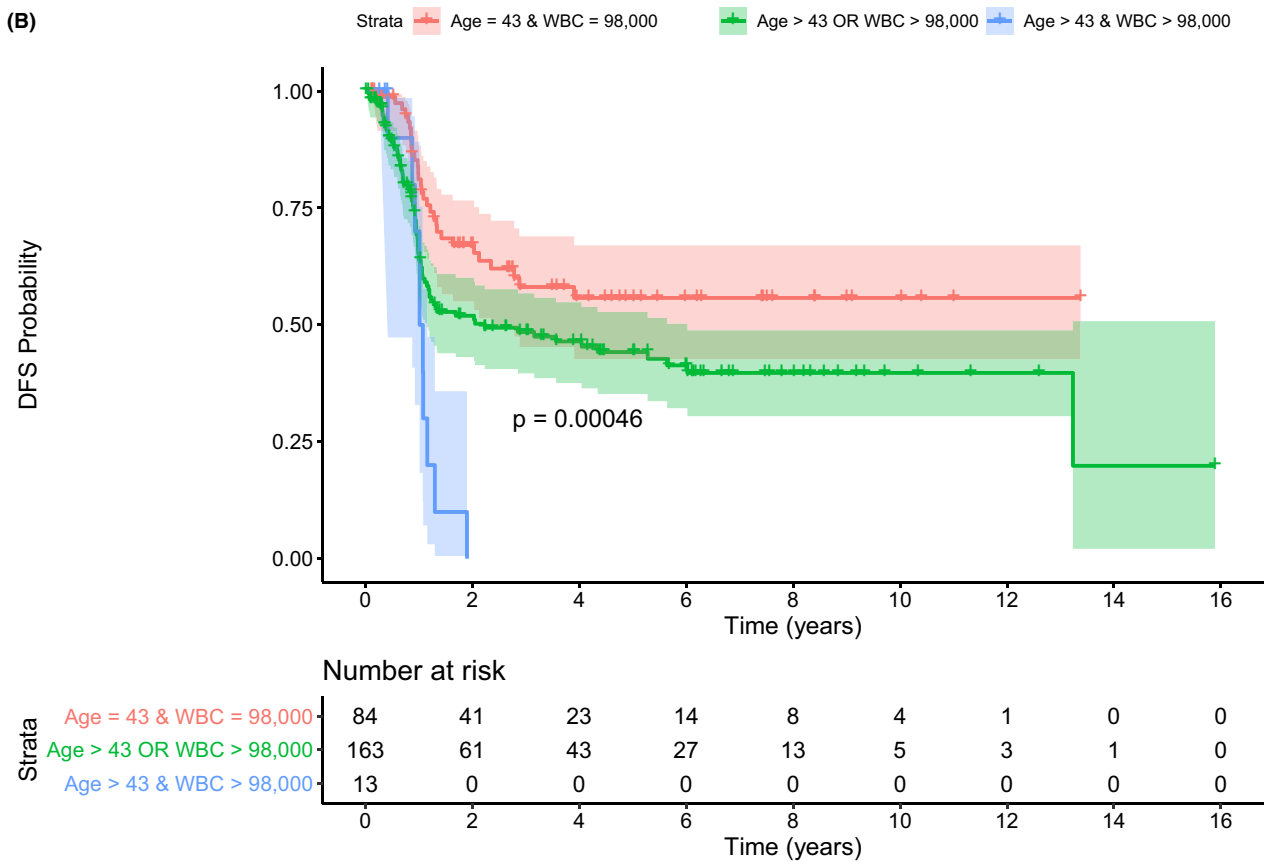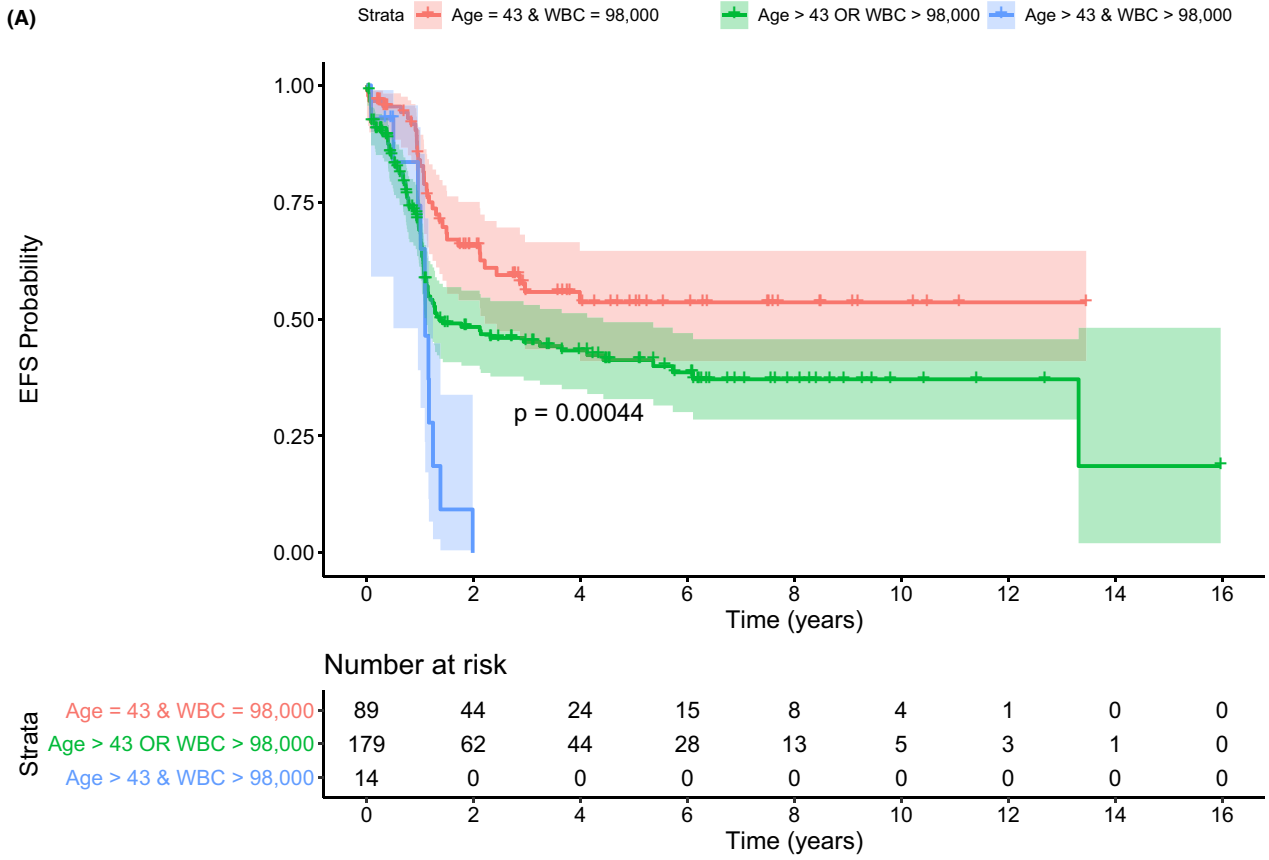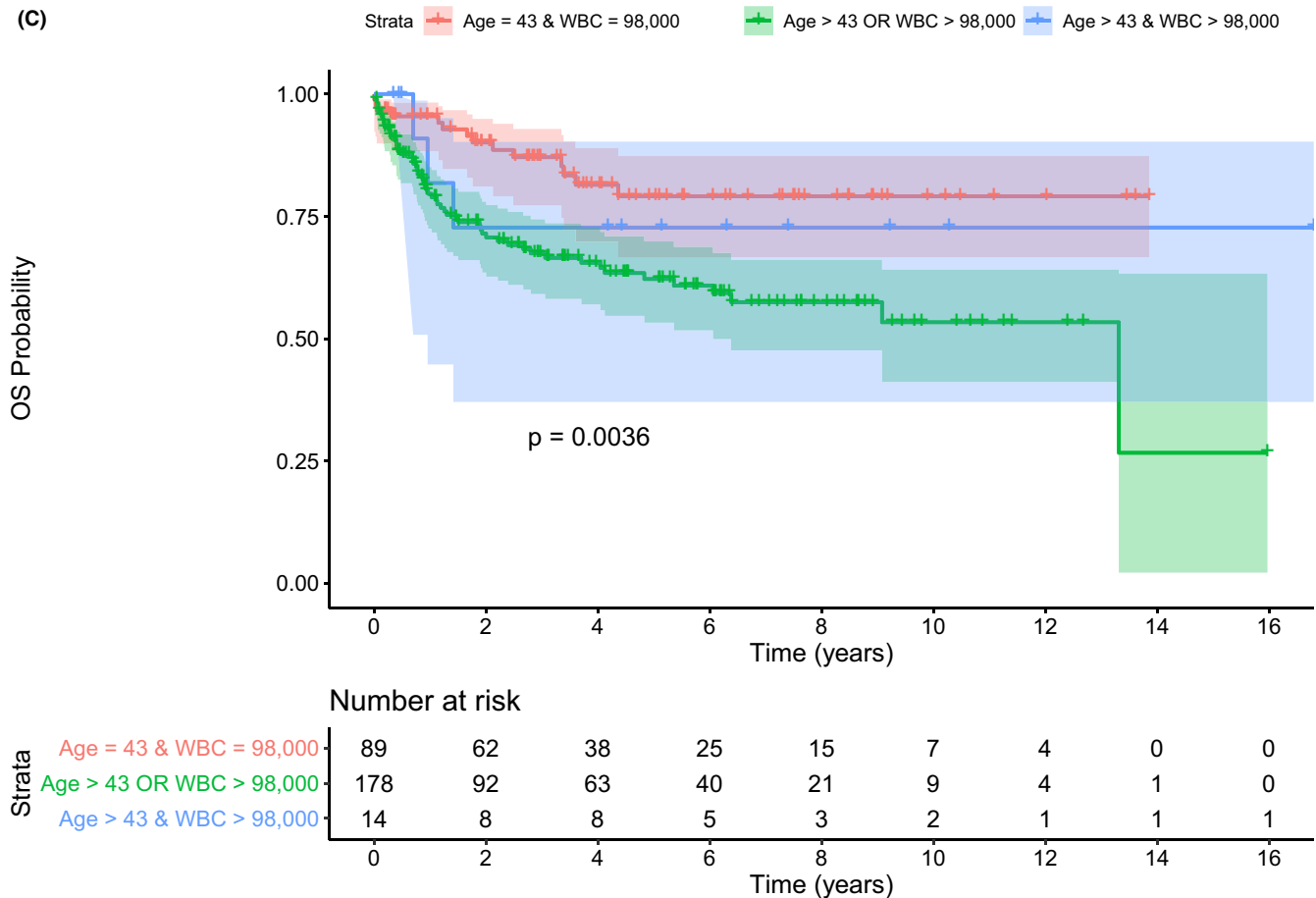
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(A)

Strata — Age = 43 & WBC = 98,000   — Age > 43 OR WBC > 98,000   — Age > 43 & WBC > 98,000

p = 0.00044

Number at risk

Strata	0	2	4	6	8	10	12	14	16
Age = 43 & WBC = 98,000	89	44	24	15	8	4	1	0	0
Age > 43 OR WBC > 98,000	179	62	44	28	13	5	3	1	0
Age > 43 & WBC > 98,000	14	0	0	0	0	0	0	0	0

(B)

Strata — Age = 43 & WBC = 98,000   — Age > 43 OR WBC > 98,000   — Age > 43 & WBC > 98,000

p = 0.00046

Number at risk

Strata	0	2	4	6	8	10	12	14	16
Age = 43 & WBC = 98,000	84	41	23	14	8	4	1	0	0
Age > 43 OR WBC > 98,000	163	61	43	27	13	5	3	1	0
Age > 43 & WBC > 98,000	13	0	0	0	0	0	0	0	0

FIGURE 2 (Continued)

(C)

FIGURE 2 EFS (A), DFS (B), and OS (C) were shown among patients with no (age ≤43 y and WBC ≤ 98 × 10^9/L), 1 (age ≤ 43 y or WBC ≤ 98 × 10^9/L) and 2 risk factors (age >43 y and WBC > 98 × 10^9/L)

computed. Separately for age and WBC, a ROC curve was generated for EFS for generated at 2 years after diagnosis using the survivalROC package in R. Cutoff points were chosen to maximize the Youden Index (Specificity + Sensitivity – 1). Kaplan-Meier curves and log-rank test were used for survival analyses in patients who had known diagnostic WBC and age (n = 282).

Of the 290 patients with inv16 (median age of 49 years), 264 achieved CR1 (Table 1). The most common additional cytogenetic abnormalities were +8 and +22. Hyperdiploidy (23.5%) was more frequent. *KIT* D816V mutation was present in 17.7% of tested patients.

Relapse or death occurred in 106 patients (40.2%) within 2 years after CR1 at a median of 10.7 months (range 0.8 to 22.8 months). The median EFS and DFS were 25.5 and 29.5 months, respectively while OS was not reached. (Table 1 and Figure 1). In multivariate analysis (MVA) for survival at 2-year, older age (≥43 years) was associated with inferior EFS [(Hazard ratio (HR): 1.017, 95% Confidence Interval (CI): (1.007-1.028), P = .0014], DFS [HR: 1.014, 95% CI (1.003-1.026), P = .0123], and OS [HR: 1.034, 95% CI (1.018-1.049), P = .00002]. Higher white blood cell counts (WBC ≥ 98 × 10^9/L) were associated with a poorer EFS [HR: 1.005, 95% CI (1.002-1.008), P = .0003] and DFS [HR: 1.0006, 95% CI (1.004-1.009), P = .0001], but not with OS [HR: 1000 95% CI (0.995-1.005), P = .958].

Patients stratified by age and WBC into three groups (age ≤43 years and WBC ≤98 × 10^9/L vs age ≤43 years or WBC ≤98 × 10^9/L vs age >43 years and WBC >98 × 10^9/L). Regarding EFS, younger patients with a lower WBC (no risk group, age ≤43 years and WBC ≤98 × 10^9/L) did not reach median survival whereas those with 1 risk (age ≤43 years or WBC ≤98 × 10^9/L) and patients with 2 risks (age >43 years and WBC > 98 × 10^9/L) had a median of EFS of 16.3 months and 13.1 months, respectively, P = .00044 (Figure 2A). Regarding DFS, patients with no risk did not reach median survival whereas those with 1 risk and those 2 risks (age >43 years and WBC >98 × 10^9/L) had a median DFS of 26.8 months and 12.5 months, respectively, P = .00046 (Figure 2B). Regarding OS, patients with no risk or with both risks did not reach median survival whereas those with 1 risk had a median OS of 160 months, P = .0036 (Figure 2C).

Although this multicenter study had limitations (due to retrospective in nature and thus missing detailed molecular data and no data on minimal residual disease), the data from a large number of patients with long-term follow-up allowed us to identify clear risk factors for treatment failure; older patients and higher WBC counts. Hoyos et al also showed that higher WBC count (> 20 × 10^9/L) had higher relapse at 5-year (40% vs

16%, $P = .001$) in 150 patients with CBF-AML (76 with CBFB-MYH11).[7] When patients were stratified by age and WBC (age >50 years and WBC > 20×10^9/L), similar to our results, patients who had no risk factor had a superior 5-year OS (80%). Other studies have also supported that WBC (one defined it similar to ours, 100×10^9/L) is associated with poor DFS, mainly due to increased relapse..[8,9] Pashka et al reported that patients with higher WBC had decreased DFS (HR = 1.33, $P = .02$) and more mutated *KIT* and *FLT3*.[8] De Jonge et al showed low WBC ($<20 \times 10^9$/L) was associated with a higher CR rate ($P = .024$), improved EFS (median 77.2 vs 9.7 months) and improved OS (median 85.5 vs 28.9 months, $P = .001$) in favorable prognostic AML.[10] Delauney et al found that older aged (>36 years), +22, and severe thrombocytopenia ($<30 \times 10^9$/L) were associated with poor DFS in 110 patients with inv(16).[11] In MVA, age was the only risk factor for poor DFS. The impact of +22 on survival is conflicting[2,3,8,11] and might be more prominent in patients with lower WBC.[3]

A *KIT* mutation (exons 8-13 & 17), present in up to 37% of patients, was associated with poor prognosis for survival in CBF-AML.[4,8] *KIT* mutations seem to have an impact in patients with t(8;21), but not in those with inv16.[6,12] However, in the current study of patients with inv16, *KIT* mutations could not be tested due to excessive missing data. Similar to our findings, complex karyotype[2,5,11] and secondary AML did not have effect in treatment failure.[13]

Prospective studies are warranted to improve survival in older inv(16) patients with a higher WBC at diagnosis.

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CONFLICT OF INTEREST

The authors have no conflict of interest relevant to the study to disclose.

AUTHOR CONTRIBUTIONS

CU conceived the idea, collected data, analyzed data, searched literature, wrote the manuscript, and edited the manuscript. ER and TB involved in execution of data analysis, writing and editing article. CA, PV, JG, HJD, and DW conceived the idea, writing and editing the manuscript. TG and H-PH involved in pathologic examination of BMs as needed, identifying patients, data collection, wrote the manuscript, and edited the manuscript. KM involved in cytogenetic confirmation, writing and editing the manuscript. EAM, SP, YK, RO, HV, CY, CAY, ML, SN, JLH, TKK, LS, LBB, MBM, WS, VP, NKK, DC, VP, AR, SK, GH, CDB, JK, GM, GB, CB, CC, JK, MRL, MAP, AS, RN, SYH, SBO, and A-IS involved in identifying patients, data collection, wrote the manuscript, and edited the manuscript.

Celalettin Ustun[1,2] iD
Elizabeth A. Morgan[3] iD
Ethan M Ritz[4]
Hanne Vestergaard[5]
Sheeja Pullarkat[6]
Philip M. Kluin[7]
Robert Ohgami[8]
Linda B. Baughn[9]
Young Kim[10]
Nam K. Ku[6]
David Czuchlewski[11]
Michael Boe Møller[5,12,13]
Ana-Iris Schiefer[14]
Krzysztof Mrózek[15]
Hans-Peter Horny[16]
Tracy I. George[11,17] iD
Thomas Kielsgaard Kristensen[12,18]
Todd Beck[4]
Sunita Nathan[1]
Cecilia Arana Yi[11]
Cecilia Yeung[19,20]
Vinod Pullarkat[21] iD
Jason Gotlib[22]
Cem Akin[23]
Jessica Kohlschmidt[15,24]
Amandeep Salhotra[10]
Lori Soma[20] iD
Dong Chen[9]
Se Y. Han[2]
Christina Cho[25,26]
Wolfgang Sperr[27] iD
Sigurd Broesby-Olsen[13,28]
Michael A. Linden[29]
Michelle Dolan[29]
Gregor Hoermann[30]
Jason L Hornick[3] iD
Clara Bloomfield[15]
Ryo Nakamura[21]
H. Joachim Deeg[19,20]
Mark R. Litzow[31] iD
Gautam Borthakur[32] iD
Daniel Weisdorf[2]
Gerwin Huls[33]
Miguel-Angel Perales[26]
Peter Valent[27]
Guido Marcucci[21]

[1]Division of Hematology, Oncology and Cellular Therapy, Rush University, Chicago, IL, USA
[2]Division of Hematology, Oncology and Transplantation, Department of Medicine, University of Minnesota, Minneapolis, MN, USA
[3]Department of Pathology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

[4]Rush Bioinformatics and Biostatistics Core, Rush University, Chicago, IL, USA

[5]Department of Hematology, Odense University Hospital, Odense, Denmark

[6]Department of Pathology, University of California, Los Angeles, CA, USA

[7]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[8]Department of Pathology, Stanford University, Stanford, CA, USA

[9]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, USA

[10]Department of Pathology, City of Hope National Medical Center, Duarte, CA, USA

[11]Department of Pathology, University of New Mexico, Albuquerque, NM, USA

[12]Department of Pathology, Odense University Hospital, Odense, Denmark

[13]Mastocytosis Center Odense University Hospital, Odense, Denmark

[14]Department of Pathology, Medical University of Vienna, Vienna, Austria

[15]The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA

[16]Institute of Pathology, Ludwig-Maximilians-University, Munich, Germany

[17]Department of Pathology, University of Utah, Salt Lake City, UT, USA

[18]Mastocytosis Center, Odense University Hospital, Odense, Denmark

[19]Fred Hutchinson Cancer Research Center, Seattle, WA, USA

[20]University of Washington School of Medicine, Seattle, WA, USA

[21]Division of Hematology and HCT, City of Hope, Duarte, CA, USA

[22]Stanford Cancer Institute, School of Medicine, Stanford University, Stanford, CA, USA

[23]Division of Allergy and Clinical Immunology, University of Michigan, Ann Arbor, MI, USA

[24]Alliance Statistics and Data Center, The Ohio State University Comprehensive Cancer Center, Columbus, OH, USA

[25]Department of Medicine, Adult Bone Marrow Transplant Service, Memorial Sloan Kettering Cancer Center, New York, NY, USA

[26]Department of Medicine, Weill Cornell Medical College, New York, NY, USA

[27]Department of Internal Medicine I, Division of Hematology & Hemostaseology and Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Vienna, Austria

[28]Department of Dermatology and Allergy Centre, Odense Research Center for Anaphylaxis, Odense, Denmark

[29]Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN, USA

[30]Division of Medical and Chemical Laboratory Diagnostics - Department of Laboratory Medicine, Medical University of Vienna, Vienna, Austria

[31]Department of Internal Medicine and Division of Hematology, Mayo Clinic, Rochester, MN, USA

[32]Department of Leukemia, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA

[33]Department of Hematology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Correspondence

Celalettin Ustun, Division of Hematology Oncology and Cellular Therapy, Department of Medicine, Rush University, 1725 W Harrison St, Suite 340, Chicago, IL 60612, USA.
Email: celalettin_ustun@rush.edu

ORCID

Celalettin Ustun https://orcid.org/0000-0001-6896-6213
Elizabeth A. Morgan https://orcid.org/0000-0001-5880-9337
Tracy I. George https://orcid.org/0000-0001-5478-7847
Vinod Pullarkat https://orcid.org/0000-0001-9129-3424
Lori Soma https://orcid.org/0000-0003-0606-899X
Wolfgang Sperr https://orcid.org/0000-0003-3288-8027
Jason L Hornick https://orcid.org/0000-0001-6475-8345
Mark R. Litzow https://orcid.org/0000-0002-9816-6302
Gautam Borthakur https://orcid.org/0000-0001-7679-6453

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