Old and new prognostic factors in acute myeloid leukemia with deranged core-binding factor beta

Roberto Cairoli,[1,2]* Alessandro Beghini,[3] Mauro Turrini,[1] Giambattista Bertani,[4] Gianpaolo Nadali,[5] Francesco Rodeghiero,[6] Carlo Castagnola,[7] Francesca Lazzaroni,[3] Michele Nichelatti,[1] Felicetto Ferrara,[8] Giovanni Pizzolo,[5] Enrico Pogliani,[9] Giuseppe Rossi,[10] Giovanni Martinelli,[11] and Enrica Morra[1]

Acute myeloid leukemia (AML) with deranged core-binding factor beta (CBFβ) is usually associated with a favorable prognosis with 50–70% of patients cured using contemporary treatments. We analyzed the prognostic significance of clinical features on 58 patients with CBFβ-AML aged ≤60 years. Increasing age was the only predictor for survival ($P < 0.001$), with an optimal cut-point at 43 years. White blood cells (WBCs) at diagnosis emerged as an independent risk factor for relapse incidence ($P = 0.017$), with 1.1% increase of hazard for each 1.0 × 10^9/L WBC increment. *KIT* mutations lacked prognostic value for survival and showed only a trend for relapse incidence ($P = 0.069$). Am. J. Hematol. 88:594–600, 2013. © 2013 Wiley Periodicals, Inc.

Introduction

Among acute myeloid leukemias (AMLs) with recurrent genetic abnormalities, patients with t(8;21)(q22;q22), inv(16)(p13q22), or t(16;16)(p13;q22), are referred to as core-binding factor (CBF)-AML. Although CBF-AML patients share a common molecular pathogenetic event, nominally the creation of a fusion protein involving a CBF gene unit, these two types of AML differ with regard to morphologic presentation, immunophenotypic marker expression, prognostic factors, and response to treatments, and should be considered as distinct clinical entities [1,2].

In the inv(16)/t(16;16) group, the CBF-beta (CBFβ) gene located on 16q22 fuses to the *MYH11* gene on 16p13, resulting in a chimeric protein. The translocation t(16;16)(p13;q22) is an equivalent rearrangement with lower incidence. Cytogenetically, the CBFβ–MYH11 rearrangement may be associated with trisomies of the chromosomes 8, 21, and 22 or with deletion of the chromosome 7 [3,4]. Patients with CBFβ-AML account for about 5–8% of adults with *de novo* AML and they are frequently associated with specific characteristics. This AML subset is morphologically associated with the French–American–British (FAB) M4 subtype with an abnormal eosinophil component (M4eo) and extramedullary involvement may be present [3–9].

Clinically, patients with CBFβ-AML are closely associated with a favorable outcome as compared with other AML subtypes [10–15]. High-complete remission (CR) rate and prolonged disease-free survival may be achieved when patients are treated with standard induction therapy followed by high-dose cytarabine (HD-AraC) post-remission therapies [16]. Despite these results, the outcome of CBFβ-AML patients does not appear to be as homogeneous as their cytogenetic definition, because only 54–74% are cured using contemporary treatment [17]. Recurrent disease occurs in 30–40% of patients, with a significant number of them subsequently dying from disease progression. Prognostic factors of relapse risk in CBFβ-AML subset are still a matter of debate. Female gender, older age, and low-platelet count have been reported as predictors for inferior outcome and/or shorter disease-free survival in patients enrolled in prospective trials [1,17,18]. Furthermore, higher white blood cells (WBCs) and low-platelet counts have been identified as bad predictor factors for CR achievements [19,20]. Conversely, nonrandom additional cytogenetic abnormalities such as trisomy +22, and male sex predicted better outcome [1,21].

Gene mutations represent novel prognostic markers in CBFβ-AML. The most common gene mutations in the inv(16)/t(16;16) group are those involving the *KIT* gene, that are observed approximately in 20–30% of patients [22–24]. Retrospective studies have demonstrated that the presence of *KIT* mutations in exon 17 have been associated with a poor outcome in CBF-AML and, for that reason, *KIT* mutation testing has been recently incorporated into National Cancer Guidelines to better stratify such patients in different prognostic subgroups [25]. However, while several studies showed that activating *KIT* mutations confer a significantly lower survival in AML with t(8;21)(q22;q22), the negative prognostic impact of *KIT* mutations in CBFβ-AML remains controversial [24,26–33].

In this study, we analyze the prognostic significance of clinical and genetic features such as age, gender, WBC count, presence of extramedullary leukemia (EML), additional cytogenetic abnormalities, and *KIT* mutations on long-term outcome of a large group of adult patients with inv(16)/t(16;16). Our results indicate that increasing age is the best predictor for survival of CBFβ-AML patients aged less than or equal to 60 years at univariate and multivariate analysis. Interestingly, *KIT* mutations lack prognostic value

[1]Division of Haematology, Niguarda Hospital, Milan, Italy; [2]Division of Haematology, Department of Internal Medicine, Valduce Hospital, Como, Italy; [3]Department of Medical Biotechnology and Translational Medicine, University of Milan, Milan, Italy; [4]Division of Transfusion Medicine, Niguarda Hospital, Milan, Italy; [5]Department of Clinical and Experimental Medicine, University of Verona, Verona, Italy; [6]Department of Haematology, San Bortolo Hospital, Vicenza, Italy; [7]Department of Haematology Oncology, University of Pavia & Fondazione IRCCS Policlinico San Matteo, Pavia, Italy; [8]Division of Haematology and Stem Cell Transplantation Unit, Cardarelli General Hospital, Naples, Italy; [9]Division of Hematology and Bone Marrow Transplantation Unit, San Gerardo University Hospital, Monza, Italy; [10]Department of Haematology, Spedali Civili, Brescia, Italy; [11]Division of Haematology and Stem Cell Transplantation Unit, Bologna, Italy

Conflict of interest: Nothing to report

*Correspondence to: Roberto Cairoli, Division of Haematology, Department of Internal Medicine, Valduce Hospital, 11, via Dante Alighieri, 22100 Como, Italy. E-mail: rcairoli@valduce.it

Received for publication 27 March 2013; Revised 4 April 2013; Accepted 8 April 2013

Am. J. Hematol. 88:594–600, 2013.

Published online 26 April 2013 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/ajh.23461

in terms of both survival and relapse incidence (RI). This result contrasts with the observation done in AML with t(8;21)(q22;q22), suggesting differences in biology of CBF-AMLs.

Design and Methods

Patients' characteristics, data collection, and treatment protocols

Fifty-eight patients aged less than or equal to 60 years with untreated AML presenting inv(16)(p13q22) or t(16;16)(p13;q22) diagnosed in eight Italian centers were included in this study (see Table I for patients' characteristics).

Each patient gave his/her informed consent for collection of clinical data, the cryopreservation of bone marrow samples, and the performance of DNA-analysis for scientific purposes, in accordance with institutional guidelines.

Bone marrow samples from each patient were collected and cryopreserved at diagnosis and then centrally analyzed for *KIT* gene mutational status at the Department of Biology and Genetics for Medical Sciences, University of Milan, Italy.

For each patient, data regarding hematologic parameters, bone marrow morphology, immunophenotype, cytogenetic, molecular analysis, diagnosis of EML, treatment schedule, and outcome were recorded. The study started in January 2001. Until January 2010, patients' data were periodically updated from the participating centers, centrally verified for consistency and completeness, and subsequently submitted for statistical analysis. The study design adhered to the Declaration of Helsinki and approval for this study was obtained from the Niguarda Hospital Review Board.

Patients were enrolled in intensive chemotherapy protocols, as previously described [30]. In brief, they received a standard induction therapy with an anthracycline-containing regimen, most commonly the "7+3" regimen with cytarabine in 7-day continuous intravenous infusion and three doses of anthracycline (idarubicin 12 mg/m^2/day or daunorubicin 60 mg/m^2/day) or the "ICE" schedule, including etoposide 100 mg/m^2/day on days 1–5.

The post-remission chemotherapy consisted of three consolidation courses. In patients treated with the "7+3" regimen, the first cycle was with high-dose cytarabine (3,000 mg/m^2 every 12 hr for 3 consecutive days, with patients older than 50 years receiving a reduced dose at 2,000 mg/m^2) and idarubicin 10 mg/m^2/day on days 1 and 3, while patients treated with ICE schedule received a NOVIA course (mitoxantrone 12 mg/m^2/day on days 1–4 and cytarabine 500 mg/m^2 every 12 hr for 6 doses). The second and third consolidation courses consisted of high-dose cytarabine.

The conditioning regimen for both autologous stem cell transplantation (ASCT) and allogeneic stem cell transplantation (allo-SCT) was with cyclophosphamide 60 mg/kg/day for 2 days and total-body irradiation in 6 fractions of 200 cGy (1,200 cGy) or busulfan 16 mg/kg over 4 days and cyclophosphamide 50 mg/kg over 4 days.

TABLE I. Clinical Characteristics at Presentation of Patients with inv(16)/t(16;16)

Parameter	$n = 58$	
Median age at diagnosis (range, yr)	42	(15–60)
Sex (male/female)	40/18	
Median WBC (range, $\times 10^9$/L)	24.7	(1.8–277)
Median marrow blast (range, %)	77.5	(26–95)
Extramedullary disease, no. (%)	7	(12.0)
Cytogenetic features		
Without additional abnormalities (%)	43	(74.1)
No. abnormalities (%)	13*	(22.4)
Including +8	2	
Including +22	6	
Including LOS	1	
Structure abnormalities (%)	3*	(5.2)
Including del(7q)	2	
KIT mutational status		
KIT mutated cases, no. (%)	15	(25.9)
Exon 17	12	
Exon 8	2	
Exon 10	1	

WBC, white blood cells; LOS, losses of a sexual chromosome.

Definitions and criteria for treatment response

CR was defined as less than 5% of bone marrow blasts, regression of extramedullary disease, transfusion independency with peripheral neutrophil count greater than 1.0 $\times$ 10^9/L and platelet count greater than 100 $\times$ 10^9/L and disappearance of the cytogenetic and molecular markers [34,35]. Recurrent disease is defined as the reappearance of more than or equal to 5% blasts in the bone marrow or in the peripheral blood or as the appearance of a new extramedullary site of disease in patients with a previously documented CR.

Extramedullary disease was defined as any leukemic collection outside the bone marrow and its presence was documented either by histological, cytological, or radiological criteria.

Overall survival (OS) was calculated from the date of diagnosis until death, where all living patients were censored at the time of last contact. The duration of CR was calculated from the date of the first CR until the date of the first relapse. RI was calculated from the date of the first CR until the date of the first relapse, where patients were censored at the time of last contact or death not because of recurrent disease.

Screening of mutations in the coding region of KIT gene

Bone marrow samples were submitted for a centralized analysis for *KIT* gene mutations in exon 2, 8, 10, 11, and 17. Mutations of exon 17 were detected using sequencing and other sensitive assays such as enzymatic digestion with *Hin*fI for Asp816Val and with Tsp509I for Asn822Lys and ARMS (amplification refractory mutation system) polymerase chain reaction (PCR) for Asp816Tyr and Asp816His [22,36,37]. Direct sequencing of DNA and cDNA products was performed using Thermo Sequence Dye Terminator sequencing reaction and ABI Prism 3100 sequencing analyzer (Applied Biosystems, Warrington, United Kingdom).

Statistical analyses

All collected variables were submitted to usual descriptive methods. In particular, for continuous variables the distribution was first evaluated by the Shapiro–Wilk test, so that normally distributed variables were summarized with mean and standard deviation, while nonnormal variables were summarized with median and range.

The Pearson's chi-square test with Yates' correction for continuity and the Fisher's exact test (if applicable) were used to check the association between categorical data, after crosstabulation. Comparisons of normally distributed continuous variables were carried out by Student's *t*-test or by Welch test (in the case of nonhomogeneous variances between groups, previously verified by Levene's test). The Mann–Whitney *U* test was used for comparison of continuous non-normally distributed variables.

The survival analysis was carried out using the Kaplan–Meier product limit method, followed by the log-rank test, to evaluate the possible differences in survival between groups.

Cox univariate and multivariate regression models were also used to analyze the effects of continuous variables on survivorship. The optimal multivariate model was chosen

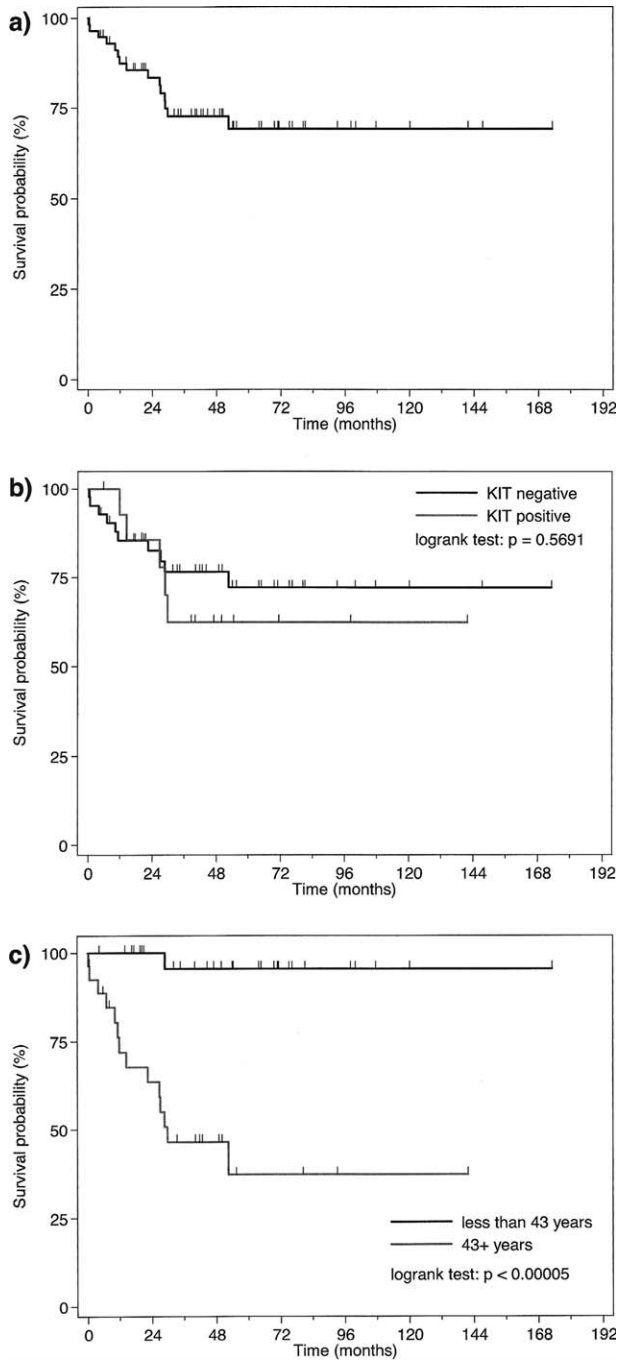

Figure 1. Kaplan–Meier plots showing the probability of survival. (A) Adult patients aged less than or equal to 60 years with inv(16)/t(16;16). The estimated 5-year OS resulted 69.2%. (B) *KIT*-negative (black line) versus *KIT*-positive (gray line) patients. OS was not affected by *KIT* mutational status ($P = 0.5691$), with an estimated 5-year OS of 72.1% and 62.3% for *KIT*-negative and *KIT*-positive patients, respectively. (C) Patients aged less than 43 years (black line) or older than or equal to 43 years (gray line). Age showed prognostic significance for OS at a cut-off point set at 43 years ($P < 0.00005$), with an estimated 5-year OS of 95.5% and 37.2%, respectively. OS: overall survival.

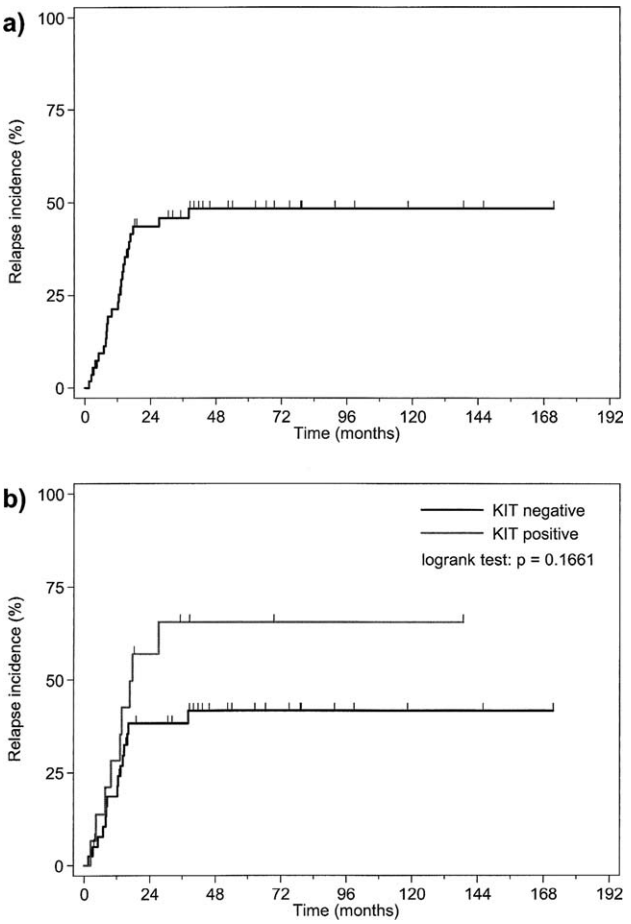

Figure 2. Kaplan–Meier plots showing the relapse incidence. (A) Adult patients aged less than or equal to 60 years with inv(16)/t(16;16). The estimated 5-year RI resulted 48.4%. (B) *KIT*-negative (black line) versus *KIT*-positive (gray line) patients. No difference was seen in term of RI between *KIT*-negative and *KIT*-positive patients ($P = 0.166$), with an estimated 5-year RI of 41.8% and 65.5%, respectively. RI: relapse incidence.

using a backward stepwise elimination after inserting all variables showing $P < 0.20$ at univariate analysis.

The receiver operating characteristics (ROC) curve was traced to analyze the role of patients' age on survivorship and to search for an optimal cut-off value for age itself. For all possible cut-off points, the total accuracy was considered together with sensitivity, specificity, positive predictive value and negative predictive value and the cut-off choice was made according to Youden.

Statistical analysis was done using Stata/SE 11.1 (The StataCorp, College Station, TX). Statistical significance was assumed for all tests with $P < 0.05$. The Bonferroni method was used to adjust significance in case of multiple comparisons.

Results

Overall results of treatments

Fifty-eight patients, aged between 15 and 60 years (median age: 42 years; male/female: 40/18), underwent treatment as described and were assessed for response. CR was obtained from 56 out of 58 (96.5%) patients. Primary refractory disease and one infectious complication during post-chemotherapy aplasia accounted for the two *KIT*-negative patients (aged 57 and 60 years, respectively) who did not achieve CR. A toxic death was subsequently recorded during the consolidation therapies. Twelve patients underwent ASCT instead of the third consolidation course and two *KIT*-negative patients received an allo-SCT in the first complete remission (CR1) from a sibling donor.

The median follow-up time for patients was 50 months based on the reverse Kaplan–Meier method. The estimated 5-year OS and RI resulted in 69.2% and 48.4%, respectively, with 32 patients alive in CR1 and 11 patients alive in second or subsequent CR (Figs. 1A and 2A; Table II).

TABLE II. Clinical Characteristics and Outcome of Patients with inv(16)/t(16;16) and Recurrent Disease

Age (Yr)/ sex	Cytogenetic at diagnosis	*KIT* status	WBC, $\times 10^9$/L	EML	Status at ASCT	Status at allo-SCT	Outcome	Survival (months)
24/M	46,XY,inv(16)(p13q22)	w/t	169,8	Absent	ND	ND	A/1st rel	16,6
26/M	46,XY,inv(16)(p13q22), t(11;12)	V503I	7,6	Absent	ND	ND	A/2nd CR	97,9
29/F	46,XX,inv(16)(p13q22)	D816V	11,1	Gastric mass	ND	2nd CR	A/2nd CR	46,7
32/F	46,XY,inv(16)(p13q22)	w/t	52,3	Absent	ND	2nd CR	A/2nd CR	34,3
36/M	46,XY,inv(16)(p13q22)	w/t	19,0	Absent	ND	2nd CR	A/2nd CR	74,8
36/M	46,XY,inv(16)(p13q22)	w/t	30,4	Absent	ND	2nd CR	A/2nd CR	70,5
36/M	46,XY,inv(16)(p13q22)	w/t	11,3	Absent	ND	ND	A/2nd CR	21,1
36/M	46,XY,inv(16)(p13q22)	D816V	141,0	Absent	1st CR	2nd CR	A/2nd CR	54,0
38/F	46,XX,inv(16)(p13q22)	Exon 8	4,4	Absent	ND	2nd CR	A/2nd CR	49,6
39/M	47,XY,inv(16)(p13q22),+6	w/t	96,2	Absent	ND	2nd CR	A/2nd CR	64,3
42/M	46,XY,inv(16)(p13q22)	w/t	11,7	Mesenteric mass	1st CR	2nd CR	A/2nd CR	107,2
56/F	46,XX,inv(16)(p13q22)	w/t	13,4	Absent	1st CR	ND	A/3rd CR	50,0
43/F	46,XX,inv(16)(p13q22)	w/t	130,0	Absent	ND	2nd CR	D/TRM 2nd CR	27,1
46/M	46,XY,inv(16)(p13q22)	w/t	23,8	Absent	ND	2nd CR	D/TRM 2nd CR	52,6
18/M	46,XY,inv(16)(p13q22)	D816V	12,8	Absent	2nd CR	ND	D/TRM 2nd CR	28,8
60/M	46,XY,inv(16)(p13q22)	w/t	27,7	Absent	ND	ND	D/1st res rel	11,2
47/M	47,XY,inv(16)(p13q22), +22,del(7)	D816H	12,3	Absent	ND	ND	D/1st res rel	29,8
54/F	48,XX, inv(16)(p13q22), +8,+21	w/t	49,8	Absent	ND	ND	D/1st res rel	7,1
52/M	46,XY,inv(16)(p13q22)	w/t	10,9	Ileal mass	ND	ND	D/1st res rel	10,3
55/M	46,XY,inv(16)(p13q22)	D816V	150,0	Absent	ND	ND	D/1st res rel	14,4
58/F	46,XX,inv(16)(p13q22)	D816V	122,0	Absent	ND	ND	D/1st res rel	11,8
50/M	46,XY,inv(16)(p13q22)	w/t	12,0	Absent	1st CR	ND	D/2nd rel	28,6
60/M	48,XY,inv(16) (p13q22), +9,+22	w/t	14,9	Absent	ND	ND	D/2nd res rel	22,4
54/M	46,XY,inv(16)(p13q22)	D816V	110,0	Absent	ND	ND	D/2nd res rel	26,8

allo-SCT, allogeneic stem cell transplantation; ASCT, autologous stem cell transplantation; CR, complete remission; EML, extramedullary leukemia; WBC, white blood cells; ND, not determined; w/t, wild type; TRM, transplant related mortality.

Relapse incidence and survival after relapse

Twenty-four out of 56 patients who achieved CR experienced relapse, including 4 patients who received an ASCT in CR1. The RI plot grew up rapidly to 43.6% within 17.7 months and reached 48.4% at 38.2 months (Fig. 2A). Twenty-three out of 24 patients underwent salvage chemotherapy, while 1 patient was lost at follow-up. The median survival time after relapse was 14.7 months (range: 1.1–92.4), with 17 (74%) patients achieving a second complete remission (CR2) and 6 (26%) dying for resistant relapse. Eleven patients underwent a stem cell transplantation (1 ASCT, 10 allo-SCT) in CR2. Of them, eight allo-transplanted patients were alive and disease-free with a median CR2 duration of 30.8 months (range: 1.0–91.4), and three patients died for transplant-related mortality. The remaining six patients who entered the CR2 received intensive consolidation chemotherapy courses: four patients presented a second relapse and three of them subsequently died from disease progression (Table II). Overall, 11 out of 23 (47.8%) relapsed patients are still alive and disease-free, with a median CR2 duration of 19.0 months (range: 1.0–91.4).

Incidence of KIT mutations and correlation between KIT status and clinical characteristics

Mutational screening reported *KIT* gene mutations in 15 of 58 patients (25.9%): 12 (20.6%) patients showed a D816 missense mutation (TKD^{816}), 2 (3.4%) patients presented an Exon 8 in-frame deletion plus insertion mutations, and 1 (1.7%) patient had an Exon 10 (V530I) transmembrane mutation (Table I). Patients with *KIT* gene mutations were classified as "*KIT*-positive" (*KIT*$^+$), while the remaining 43 patients who showed no mutations were classified as "*KIT*-negative" (*KIT*$^-$). Statistical analysis showed no significant difference in terms of age ($P = 0.368$), sex ratio ($P = 0.756$), and WBC count at diagnosis ($P = 0.765$) between *KIT*$^+$ and *KIT*$^-$ patients.

Seven patients out of the 58 cases included in this study (12.0%) had EML at presentation. In all cases the EML manifested in the form of myeloid sarcoma involving a variety of sites (spinal masses, gastrointestinal tract, lungs) except for skin. The association between the *KIT* mutational status and EML turned out to be not significant ($P = 0.360$).

Treatment outcome by KIT mutational status

CR was achieved in 100.0% (15/15) of *KIT*$^+$ patients after induction therapy. Recurrent disease was observed in 9 (60.0%) and 15 (34.9%) patients of *KIT*$^+$ and *KIT*$^-$ groups, respectively. No difference was seen in terms of RI between *KIT*$^+$ and *KIT*$^-$ patients ($P = 0.166$), with an estimated 5-year RI of 65.5% and 41.8%, respectively (Fig. 2B). Similarly, OS was not affected by *KIT* mutational status ($P = 0.569$), with an estimated 5-year OS of 62.3% and 72.1% for *KIT*$^+$ and *KIT*$^-$ patients, respectively (Fig. 1B). Resistant relapses (four patients) and one transplant-related death accounted for the five *KIT*$^+$ deceased patients (Table III).

Prognostic factors for overall survival and relapse incidence

Cox univariate and multivariate regression models were performed to evaluate the role of different clinical variables as predictors for relapse or survival. The following potential prognostic parameters were evaluated, namely, age, sex, WBC count at diagnosis, EML, *KIT* status, and presence at standard cytogenetic of trisomy of chromosome 22. For continuous variables (age and WBC), an ROC curve analysis was performed toward survival in search of possible cut-off values. Age distribution showed an optimal cut-point at 43 years (AUC 0.827, sensitivity 93.3%, specificity 68.3; $P = 0.0001$), while no possible cut-off points for WBC were identified.

In univariate analyses, only age, both as continuous or dichotomous variable with cut-off point set at 43 years,

TABLE III. Clinical Characteristics and Outcome of *KIT*-Positive Patients with inv(16)/t(16;16)

Age (Yr)/ Sex	Cytogenetic at diagnosis	*KIT* status	WBC, $\times 10^9$/L	EML	Status at ASCT	Status at allo-SCT	Outcome	Survival (months)
18/M	46,XY,inv(16)(p13q22)	D816V	12.8	Absent	2nd CR	ND	D/TRM 2nd CR	28,8
20/M	47,XY,inv(16)(p13q22),+22	D816Y	18.0	Absent	ND	ND	A/1st CR	39,6
26/M	46,XY,inv(16)(p13q22),t(11;12)	V503I	7.6	Absent	ND	ND	A/2nd CR	97,9
29/F	46,XX,inv(16)(p13q22)	D816V	11.1	Gastric mass	ND	2nd CR	A/2nd CR	46,7
36/M	46,XY,inv(16)(p13q22)	D816V	141.0	Absent	1st CR	2nd CR	A/2nd CR	54,0
38/F	46,XX,inv(16)(p13q22)	Exon 8	4.4	Absent	ND	2nd CR	A/2nd CR	49,6
38/M	47,XY,inv(16)(p13q22),+8	D816Y	13.6	Mesenteric mass	ND	ND	A/1st CR	71,0
41/M	46,XY,inv(16)(p13q22)	D816F	13.2	Absent	ND	ND	A/1st CR	19,6
47/M	47,XY,inv(16)(p13q22),+22,del(7)	D816H	12.3	Absent	ND	ND	D/1st res rel	29,8
50/F	46,XY,inv(16)(p13q22)	Exon 8	277.5	Lung	ND	ND	A/1st CR	38,2
51/M	46,XY,inv(16)(p13q22),del(7)	D816F	74.2	Absent	ND	ND	A/1st CR	5,4
53/M	46,XY,inv(16)(p13q22)	D816V	27.3	Absent	ND	ND	A/1st CR	141,5
54/M	46,XY,inv(16)(p13q22)	D816V	110.0	Absent	ND	ND	D/2nd res rel	26,8
55/M	46,XY,inv(16)(p13q22)	D816V	150.0	Absent	ND	ND	D/1st res rel	14,4
58/F	46,XX,inv(16)(p13q22)	D816V	122.0	Absent	ND	ND	D/1st res rel	11,8

allo-SCT, allogeneic stem cell transplantation; ASCT, autologous stem cell transplantation; CR, complete remission; EML, extramedullary leukemia; WBC, white blood cells; ND, not determined; TRM, transplant related mortality.

TABLE IV. Multivariate Analysis for OS and RI

Variable	HR	*P*	95% CI	
Overall survival			($P > \chi^2 = 0.0003$)	
AGE	1.148	**0.0003**	1.065	1.237
WBC	1.008	0.252	0.994	1.023
EML	4.605	0.090	0.787	26.930
KIT	1.354	0.651	0.364	5.038
Relapse incidence			($P > \chi^2 = 0.0271$)	
AGE	1.031	0.105	0.994	1.070
WBC	1.011	**0.017**	1.002	1.020
KIT	2.172	0.069	0.940	5.017

EML, extramedullary leukemia; WBC, white blood cells; AGE, age at diagnosis. Significant *P*-values are showed in bold.

showed prognostic significance for OS ($P < 0.00005$) (Fig. 1C), whereas no statistical significance was found for all the other variables. When combined in the multivariate analyses, only age both as continuous or dichotomous variable was a significant part of the Cox model and proved to be an independent risk factor for OS ($P < 0.001$). Any increase of 1 year in age led to a 15% increase ($P < 0.0005$) of the hazard of death, while being 43 years old or more implied a hazard ratio of 47.41 (95% CI; 4.87–461.39; $P = 0.004$), adjusting by WBC, EML, and*KIT* mutational status (Table IV).

In the multivariate Cox model with backward elimination of factors, WBC emerged as an independent risk factor for RI ($P = 0.017$) and any 1.0 $\times$ 10^9/L increment of WBC meant a 1.1% increase of the hazard of relapse (95% CI; 1.002–1.020; $P = 0.017$), adjusting by age and *KIT* mutational status (Table IV). *KIT* mutations showed a trend for RI but did not reach a significative value ($P = 0.069$).

Discussion

In this study, we have evaluated the impact of clinical and genetic features on the prognosis of *de novo* AML with inv(16)/t(16;16) in 58 patients with age less than or equal to 60 years, treated according to standard chemotherapy protocols. Overall, we observed a high CR rate (96.5%), a RI of 48.4% at 38.2 months after the first CR and, an estimated 5-year OS of 69.2%, according to outcome data reported in the recent literature. In this relative large cohort of homogeneously treated patients, we found that only WBC at presentation and age emerged as an independent risk factor for relapse ($P = 0.017$) and OS ($P < 0.001$), respectively.

A high-peripheral WBC count together with a raised serum LDH, the presence of hepatosplenomegaly, and EML, may reflect an increased tumor burden in AML even in the setting of "good risk" acute leukemias, such as acute promyelocytic leukemia or CBF-AML [19,38–40]. In AML with inv(16), Delaunay et al. [20] reported that bad prognosis factors for CR achievement were a high WBC count, with an optimal cut-off point at 120 $\times$ 10^9/L, and lower platelet count. Martin et al. [19], in a small study, found that presenting WBC count had a significant negative influence on disease-free survival. Our data are substantially in line with reported data, therefore we found an increase of 1.1% hazard of relapse for each 1.0 $\times$ 10^9/L increment of WBC count. However, we were not able to identify any possible WBC cut-off value by means of the ROC analysis.

Activating *KIT* mutations are frequently found in CBF leukemia [41]. We recorded here an incidence of 25.9% of *KIT* mutations, with most patients showing D816 missense mutations (Table I). From this aspect, it is of interest that expression levels of both *KIT* mRNA and proteins is much higher in CBF-AML, with either wild type or mutant *KIT*, than in leukemia cells negative for CBF rearrangements. Moreover, we recently reported that CBF genetic abnormalities, in addition to directly targeting and down-regulating the expression of hematopoietic protein-coding genes containing AML1 consensus sequences, can target microRNA genes (Mir222/221) involved in the regulation of the *KIT* receptor leading to *KIT* overexpression in CBF-AML [42]. Furthermore, it has been postulated that mutations of the *KIT* gene may drive the WBC proliferation in CBF leukemia. Recently, Luck et al., showed that *KIT* mutations confer a distinct gene expression signature in CBF-AML and that one of the most significantly differentially expressed gene is LRP6 that is essential for noncanonical WNT5A signaling and thus for the maintenance of stem and progenitor cells [43,44]. Authors suggested that the different gene profiling may lead to an enhancement of proliferation in the *KIT*-mutated cases, which may be reflected in the higher blast counts of those patients [43]. The clinical observations that affected patients with t(8;21) appear to have a higher WBC count and WBC-index at presentation and a higher frequency of EML might support this hypotheses [30,39,45].

However, despite these reports on AML with t(8;21), we did not find any difference in WBC count ($P = 0.765$) and incidence of EML ($P = 0.360$) between the 15 KIT^+ and the 43 KIT^- cases harboring the inv(16)/t(16;16) recruited in this study. Similarly, regarding the impact on outcome, this

study showed that *KIT* mutations did not reach a significative value as independent prognostic factor for relapse and survival neither in the multivariate nor in the Kaplan–Meier analysis, in contrast to those reported in adult patients with t(8;21) (Figs. 1B–2B; Tables III and IV) [24,26–28,30–32].

Accumulating evidence suggests that a high degree of similarity is identified between the two major subtypes of CBF leukemias. However, important differences on clinical and biological ground are reported [1,2]. A recent study using Drosophila as a model showed that AML1-ETO-expressing precursor cells express high levels of reactive oxygen species (ROS), and that ROS plays a central role in the proliferation of these precursors [46]. As for CBFβ–MYH11 leukemia, gene expression profiling of AML-M4 subtype suggested a highly activated NF-kB pathway in inv(16) patients [47]. Given that these pathways, particularly Notch, Wnt, and Cox/PGE2 signaling, are essential for stem cell self-renewal, they could contribute to a different transforming activity of AML1-ETO and CBFβ–MYH11 in CBF-AML.

Although our data suggest that a high WBC count is an unfavorable prognostic factor, because it increases the risk of relapse in multivariate analysis, it shows no significant effect on overall survival. In fact, after salvage and subsequent therapy including allo-SCT, we found that 11 out of 23 relapsed patients who underwent salvage chemotherapy are still alive and disease-free, with a median CR2 duration of 19 months.

In this study, only age as continuous or dichotomic value, with a best calculated cut-off point at 43 years, emerges as a prognostic factor affecting survival in both univariate or multivariate analysis. It is to be noted that, among the 30 patients aged 42 years or younger, we recorded only one death (transplant related), leading to a Kaplan–Meier plot of 95.5% for OS ($P < 0.00005$) (Table II; Fig. 1C). By contrary, focusing on 28 patients aged 43 years or older, we recorded 14 deaths (2 early deaths, 1 death in aplasia, 2 deaths for transplant-related complications, and 9 for first or second resistant relapse). Overall, 50% of patients aged 43 years or older are disease-free at 27.1 months (Table II; Fig. 2C).

Our data confirm that the strategy to perform an allogeneic SCT in CR >1 lead to encouraging results. In fact, of the 10 patients allo-transplanted while in second CR, 8 (80%) are alive and disease-free, with a median follow-up of 30.8 months. As in our study, Kuwatsuka et al., of 66 patients with inv(16) undergoing allo-SCT, reported an OS of 86% at 3 years in CR2 or CR3 and identified only age to be a significant prognostic factor. The Japanese study concluded that allo-SCT is not necessarily recommended for inv(16) in CR1 and that inv(16) patients who received an allo-SCT not in CR did significantly better than those with t(8;21) [2]. Furthermore, a French survey reported that age, with a best cut-off at 35 years, was the only factor for shorter disease-free survival in AML with inv(16) [20].

It has been repeatedly demonstrated that prognosis worsens with increasing age in AML [48,49]. This may reflect concurrent comorbidities in addition to different disease biology such as multidrug resistance protein (MDR-1) positivity or stem cell phenotype adversely affecting both attainment of remission and refractory relapse risk [49,50].

Paschka et al. reported that in inv(16) patients the cumulative incidence of relapse (CIR) was higher for *KIT*-positive patients, especially if presenting exon 17 mutation, compared with *KIT*-negative patients (5-year CIR 80% vs. 29%; $P = 0.002$). Furthermore, the authors reported that *KIT* mutations predicted worse survival when adjusted for sex [24]. Anyway, it has to be noted that in the CALGB study the *KIT*-mutated patients were significantly older (median age: 38 vs. 49 years; $P < 0.001$) and were more frequently male ($P < 0.05$) compared with nonmutated patients. Moreover, in the reviewed literature, all the focused studies on the prognostic significance of *KIT* mutations in the CBFβ-MYH11 adult patients have been unable to demonstrate any role of such mutations on survival; furthermore, to our knowledge, all studies but one [26] do not show any influence of *KIT* mutations on relapse [27,30–32].

All the results reported in these different studies are based on a relatively small population, principally because of the fact that AML is a rare disease and that the CBFβ subtype accounts for about 5–8% of adults with *de novo* AML. In a 9-years period (January 2001–January 2010), we considered 58 patients aged less than or equal to 60 years belonging from 8 Italian centers. At present, this is one of the studies with the largest number of adult CBFβ AML patients, second only to the one of Paschka and colleagues (counting 61 patients). Incrementing the number of patients surely would be of interest, but when it results in an excessive accrual time statistical analysis it is more likely to be biased.

In conclusion, while the prognostic significance of *KIT* mutations remains unclear with several studies yielding contrasting results [24,26,27,30–32], our data showed that only "old" prognostic factors, such as age and the WBC count at diagnosis, are important predictors of outcome in AML adult patients with inv(16)/t(16;16).

Acknowledgments

Contributions. RC was the principal investigator and takes primary responsibility for the paper. MT, GB, GN, FR, CC, FF, GP, EP, GR, GM, and EM recruited the patients. AB and FL performed the laboratory work for this study. MT and GB verified patients' data for consistency and completeness. MT and MN participated in the statistical analysis. RC, MT, and AB wrote the paper. We are grateful to Como Hematolgy and Oncology ODV for organizational support.

References

1. Marcucci G, Mrozek K, Ruppert AS, et al. Prognostic factors and outcome of core binding factor acute myeloid leukemia patients with t(8;21) differ from those of patients with inv(16): A Cancer and Leukemia Group B study. J Clin Oncol 2005;23:5705–5717.
2. Kuwatsuka Y, Miyamura K, Suzuki R, et al. Hematopoietic stem cell transplantation for core binding factor acute myeloid leukemia: t(8;21) and inv(16) represent different clinical outcomes. Blood 2009;113:2096–2103.
3. Wong KF, Kwong YL. Trisomy 22 in acute myeloid leukemia: A marker for myeloid leukemia with monocytic features and cytogenetically cryptic inversion 16. Cancer Genet Cytogenet 1999;109:131–133.
4. Le Beau MM, Larson RA, Bitter MA, et al. Association of an inversion of chromosome 16 with abnormal marrow eosinophils in acute myelomonocytic leukemia: A unique cytogenetic clinicopathological association. N Engl J Med 1983;309:630–636.
5. Byrd JC, Mrozek K, Dodge RK, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: Results from Cancer and Leukemia Group B (CALGB 8461). Blood 2002;100:4325–4336.
6. Mrozek K, Heinonen K, de la Chapelle A, Bloomfield CD. Clinical significance of cytogenetics in acute myeloid leukemia. Semin Oncol 1997;24:17–31.
7. Marlton P, Keating M, Kantarjian H, et al. Cytogenetic and clinical correlates in AML patients with abnormalities of chromosome 16. Leukemia 1995;9:965–971.
8. Bloomfield CD, de la Chapelle A. Chromosome abnormalities in acute nonlymphocytic leukemia: Clinical and biologic significance. Semin Oncol 1987.14:372–383.
9. Billstrom R, Ahlgren T, Bekassy AN, et al. Acute myeloid leukemia with inv(16)(p13q22): Involvement of cervical lymph nodes and tonsils is common and may be a negative prognostic sign. Am J Hematol 2002;71:15–19.
10. Appelbaum FR, Kopecky KJ, Tallman MS, et al. The clinical spectrum of adult acute myeloid leukaemia associated with core binding factor translocations. Br J Haematol 2006;135:165–173.
11. Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: Analysis of 1,612 patients entered into the MRC AML 10 trial. Blood 1998;92:2322–2333.
12. Larson RA, Williams SF, Le Beau MM, et al. Acute myelomonocytic leukemia with abnormal eosinophils and inv(16) or t(16;16) has a favorable prognosis. Blood 1986;68:1242–1249.

13. Bloomfield CD, Shuma C, Regal L, et al. Longterm survival of patients with acute myeloid leukemia: A third follow-up of the Fourth International Workshop on Chromosomes in Leukemia. Cancer 1997;80:2191–2198.
14. Bloomfield CD, Lawrence D, Byrd JC, et al. Frequency of prolonged remission duration after high-dose cytarabine intensification in acute myeloid leukemia varies by cytogenetic subtype. Cancer Res 1998;58:4173–4179.
15. Marcucci G, Caligiuri MA, Bloomfield CD. Molecular and clinical advances in core binding factor primary acute myeloid leukemia: A paradigm for translational research in malignant hematology. Cancer Invest 2000;18:768–780.
16. Byrd JC, Ruppert AS, Mrozek K, et al. Repetitive cycles of high-dose cytarabine benefit patients with acute myeloid leukemia and inv(16)(p13q22) or t(16;16)(p13;q22): Results from CALGB 8461. J Clin Oncol 2004;22:1087–1094.
17. Mrozek K, Bloomfield CD. Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. Hematology Am Soc Hematol Educ Program 2006,169–177.
18. Schlenk RF, Benner A, Hartmann F, et al. Risk-adapted postremission therapy in acute myeloid leukemia: Results of the German multicenter AML HD93 treatment trial. Leukemia 2003;17:1521–1528.
19. Martin G, Barragan E, Bolufer P, et al. Relevance of presenting white blood cell count and kinetics of molecular remission in the prognosis of acute myeloid leukemia with CBFbeta/MYH11 rearrangement. Haematologica 2000;85:699–703.
20. Delaunay J, Vey N, Leblanc T, et al. Prognosis of inv(16)/t(16;16) acute myeloid leukemia (AML): A survey of 110 cases from the French AML Intergroup. Blood 2003;102:462–469.
21. Schlenk RF, Benner A, Krauter J, et al. Individual patient data-based meta-analysis of patients aged 16 to 60 years with core binding factor acute myeloid leukemia: A survey of the German Acute Myeloid Leukemia Intergroup. J Clin Oncol 2004;22:3741–3750.
22. Beghini A, Peterlongo P, Ripamonti CB, et al. C-kit mutations in core binding factor leukemias. Blood 2000;95:726–727.
23. Longley BJ, Reguera MJ, Ma Y. Classes of C-kit activating mutations; proposed mechanisms of action and implication for disease classification and therapy. Leuk Res 2001;25:571–576.
24. Paschka P, Marcucci G, Ruppert AS, et al. Adverse prognostic significance of KIT mutations in adult acute myeloid leukemia with inv(16) and t(8;21): A Cancer and Leukemia Group B study. J Clin Oncol 2006;24:3904–3911.
25. The NCCN Clinical Practice Guidelines in Oncology. Acute Myeloid Leukemia (Version 2.2011), 2011 National Comprehensive Cancer Network, Inc. http:www.nccn.org.
26. Care RS, Valk PJ, Goodeve AC, et al. Incidence and prognosis of c-KIT and FLT3 mutations in core binding factor (CBF) acute myeloid leukaemias. Br J Haematol 2003;121:775–777.
27. Boissel N, Leroy H, Brethon B, et al. Incidence and prognostic impact of c-KIT, FLT3, and RAS gene mutations in core binding factor acute myeloid leukemia (CBF-AML). Leukemia 2006;20:965–970.
28. Schnittger S, Kohl TM, Haferlach T, et al. KIT-D816 mutations in AML1-ETO-positive AML are associated with impaired event-free and overall survival. Blood 2006;107:1791–1799.
29. Fuster O, Barragan E, Bolufer P, et al. Rapid detection of KIT mutations in core-binding factor acute myeloid leukemia using high-resolution melting analysis. J Mol Diagn 2009;11:458–463.
30. Cairoli R, Beghini A, Grillo G, et al. Prognostic impact of c-KIT mutations in core binding factor leukemia: An Italian retrospective study. Blood 2006;107:1791–1799.
31. Mrozek K, Marcucci G, Paschka P, Bloomfield CD. Advances in molecular genetics and treatment of core-binding factor acute myeloid leukemia. Curr Opin Oncol 2008;20:711–718.
32. Patel JP, Gonen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med 2012;366:1079–1089.
33. Shih LY, Liang DC, Huang CF, et al. Cooperating mutations of receptor tyrosine kinases and Ras genes in childhood core-binding factor acute myeloid leukemia and a comparative analysis on paired diagnosis and relapse samples. Leukemia 2008;22:303–307.
34. Morra E, Barosi G, Bosi A, et al. Clinical management of primary non-acute promyelocytic leukemia acute myeloid leukemia: Practice Guidelines by the Italian Society of Hematology, the Italian Society of Experimental Hematology and the Italian Group for Bone Marrow Transplantation. Haematologica 2009;94:102–112.
35. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol 2003;21:4642–4649.
36. Beghini A, Ripamonti CB, Cairoli R, et al. KIT activating mutations: Incidence in adult and pediatric AML, and identification of an internal tandem duplication. Haematologica 2004;89:920–925.
37. Beghini A, Magnani I, Ripamonti CB, Larizza L. Amplification of a novel c-Kit activating mutation Asn(822)-Lys in the Kasumi-1 cell line: A t(8;21)-Kit mutant model for acute myeloid leukemia. Hematol J 2002;3:157–163.
38. Dalley CD, Lister TA, Cavenagh JD, Rohatiner AZ. Serum LDH, a prognostic factor in elderly patients with acute myelogenous leukemia. Br J Cancer 2001;84:147.
39. Nguyen S, Leblanc T, Fenaux P, et al. A white blood cell index as the main prognostic factor in t(8;21) acute myeloid leukemia: A survey of 161 cases from the French AML intergroup. Blood 2002;99:3517–3523.
40. Burnett AK, Grimwade D, Solomon E, et al. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocitic leukemia treated with all-trans retinoic acid: Result of the randomized MRC trial. Blood 1999;93:4131–4143.
41. Smith ML, Hills RK, Grimwade D. Independent prognostic variable in acute myeloid leukemia. Blood rev 2011;25:39–51.
42. Brioschi M, Fischer J, Cairoli R, et al. Down-regulation of MicroRNAs 222/221 in Acute Myelogenous Leukemia with Deranged Core-Binding Factor Subunits. Neoplasia 2010;12:866–876.
43. Luck SC, Russ AC, Du J, et al. KIT mutations confer a distinct gene expression signature in core binding factor leukaemia. Br J Haematol 2010;148:925–937.
44. Katoh M, Katoh M. STAT3-induced WNT5A signaling loop in embryonic stem cells, adult normal tissues, chronic persistent inflammation, rheumatoid arthritis and cancer (Review). Int J Mol Med 2007;19:273–278.
45. Cairoli R, Grillo G, Beghini A, et al. C-Kit point mutations in core binding factor leukemias: Correlation with white blood cell count and the white blood cell index. Leukemia 2003;17:471–472.
46. Sinenko SA, Hung T, Moroz T, et al. Genetic manipulation of AML1-ETO-induced expansion of hematopoietic precursors in a Drosophila model. Blood 2010;116:4612–4620.
47. Sun X, Zhang W, Ramdas L, et al. Comparative analysis of genes regulated in acute myelomonocytic leukemia with and without inv(16)(p13q22) using microarray techniques, real-time PCR, immunohistochemistry, and flow cytometry immunophenotyping. Mod Pathol 2007;20:811–820.
48. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. Blood 2006;107:3481–3485.
49. Sekeres MA, Stone RM. The challenge of acute myeloid leukemia in older patients. Curr Opin Oncol 2002;14:24–30.
50. Sorror ML, Giralt S, Sandmaier BM, et al. Hematopoietic cell transplantation specific comorbidity index as an outcome predictor for patients with acute myeloid leukemia in first complete remission: Combined FHCRC and MDACC experiences. Blood 2007;110:4606–4613.