

Complex karyotype, older age, and reduced first-line dose intensity determine poor survival in core binding factor acute myeloid leukemia patients with long-term follow-up



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Approximately 40% of patients affected by core binding factor (CBF) acute myeloid leukemia (AML) ultimately die from the disease. Few prognostic markers have been identified. We reviewed 192 patients with CBF AML, treated with curative intent (age, 15–79 years) in 11 Italian institutions. Overall, 10-year overall survival (OS), disease-free survival (DFS), and event-free survival were 63.9%, 54.8%, and 49.9%, respectively; patients with the t(8;21) and inv(16) chromosomal rearrangements exhibited significant differences at diagnosis. Despite similar high complete remission (CR) rate, patients with inv(16) experienced superior DFS and a high chance of achieving a second CR, often leading to prolonged OS also after relapse. We found that a complex karyotype (i.e., ≥ 4 cytogenetic anomalies) affected survival, even if only in univariate analysis; the KIT D816 mutation predicted worse prognosis, but only in patients with the t(8;21) rearrangement, whereas FLT3 mutations had no prognostic impact. We then observed increasingly better survival with more intense first-line therapy, in some high-risk patients including autologous or allogeneic hematopoietic stem cell transplantation. In multivariate analysis, age, severe thrombocytopenia, elevated lactate dehydrogenase levels, and failure to achieve CR after induction independently predicted longer OS, whereas complex karyotype predicted shorter OS only in univariate analysis. The achievement of minimal residual disease negativity predicted better OS and DFS. Long-term survival was observed also in a minority of elderly patients who received intensive consolidation. All considered, we identified among CBF AML patients a subgroup with poorer prognosis who might benefit from more intense first-line treatment.

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Introduction

Core binding factor (CBF) acute myeloid leukemia (AML), defined by the presence of t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) [1] represents approximately 15% of all AML in younger patients, and 7% in patients older than age 60 years [2–5]. It is debated whether t(8;21) and inv(16) should be considered distinct entities [6–9]. Despite the common finding of additional cytogenetic abnormalities, such as loss of a sex chromosome and/or deletions of chromosome 9q for t(8;21) [2,3,10], and trisomies of chromosomes 22, 8, and 21 for inv(16) [8–10], their role in the pathogenesis and prognosis of CBF AML is still uncertain [3,8,11].

This type of leukemia is usually considered “favorable” [3,12–14], when patients are treated with induction chemotherapy followed by multiple cycles of high-dose cytarabine (HiDAC) [13,14]. Therefore, CBF AML patients are not considered candidates for allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1) by the National Comprehensive Cancer Network (NCCN) [15] or the European LeukemiaNet (ELN) [16]. However, relapse, in the order of 40–50%, remains the main cause of treatment failure [8,13]. Patients with the t(8;21) translocation seem to have a worse disease-free survival (DFS) and overall survival (OS) than those with inv(16) [4,5,13]. Growing evidence of genetic heterogeneity of CBF AML [13], e.g. involving tyrosine kinases, such as *KIT*, *FLT3*, and *RAS*, could partly explain such disparity [11,17–20]. In particular, the *KIT* D816 mutation has been associated with unfavorable DFS and OS, mostly in the case of t(8;21) patients [11]. Despite recent studies [21–24] proving its prognostic value in CBF AML patients, the use of minimal residual disease (MRD) monitoring by quantitative RT-PCR to determine clinical decisions for patients at high risk of relapse is still mainly limited to ongoing trials [21,23,24]. Consequently,

Additional Supporting Information may be found in the online version of this article.

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TABLE I. Patient Characteristics

	All (n = 192)	AML t(8;21) (n = 80)	AML inv(16) (n = 112)	P
Age, median (range), years	44 (15–79)	41.8 (15–79)	45.1 (15–73)	0.13
Patients >60 years, n (%)	26 (13.5)	9 (11.3)	17 (15.2)	0.43
Male:female ratio	1.37	1.16	1.55	0.34
AML type, n (%)				
De novo	181 (94.3)	73 (91.3)	108 (96.4)	0.21
Secondary	11 (5.7)	7 (8.7)	4 (3.6)	0.21
Splenomegaly, n (%)	30 (15.6)	6 (7.5)	24 (21.4)	0.008
Hepatomegaly, n (%)	41 (21.4)	13 (16.3)	28 (25.0)	0.13
Lymphadenopathy, n (%)	35 (18.2)	7 (8.8)	28 (25.0)	0.005
Extramedullary disease, n (%)	15 (7.8)	3 (3.8)	12 (10.7)	0.10
Granulocytic sarcoma, n (%)	6 (3.1)	4 (5.0)	2 (1.8)	0.40
WBC (range), $\times 10^3/\text{mm}^3$	18.9 (1.2–656.0)	10.5 (1.2–289.4)	32.2 (1.7–656.0)	<0.001
WBC $\geq 30 \times 10^3/\text{mm}^3$, n (%)	67 (34.9)	11 (13.8)	56 (50.0)	<0.001
WBC $\geq 100 \times 10^3/\text{mm}^3$, n (%)	15 (7.8)	2 (2.5)	13 (11.6)	0.017
Platelets (range), $\times 10^3/\text{mm}^3$	38.0 (4.0–586.0)	31 (4–586)	41.5 (6–331)	0.04
Platelets $\leq 20 \times 10^3/\text{mm}^3$, n (%)	50 (26.0)	27 (33.8)	23 (20.5)	0.016
Hemoglobin (range), g/dL	8.9 (3.1–15.0)	8 (3.4–13.6)	9.2 (3.1–15.0)	0.002
Packed marrow (>80%), n (%)	88 (45.8)	29 (36.3)	59 (52.7)	0.021
Elevated LDH, n (%)	138 (71.9)	53 (66.3)	85 (75.9)	0.62

AML, acute myeloid leukemia; LDH, lactate dehydrogenase; WBC, white blood cells.

until MRD monitoring overcomes its present issues regarding standardization and wide-spread availability, up-front risk stratification based on clinical and biological markers remains a useful tool to refine clinical decisions.

In this study, we retrospectively evaluated a large series of patients with CBF AML diagnosed and treated at 11 different Italian hematology institutions in the last two decades.

Methods

Patients. We retrospectively reviewed 192 patients treated with curative intent in 11 Italian hematology institutions from 1987 to 2012. Minimal required follow-up was 6 months. Consent to use the medical records was obtained from all patients according to the existing regulations at diagnosis.

In eight patients, the diagnosis of AML followed a previous cancer treated with either chemotherapy or radiotherapy (four non-Hodgkin lymphomas, two Hodgkin lymphomas, one colon cancer, and one breast cancer); in two more patients, a previous history of myelodysplastic syndrome lasting >6 months was present; and in one patient, AML emerged in the context of chronic myeloid leukemia from a Philadelphia-negative clone.

We defined “granulocytic sarcoma” as a mass-forming extramedullary localization of AML with histological confirmation; when extramedullary localization was suspected based on imaging but without measurable masses or histological confirmation, we named it “extramedullary disease.”

Laboratory, cytogenetic, and molecular data. All patients had clinical examination, complete laboratory profile, and morphologic and immunophenotypic characterization of leukemic blasts evaluated at diagnosis.

Cytogenetics was performed at diagnosis according to the International System for Human Cytogenetic Nomenclature [25]. Chromosome banding analysis was performed on bone marrow cells after short-term culture (24–48 hr). A total of 20 metaphase cells were analyzed for each patient, and subclone analysis was provided when different pathologic clones coexisted, or the CBF AML clone was present together with cytogenetically normal hematopoiesis.

Molecular analysis in most recent years included data for mutations in *KIT* ($n = 59$; 30.7%) [11], *FLT3* ($n = 101$; 52.6%) [26], and *NPM1* ($n = 79$; 41.1%) [27], as per previously described methods. MRD analysis for *RUNX1/RUNX1T1* and *CBFB/MYH11* at regular time points (end of induction, end of consolidation, post-HSCT) was performed on 60 patients as per previously described methods [28,29], with a cutoff of transcript level to define MRD-negativity of 12 copies after normalization to 10^4 copies of ABL.

Chemotherapy. Induction regimens in patients aged 18–60 years were categorized into: (1) D3A7 regimen, consisting of Daunorubicin 45 mg/m² days 1–3 + intravenous cytarabine continuous infusion 100 mg/m² days 1–7 or other similar 2-drug regimens consisting of an anthracycline plus standard-dose cytarabine; (2) similar 2-drug regimens with intermediate-dose cytarabine (IDAC; 1–1.5 g/m² bid days 1–4) or HiDAC (3 g/m² bid days 1–4) plus an anthracycline (e.g., HAM, HiDAC + idarubicin); (3) 3-drug regimens, adding etoposide 50 mg/m² days 1–5 or other drugs (e.g., thioguanine 200 mg/m² days 1–5 in the ETI and days 1–7 in the AAT regimens), excluding purine nucleoside analogues to anthracycline and

cytarabine (e.g., ICE, MICE, DAV/DAE/DCE, MEC, BARTS, ETI, AAT); (4) 3-drug fludarabine-based regimens, with fludarabine 25–30 mg/m² days 1–5 or, in a few cases, other purine analogues as the third drug together with cytarabine 1–2 g/m² qid days 1–5 and an anthracycline (e.g., FLAI5, FLAIRG, FLAN, FLAIE); (5) 3-drug fludarabine-based similar regimens with the addition of anti-CD33 Gemtuzumab Ozogamicin (e.g., My-FLAI).

Following induction, patients achieving CR1 were consolidated with ≥ 1 IDAC/HiDAC-based consolidation courses (median: two cycles; range, 1–4; median dose of cytarabine given overall in consolidation: 24 g/m²; range, 6–94). After 2–3 courses, autologous HSCT (ASCT) was implemented in (1) patients considered at high risk of relapse because of adverse clinical or laboratory findings at diagnosis (e.g., hyperleukocytosis, i.e., $>10^5/\text{mm}^3$ white blood cells [WBC]; secondary CBF AML; extensive bone marrow, hepatic, and splenic infiltration; granulocytic sarcoma); (2) patients failing to achieve CR1; (3) patients who achieved hematologic but not cytogenetic CR1 after induction therapy; (4) patients with persisting or later relapsing molecular transcripts. A small group of patients with available HLA-matched donors ($n = 29$) was treated with allogeneic HSCT at the end of first-line treatment for the same reasons. BuCy, BuMel, BAVC, fTBI + CTX, fTBI + CTX + ATG, and Flu-CTX were used as conditioning regimens for both autologous or allogeneic HSCT, with BuCy as the most common regimen (62%). All first-line allogeneic HSCT but 1 was performed after myeloablative conditioning.

In relapsing patients, rescue therapy mainly included 3-drug fludarabine-based regimens, and responding patients were consolidated whenever possible. After that, second-line allogeneic HSCT was the treatment of choice in all patients with a potential HLA-matched donor.

Patients aged >60 years were still intensively treated with curative intent, using either the same regimen as younger patients or their reduced versions, when available in the literature.

Statistics. To test the differences in proportions, the Fisher exact and Pearson χ^2 tests were used. The Mann-Whitney and two-way Student *t* tests were used to compare nonparametric/parametric variables between two groups, whereas the Kruskal-Wallis, 1-way ANOVA, and Holm-Sidak tests were used for multiple groups. The Shapiro-Wilk test was preferred to test normal distribution. Differences were considered statistically significant for $P \leq 0.05$.

As per February 2014, 134 patients (69.8%) were alive, with a median follow-up of 73.4 months (6–294). OS was defined as the time from diagnosis to death from all causes, or last follow-up. DFS was defined only in patients achieving CR1 as the time from assessment of CR1 until relapse of leukemia, death from all causes, or last follow-up. Event-free survival (EFS) was defined as the time from diagnosis to any adverse event, including death from all causes, relapse, and treatment-related death. With a competing risk survival approach, relapse mortality (RM) was defined as death due to leukemia relapse, and non-relapse mortality (NRM) was defined as death from any cause in the absence of leukemia. We used a Mantel-Bayar approach, treating allogeneic HSCT as a time-varying covariate, to test the effects of allogeneic HSCT.

Survival curves were calculated according to the method by Kaplan-Meier, and differences were tested using the log-rank test. We then applied Cox proportional hazard modeling to evaluate potential prognostic factors on OS. Multivariate analysis was carried out for those factors resulting in significant differences (i.e., $P \leq 0.05$). *KIT* evaluation ($n = 59$) and MRD evaluation ($n = 60$) were excluded

from the multivariate analysis because of incomplete data. To determine the effects of the same factors on NRM and RM, we modeled survival analysis in a competing risk setting, using death from different causes as mutually exclusive competing events. Again, we considered allogeneic HSCT in this setting as a time-dependent covariate. Cumulative incidence functions between groups were compared by the Pepe-Mori test.

Statistical analyses were performed using Stata IC v.10.1 by StataCorp (College Station, TX).

Results

Patient characteristics

Patient characteristics are summarized in Table I. At diagnosis, splenomegaly (24 vs. 6; $P = 0.008$) and lymphadenopathy (28 vs. 7; $P = 0.005$) were more common with patients with *inv(16)* than *t(8;21)*. Patients with *inv(16)* AML had higher WBC ($P < 0.001$) and lower platelet counts ($P = 0.04$), and a higher degree of bone marrow substitution ($P = 0.02$). Hemoglobin level was lower in patients with *t(8;21)* AML ($P = 0.002$). Eleven patients presented with secondary CBF AML: their characteristics did not differ significantly (Supporting Information Table 1).

Treatment and survival

Overall, OS of our series was 67.0% at 5 years and 63.9% at 10 years; 5-year and 10-year DFS were 58.2% and 54.8%, and 5-year and 10-year EFS were 53.9% and 49.9%, respectively. We observed a better DFS rate for patients with *inv(16)* compared with *t(8;21)* ($P = 0.04$; Fig. 1).

Over the years, 25 patients were treated with the D3A7 regimen and 167 with more intensive regimens (IDAC/HiDAC based, $n = 12$; 3-drug regimens, $n = 112$; fludarabine based, $n = 43$) (Supporting Information Table 2). There was no temporal bias toward the D3A7 regimen in the first decade covered by our study compared with the more recent one (data not shown). We observed a more favorable EFS after 3-drug or fludarabine-based regimens than after D3A7 (Supporting Information Fig. 1) ($P = 0.043$).

Both *t(8;21)* and *inv(16)* AML presented high CR1 rates (92.5% vs. 93.8%), with 29 of 74 patients (39.2%) relapsing in the case of *t(8;21)* and 31 of 105 patients (29.5%) for *inv(16)*. Median DFS was 62 months vs. unreached (at 74.6 months of follow-up) for *t(8;21)* and *inv(16)* patients, respectively ($P = 0.04$). Age did not impact CR1 rates: by applying the age cutoff of 60 years at diagnosis, 23 elderly patients achieved CR1 (88.5%) vs. 156 younger patients (95.1%; $P = 0.18$). We observed an overall treatment-related mortality of four patients (2.1%). Eleven patients not achieving CR1 experienced poor survival, with a median OS of 2.2 months. We could not find any correlation between clinical, laboratory, cytogenetic, or molecular features and the chance to achieve CR1.

We then divided patients achieving CR1 according to the intensity of consolidation therapy in four groups: (1) patients treated with 1–2 consolidation courses ($n = 60$ [33.5%]); (2) ≥ 3 intensive consolidation courses ($n = 57$ [31.8%]); (3) 2–3 consolidation courses + ASCT ($n = 33$ [18.4%]); and (4) first-line allogeneic HSCT ($n = 27$ [15.1%]) (Fig. 2). Secondary CBF AML was a criterion for allogeneic HSCT; as such, 7 of these 11 patients underwent allogeneic HSCT in CR1. We recognized a distinctive trend toward better survival as the dose intensity increased. Outcome significantly improved from 5-years DFS and OS of 29.7% and 52.7%, respectively, to 61.8% and 73.0% with more intensive therapy, to 71.3% and 80.3% with ASCT and 83.7% and 91.3% with allogeneic HSCT ($P < 0.001$ and $P = 0.005$, respectively). We noted a significant difference in OS and DFS between patients consolidated with 1–2 courses as compared to 3–4 courses ($P = 0.025$ and $P = 0.002$, respectively), ASCT ($P = 0.009$ and $P < 0.001$) and allogeneic HSCT ($P = 0.003$ and $P < 0.001$). On the opposite, differences in OS were nonsignificant between 3 and 4

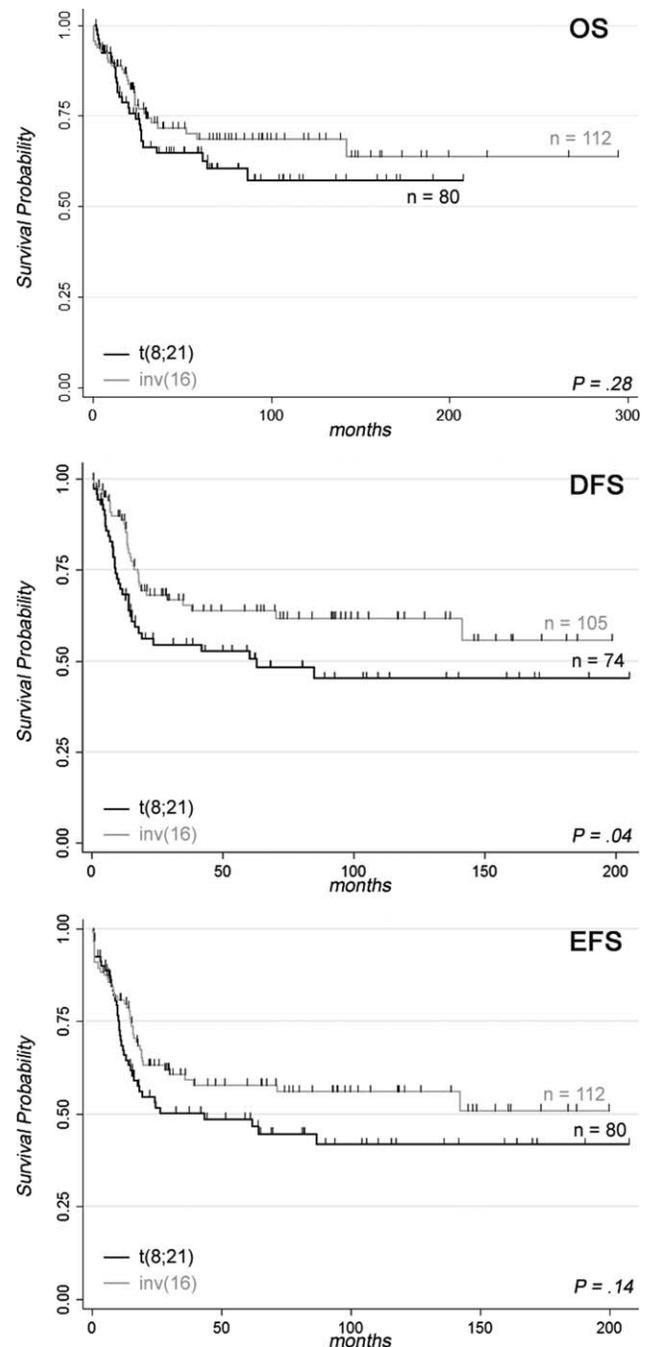


Figure 1. Survival of patients with *t(8;21)* and *inv(16)* AML. AML, acute myeloid leukemia; DFS, disease-free survival; EFS, event-free survival; OS, overall survival.

courses and ASCT ($P = 0.43$) or allogeneic HSCT ($P = 0.15$), while DFS was better for patients undergoing allogeneic HSCT as compared to 3–4 chemotherapy courses ($P = 0.011$), but equally good for patients undergoing ASCT or 3–4 courses ($P = 0.17$; Fig. 2).

Allogeneic HSCT was performed as part of first-line treatment in 27 patients achieving CR1 after induction therapy and in 2 who achieved CR only after a second reinduction course. Twenty-two more patients allotransplanted after relapse of leukemia are discussed in a separate section; no patient of this study was transplanted twice. The characteristics of patients allotransplanted during first-line treatment are provided in Supporting Information Table 3. The only significant difference in this group was the prevalence of secondary AML, which can be explained by secondary AML being one of the

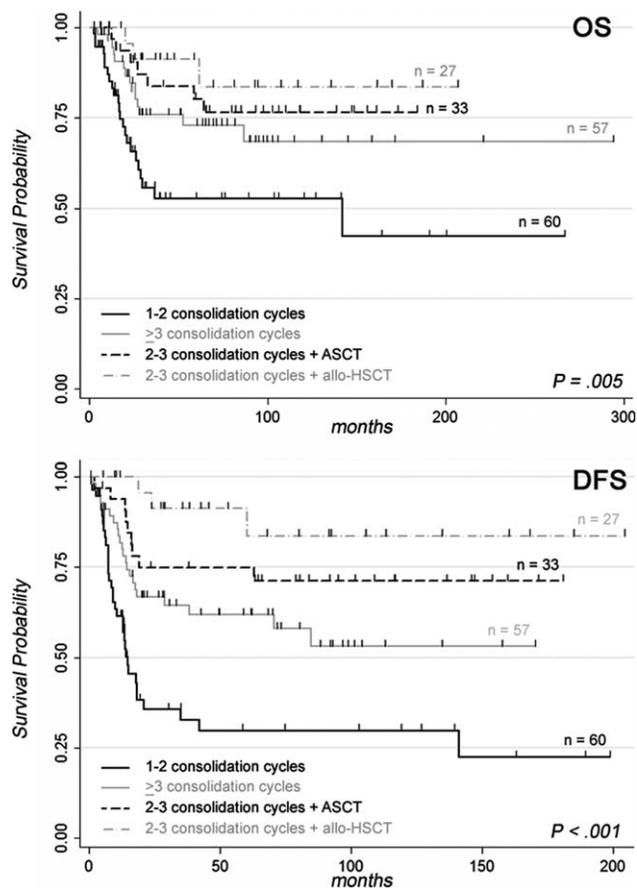


Figure 2. OS and DFS according to dose intensity of first-line treatment. Allo-HSCT, allogeneic hematopoietic stem cell transplant; ASCT, autologous hematopoietic stem cell transplant; DFS, disease-free survival; OS, overall survival.

criteria determining the choice of allogeneic HSCT. We observed a very low mortality in this group, with 25 patients (86.2%) alive at follow-up, one patient dying because of relapse of leukemia, and 3 because of extensive chronic Graft-vs-Host-Disease (GVHD) ($n = 2$) or Cytomegalovirus (CMV) reactivation ($n = 1$). Applying a time-dependent competing risk survival approach, we found that allogeneic HSCT during first-line therapy deeply reduced RM ($P < 0.001$) without significantly increasing NRM ($P = 0.81$), with 5-year OS at 88.5% and 5-year DFS at 83.7% (Fig. 3).

After relapse, patients with *inv(16)* had a slightly better chance to achieve a second CR (CR2), although not at the level of statistical significance ($n = 21$ of 25 [84.0%] vs. $n = 16$ of 24 [66.7%], respectively; $P = 0.20$), with similar final OS ($P = 0.28$; Fig. 1).

The CR1 rate was high also in elderly (i.e., >60 years at diagnosis) patients (23/26 [88.4%]). Fourteen patients of this group (53.8%) were consolidated by 1–2 courses and four patients by ≥ 3 courses (15.3%), whereas four more patients (15.3%) received ASCT and one reduced-induction conditioning allogeneic HSCT. Overall, elderly patients were less likely to undergo intensive consolidation therapy (i.e., >2 courses) than younger patients ($P = 0.042$; Supporting Information Table 4). Long-term DFS was achieved only in the more intensively treated cohort. Five-year DFS and OS ranged from 11.3% and 20.2% for patients consolidated with 1–2 courses, to 62.2% and 62.2% in those treated more intensively ($P = 0.002$ and $P = 0.019$, respectively). Notably, there were no treatment-related deaths among these patients. When relapsing, elderly patients were less likely to receive rescue therapy ($P = 0.01$; Supporting Information Table 4), but, when treated, they had the same chance to achieve RC2 ($P = 0.63$). With the cutoff of 60 years, age proved one of the most

important predictors of poorer survival, with 5-year DFS and OS dropping from 60.6% and 70.9% in younger patients to 34.5% and 35.6% in the elderly ($P = 0.004$ and $P < 0.001$; Supporting Information Fig. 2).

Eleven patients (5.7%) presented with secondary CBF AML. They all achieved CR1 (11/11, 100%), and experienced a similar relapse rate as patients with de novo leukemia (4/11 vs. 56/167, $P = 0.85$). As such, DFS and EFS did not differ ($P = 0.224$ and $P = 0.581$, respectively). Seven of these patients underwent allogeneic HSCT in CR1, while the remaining four received only chemotherapy because of the lack of a HLA-matched donor ($n = 2$) or coexisting comorbidity ($n = 2$). Eventually, OS of patients with secondary CBF AML appeared slightly worse, with difference at the limit of statistical significance ($P = 0.049$ at log-rank; $P = 0.056$ at Cox modeling).

Prognostic role of additional cytogenetic abnormalities

We detected additional cytogenetic abnormalities, listed in Table II and in Supporting Information Table 5, in 83 patients (*t*[8;21] $n = 42$ [52.5%] and *inv*[16] $n = 41$ [36.6%; $P = 0.18$]).

We found a trend toward better OS and DFS for patients with *inv*(16) and trisomy 22 and trisomy 8 (data not shown). As presented in Fig. 4, only patients with ≥ 3 additional cytogenetic abnormalities fared significantly worse than all other groups in terms of DFS ($P = 0.002$) and EFS ($P = 0.027$; Fig. 4), and also in terms of OS with Cox modeling (HR, 2.58; 95% CI, 1.02–6.49; $P = 0.044$; Table III). This subgroup consisted of nine patients (4.7% of the whole), five presenting with *t*(8;21) and four with *inv*(16); three patients were aged >60 years and two presented with secondary AML. Eight achieved CR following induction and four relapsed, with a median DFS of 15.4 months. Three relapsing patients were treated with second-line therapy, including allogeneic HSCT in two cases. The presence of ≥ 3 additional cytogenetic abnormalities still identified (at the limit of statistical significance) a subgroup with dismal prognosis in *t*(8;21), but not *inv*(16) (HR, 2.85; 95% CI, .98–8.29; $P = 0.055$; $n = 5$) (Supporting Information Tables 6 and 7).

Paradoxically, we observed a trend toward better survival in patients with two additional cytogenetic abnormalities; in this group, though, there were more patients with *inv*(16) as compared with the others (19/31 [61.3%] vs. 18/43 [41.9%] and 4/9 [44.4%], respectively) and frequent finding of trisomy 22 and trisomy 8 (45.2%), previously associated with better OS in *inv*(16) patients [7].

We did not detect any prognostic role for the presence of subclones.

Prognostic role of molecular data

Details are listed in Supporting Information Table 8. We observed the presence of mutated *FLT3* or *NPM1* in rare cases (10/101 [9.9%] and 2/79 [2.5%], respectively), whereas *KIT* was mutated in 7 of 59 patients (11.8%). *KIT* mutations predicted shorter OS at the univariate analysis in patients with *t*(8;21) (HR, 12.5; 95% CI, 1.12–139.33; $P = 0.04$) but not in patients with *inv*(16) (Table III). *FLT3* mutations did not predict worse OS or DFS, whereas *NPM1* mutations could not be analyzed ($n = 2$).

Molecular MRD

In patients where MRD was monitored ($n = 60$), we found a fundamental difference in survival between those achieving molecular CR and those failing, regardless of the time point during treatment at which MRD was negative (Supporting Information Fig. 3). Twenty-three patients (38.3%) never achieved molecular remission and had a median OS of 16.7 months (Supporting Information Fig. 3), despite the use of ASCT and allogeneic HSCT in four and three patients, respectively.

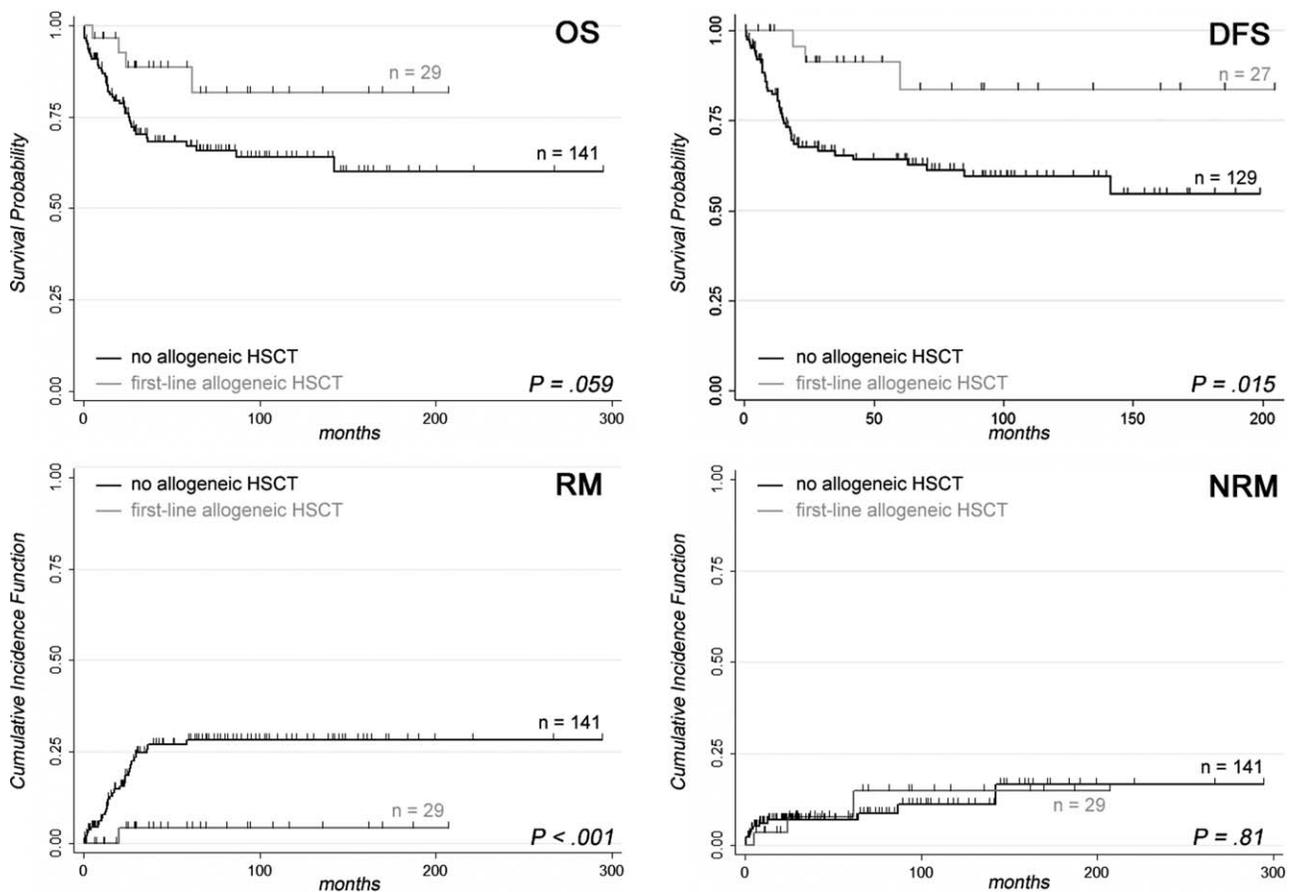


Figure 3. Survival according to first-line allogeneic HSCT. DFS, disease-free survival; HSCT, hematopoietic stem cell transplant; NRM, nonrelapse mortality; OS, overall survival; RM, relapse mortality.

TABLE II. Additional Cytogenetic Abnormalities

	All <i>n</i> = 192 (<i>n</i> = 83 [43.2%])	t(8;21) <i>n</i> = 80 (<i>n</i> = 42 [52.5%])	inv(16) <i>n</i> = 112 (<i>n</i> = 41 [36.6%])
Single additional abnormality, <i>n</i> (%)	43 (22.4)	25	18
Trisomy 22	5 (2.6)	–	5
Chromosome 7	2 (1.0)	–	2
Chromosome 9	6 (3.1)	6	–
Trisomy 8	4 (2.1)	1	3
Chromosome 21	1 (0.5)	–	1
Chromosomes X or Y	20 (10.4)	18	2
Mixed	5 (2.6)	–	5
Two additional abnormalities, <i>n</i> (%)	31 (16.1)	12	19
Three (or more) additional abnormalities, <i>n</i> (%)	9 (4.7)	5	4

Relapsing patients and second-line therapy

Overall, 60 patients (31.2%) relapsed, with similar rates in those with t(8;21) and inv(16) AML (*n* = 29 [39.2%] vs. *n* = 31 [29.5%]; *P* = 0.19), resulting in only a slight advantage in DFS for patients with inv(16) (*P* = 0.04; Fig. 1). When the intensity of treatment given as first line was tested, we did not find any significant difference between patients relapsing with t(8;21) vs. inv(16) (data not shown). In both groups, the chance of achieving CR2 after rescue treatment was good (37/49 treated, 75.5%): 21/25 patients with inv(16) achieved CR2 (84%) vs. 16/24 with t(8;21) (66.7%; *P* = 0.20).

Twenty-two of the 37 relapsing patients achieving CR2 were then consolidated by second-line allogeneic HSCT. Of these, four patients later died of relapsing leukemia, six of infectious complications, and one of chronic GVHD, leaving 11 patients (50%) alive at follow-up.

We found favorable relapse survival for the allotransplanted group (*P* = 0.044; Supporting Information Fig. 4), because of a low RM (*P* < 0.001), even if balanced by a higher NRM (*P* = 0.011; Supporting Information Fig. 4).

Survival modeling

In the univariate analysis for OS (Table III), age (>60 years; *P* < 0.001), severe thrombocytopenia ($<20 \times 10^3/\text{mm}^3$; *P* = 0.004), increased LDH levels (*P* = 0.032), ≥ 3 additional cytogenetic abnormalities (*P* = 0.044), and failure to achieve CR1 (*P* < 0.001) identified patients at higher risk. Of these, only age (*P* < 0.001), severe thrombocytopenia (*P* = 0.027), increased LDH levels (*P* = 0.038), and failure to achieve CR1 (*P* < 0.001) proved to be independent prognostic factors. The diagnosis of secondary CBF AML only approached the level of statistical significance (RR 2.30, CI 95%: 0.98–5.39, *P* = 0.056, Table

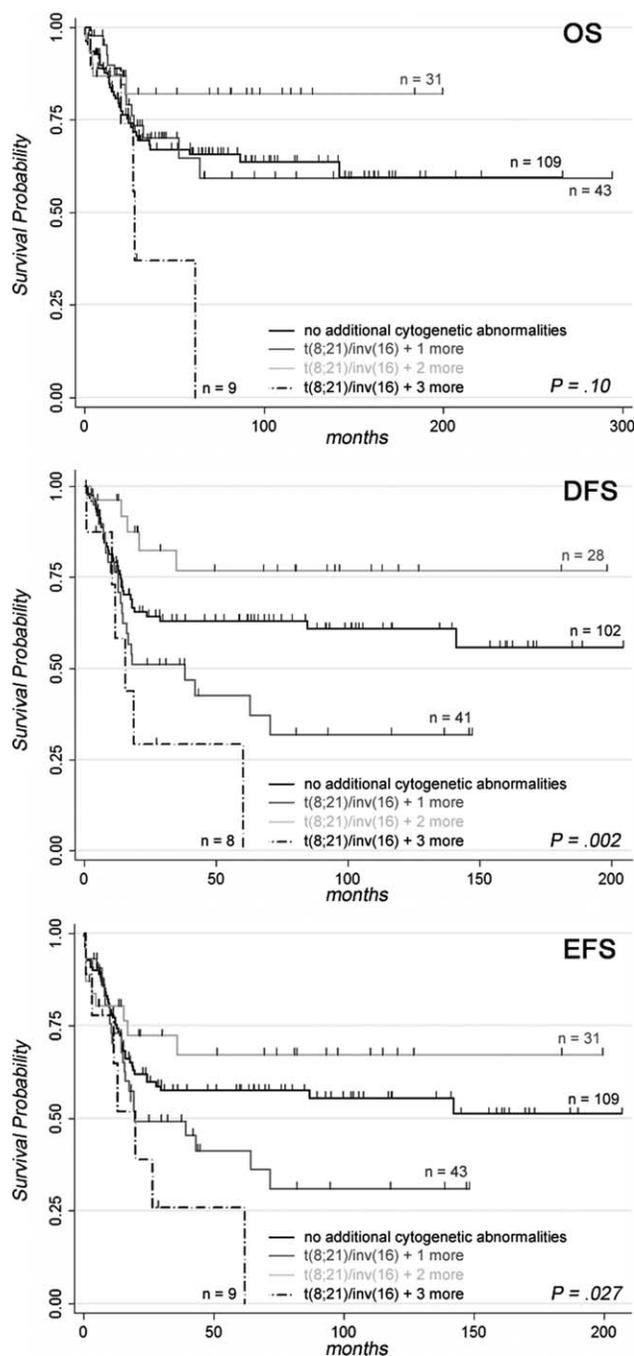


Figure 4. Survival according to additional cytogenetic abnormalities. AML, acute myeloid leukemia; DFS, disease-free survival; EFS, event-free survival; OS, overall survival.

III), and was therefore not considered in multivariate analysis. When we analyzed OS for patients with $t(8;21)$ or $inv(16)$ AML separately (Supporting Information Tables 6 and 7), we found that age, ≥ 3 additional cytogenetic abnormalities, and failure to achieve CR1 were independent prognostic factors for patients with $t(8;21)$, whereas only age and severe thrombocytopenia remained independent prognostic factors in patients with $inv(16)$. The *KIT* D816 mutation identified patients with worse prognosis only in those with $t(8;21)$ AML and only in univariate analysis (Table III and Supporting Information Table 6). Conversely, failure to achieve CR1 indicated adverse prognosis only in univariate, but not multivariate analysis for patients with $inv(16)$, thus highlighting the possibility to rescue failing patients with second-line therapies (Table III and Supporting Information Table 7).

Discussion

In our study, age proved to be a pivotal independent factor. Poor survival in elderly AML patients usually reflects more aggressive disease, as well as the effect of comorbidities preventing the administration of an adequate treatment intensity [30]. Using a cutoff of 60 years, we observed a CR rate comparable for younger and older patients, but a higher relapse rate and poorer OS in elderly patients. These results are similar to what reported in a recent study [31], in which a high induction-related toxicity prevented the administration of consolidation therapy to most older patients [31]. In our cohort, only one-third of elderly patients received intensive post-remission therapy (data not shown). Nevertheless, long-term DFS and OS could still be achieved in a significant proportion of these patients when intensive consolidation was provided. We believe that this highlight preserved chemosensitivity of CBF AML blasts also in elderly patients [6,31].

Besides age, elevated LDH levels ($P = 0.041$) and low platelet count at diagnosis ($P = 0.016$) proved to be independent predictors of shorter OS, as in other studies [4,9,21,32].

We also tested whether the presence of high tumor burden (i.e., WBC count, bone marrow substitution, or hepatosplenic involvement), might impact CR rate and survival. We found no correlation between clinical and laboratory data and CR1 rate or final OS. This is similar to what was reported by others [4,16], in which cytogenetic and molecular data proved to be more powerful prognostic factors.

We therefore addressed the role of cytogenetics. In agreement with most studies [4,8], we could not detect a prognostic value for single additional abnormalities. The group defined by the presence of two additional abnormalities showed a non-statistical advantage in survival, possibly because of the prevalence of patients with $inv(16)$ in this group, as well as by cytogenetic findings, such as trisomy 22 and trisomy 21, already linked by others [8,17] to better prognosis.

Most significantly, though, we found that ≥ 3 additional cytogenetic abnormalities, herein defined as “complex karyotype,” predicted significantly worse OS at univariate analysis. This was proved despite the relative rarity of this subgroup. Effects were more evident for patients with $t(8;21)$ than those with $inv(16)$. Differently from another report [10], complex karyotype did not retain in our series its prognostic value in multivariate analysis. So far, complex karyotype AML has been defined on a statistical basis as an indicator of poor prognosis; its definition ranged from ≥ 3 to ≥ 5 independent cytogenetic abnormalities in different clinical series [2,3,16]. A cutoff of ≥ 3 independent abnormalities is commonly used to define complex karyotype also in CBF AML [4,9,10]; as such, opposite to our own results, most of these studies failed to detect a prognostic impact of these on OS [4,9,21]. We believe this discrepancy to be possibly explained by: (1) the lack of complete karyotypic data, also due to the ever-growing use of molecular data as an alternative to cytogenetic analysis; (2) the different definition of “complex karyotype” in CBF AML that in our series, as in another one [3], required ≥ 4 independent cytogenetic abnormalities to identify patients with worse OS; (3) the relative rarity of such patients. In our opinion, these patients might deserve higher first-line intensity, possibly including ASCT. In one study [33], ASCT after Busulfan-Etoposide-cytarabine conditioning managed to reverse molecular MRD-positivity in adverse risk CBF AML patients, provided that the stem cell harvest was PCR-negative for the molecular transcripts.

With the limitation of incomplete data, we also addressed the prognostic role of *KIT* [11,17,18] *FLT3* [20,34], and *NPM1*. The *KIT* D816 mutation has been proposed to identify intermediate-risk CBF AML [11,12,17,18]; these patients are also considered to have intermediate risk by the ongoing cooperative GIMEMA AML1310 trial and two studies by the NCI (ClinicalTrials.gov NCT01238211) and

TABLE III. Univariate and Multivariate Proportional Hazard Modeling for Potential Factors Impacting Overall Survival

	Univariate analysis		Multivariate analysis	
	RR (95% CI)	P	RR (95% CI)	P
Age >60 years	3.05 (1.69–5.51)	<0.001	4.52 (2.24–9.12)	<0.001
Secondary AML	2.30 (0.98–5.39)	0.056		
Male	0.98 (0.58–1.66)	0.95		
Splenomegaly	1.02 (0.50–2.08)	0.96		
Hepatomegaly	1.13 (0.62–2.07)	0.69		
≥2 lymph nodes	0.41 (0.15–1.13)	0.084		
Extramedullary disease	1.44 (0.68–3.04)	0.50		
Granulocytic sarcoma	1.50 (0.47–4.80)	0.50		
WBC ≥30 × 10 ³ /mm ³	1.07 (0.62–1.84)	0.81		
Platelets ≤20 × 10 ³ /mm ³	2.24 (1.29–3.91)	0.004	1.99 (1.08–3.66)	0.027
Elevated LDH	3.60 (1.12–11.57)	0.032	3.52 (1.07–11.60)	0.038
DIC	0.70 (0.33–1.48)	0.35		
inv(16) vs t(8;21)	0.75 (0.45–1.26)	0.28		
≥3 additional cytogenetic abnormalities	2.58 (1.02–6.49)	0.044	1.47 (0.48–4.48)	0.50
Presence of subclones	1.15 (0.66–1.98)	0.63		
Mutated <i>KIT</i>	2.33 (0.61–8.8)	0.21		
Mutated <i>FLT3</i>	0.95 (0.28–3.17)	0.93		
Packed marrow	1.37 (0.79–2.38)	0.26		
Failure to achieve CR1 after induction therapy	6.21 (2.92–13.22)	<0.001	5.43 (2.33–12.68)	<0.001

The probability of dying while having the mentioned covariate (putative prognostic factor) is shown over the probability of dying while *not* having the covariate (hazard ratio).

AML, acute myeloid leukemia; CR, complete remission; DIC, disseminated intravascular coagulation; FLT3, fms-like tyrosine kinase 3; LDH, lactate dehydrogenase; RR, relative risk; WBC, white blood cells.

the German AMLSG (ClinicalTrials.gov NCT00850382) that both add dasatinib to standard treatment. Mixed results have been obtained in the patients with inv(16) AML [8,20,23]. In our series, the *KIT* mutation predicted independent unfavorable OS for t(8;21) but not inv(16) patients. *NPM1* mutations proved mutually exclusive with *CBF* translocations, as previously observed [20,35], whereas *FLT3* mutations did not seem to predict worse OS or EFS, similar to some studies [36] and opposite to others [37]. It has been recently proposed that the effect of *FLT3* mutations on the prognosis of CBF AML depends on the relative mutant level [20], which might explain these differences.

Besides cytogenetics, the overall dose-intensity of first-line treatment proved to be pivotal in determining the final OS and DFS. We observed a high CR rate with few deaths occurring during induction also in the case of patients undergoing the more intensive induction 3-drug regimens. A study from the MDACC [38] reported similar results comparing fludarabine-based regimens with more conventional induction protocols. The most recently published results of the MRC group on the FLAG+idarubicin regimen for younger patients, moreover, improved historical results, especially in the “favorable” and “intermediate-risk” categories [39]. However, in our series, the better control over the disease obtained by more intensive induction ultimately resulted only in a trend toward better OS, probably because of the high probability of achieving CR2 with rescue therapy. Despite this, failure to achieve CR after induction still translated into more than six times higher relative risk of dying of disease ($P < 0.001$).

Repetitive HiDAC courses given as consolidation therapy are currently considered the standard primary treatment for CBF AML, even if overall dose of cytarabine may vary [15,16]. A clear advantage in terms of DFS has been demonstrated for HiDAC (i.e., 3 g/m² bid at days 1,3,5) compared with 400 mg/m² and lower doses of cytarabine [40] or as repetitive courses compared with 1 cycle only [4]. Despite this, not all related studies eventually demonstrated a significant prolongation in OS [41], and the overall dose of cytarabine needed to achieve best results is still uncertain [13,21,41]. In our series, intensive first-line treatment, consisting of repetitive courses of HiDAC (≥3) or ASCT performed after 2–3 HiDAC-based cycles, proved to be the most important factor in determining final DFS and OS. We also

could not detect a linear relationship between the overall dose of administered cytarabine and eventual survival [13,41]; in fact, when excluding patients undergoing allogeneic HSCT during first-line treatment, the best OS in our series was found in patients treated with 15–24 g/m² of cytarabine overall (data not shown). As noted by others [41], an administered dose of 6–12 g/m² of cytarabine per cycle should pharmacocinetically saturate the target of the drug and provide the best results in terms of cytotoxicity. In fact, in our data as in other studies [4,13,41], the number of repetitive IDAC/HiDAC cycles seems more important than overall cytarabine dose in determining eventual survival: we observed a clear advantage between patients consolidated with 3–4 courses as compared to 1–2 courses, while the difference between 3–4 course and ASCT did not appear significant. Furthermore, a minority of selected patients treated with first-line allogeneic HSCT experienced a surprisingly good DFS and OS, as a consequence of low RM and unexpectedly low NRM, probably due to patient selection. All of this considered, in line with what reported by the CALGB group [4], and with the guidelines by the NCCN [15] and the ELN [16], we believe that 3–4 courses of IDAC/HiDAC should remain the standard consolidation treatment in CR1. Alternatively, ASCT might be beneficial for selected patients presenting with features of aggressive disease [42]. Prospective randomized trials would be needed to properly address this issue. The role of allogeneic HSCT for CBF AML patients in CR1 has been reviewed in a meta-analysis that included 547 AML patients with favorable cytogenetics [43]: no advantage was found in neither relapse-free (RR 1.06, 95% CI, 0.80–1.42) nor overall survival (RR 1.07; 95% CI, 0.83–1.38) [43]. Considering the retrospective non-randomized nature of our own analysis, we also do not suggest the use of first-line allogeneic HSCT, despite the good OS and DFS we observed in our series; comparable results can be achieved with less toxic approaches.

Overall intensity of first-line treatment might be rationally modulated by the use of molecular MRD monitoring [23,24,44,45]. Several studies have shown how the integration of MRD into clinical protocols holds the potential to supersede the risk assessment made at diagnosis [44,45]. At present, however, the use of MRD monitoring is still limited by the lack of interlaboratory standardization and

inconsistencies in MRD thresholds and time points to use [44]. Besides, in the case of CBF AML very few prospective studies have been published [23,24], with most information coming from the retrospective analysis of limited numbers of patients [22]. Finally, the reports of patients achieving long-term DFS while still with detectable molecular transcripts [23,46] add further complexity to the clinical translation of MRD monitoring; possible explanations of these findings imply successful immune surveillance and/or the presence of CBFβ/MYH11 or RUNX1/RUNX1T1 in persisting pre-leukemic clones. In this study, we detected a powerful advantage in survival for patients achieving molecular MRD-negativity compared to patients never achieving it. Nonetheless, we could not refine the precise time points when the achievement of MRD-negativity proved most predictive. Future ongoing trials will help to refine how MRD may be used as a tool to drive clinical decisions.

The present series included 11 patients diagnosed with secondary CBF AML: they did not differ in clinical and biological features as compared to de novo leukemia. Similar to other reports [47,48], they achieved CR1 at very high rate and experienced similar relapse rate; this resulted in comparable DFS and EFS between the two groups. Nevertheless, difference in OS almost approached statistical significance; due to limited numbers, we could not assess whether this reflected biological differences or the effect of comorbidity.

As postulated by others [4,13], we believe t(8;21) and inv(16) to be distinct biological entities: in our series, they differed in clinical pre-

sentation, DFS, and response to second-line therapy. This difference, in our opinion, becomes relevant especially in relapsing patients. Although a high CR2 rate is achievable in both types of CBF AML, in our series and in other studies [13,17], ultimate survival was significantly poorer with t(8;21). Recently, Kurosawa et al. [6] reported that patients with t(8;21) acquired more commonly additional cytogenetic abnormalities at relapse and benefited more than inv(16) patients from the use of second-line allogeneic HSCT [6].

In conclusion, we believe that our study contributes to the knowledge about CBF AML by highlighting the presence of a small group of patients, especially those with t(8;21), characterized by the presence of ≥ 3 additional cytogenetic abnormalities, who ultimately have a poor survival despite intensive chemotherapy. We also demonstrated the importance of overall dose intensity of first-line treatment in determining ultimate cure. Based on these results, we believe that proper intensive consolidation, possibly including first-line ASCT should be administered to all patients with CBF AML. Finally, a recent study by the Surveillance, Epidemiology, and End Results (SEER) demonstrated how survival in CBF AML patients sharply declined in patients older than 65 years, possibly as a consequence of undertreatment [5]. We, among others [31], found evidence indicating conserved chemosensitivity also in elderly (i.e., >60 years) CBF AML patients; this, in our opinion, prompts the definition of more precise criteria to exclude only truly unfit elderly patients from the potential benefit deriving from chemotherapy.

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