Complex karyotype, older age, and reduced first-line dose intensity are major determinants of poor final outcome in core-binding factor acute myeloid leukemia

*Running head: A **retrospective** study on CBF AML from 11 Italian institutions*

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ABSTRACT

Purpose

The aim of this study was to identify markers of poorer prognosis in patients with core-binding factor acute myeloid leukemia (AML).

Patients and Methods

A total of 192 patients were treated with curative intent (age, 18-79 years) in 11 Italian institutions.

Results

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 in terms of clinical presentation and laboratory and cytogenetic findings. Although the complete remission (CR) rate was similarly high in both groups, patients with inv(16) experienced superior DFS, a higher chance of achieving a second CR following relapse, and eventually a trend toward longer OS. Despite its rarity, we found that a complex karyotype (ie, ≥ 4 cytogenetic anomalies) strongly impacted survival. The KIT D816 mutation predicted worse prognosis only in patients with the t(8;21) rearrangement, whereas FLT3 mutations had no prognostic impact. We found increasingly better outcomes with more intense first-line therapy, with the best results achieved after 3-drug induction regimens and consolidation by repetitive high-dose cytarabine courses and/or autologous stem cell transplant (ASCT) or allogeneic hematopoietic stem cell transplant. 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 outcome. Long-term survival was also observed in a minority of elderly patients who received intensive consolidation treatment.

Conclusions

AML with the t(8;21) and inv(16) rearrangements should be considered distinct diseases. The overall intensity of first-line treatment remains the strongest predictor of ultimate cure. Complex karyotype identified patients at higher risk who might benefit from intensive consolidation therapy, possibly including ASCT.

INTRODUCTION

Core-binding factor (CBF) acute myeloid leukemia (AML) is cytogenetically defined by the presence of t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22).[1,2] These cytogenetic alterations lead to the formation of the runt-related transcription factor 1 (*RUNX1*)/*RUNX1T1* and *CBFB*/myosin heavy chain 11 (*MYH11*) fusion genes and their respective fusion proteins that disrupt the signaling of the CBF complex, a heterodimeric transcription factor involved in normal hematopoiesis.[3] Both genetic events are responsible for the loss of CBF function and result in impaired hematopoietic differentiation, a first step in the predisposition of the development of AML.[3-5] According to the World Health Organization (WHO) 2008 classification of myeloid neoplasms,[6] CBF AML is categorized as one type of AML with recurrent genetic abnormalities.[6] Among adults with de novo AML, the frequency of CBF AML decreases from approximately 15% in younger patients to only 7% in patients older than age 60 years.[7-10] Due to a common pathogenetic foundation, but different genetic, clinical, and prognostic implications, it is still debated whether t(8;21) and inv(16) should be considered distinct disease entities.[11-15] Patients with t(8;21) are frequently grouped as morphological subtype M2 according to the French-American-British (FAB) classification; they frequently also display loss of a sex chromosome and/or deletions of the long arm of chromosome 9.[6] On the other hand, patients with inv(16) are more often diagnosed with FAB subtype M4Eo; these patients are frequently associated with trisomies of chromosomes 22, 8, and 21.[6,16] Despite the common finding of these additional cytogenetic abnormalities, their role in the pathogenesis of CBF AML is still uncertain, as is their putative prognostic relevance.[7,8,10,16,17]

Considering the high remission rate and good survival of patients with CBF AML, this type of leukemia is usually considered “favorable” in most recent classifications based on cytogenetics.[7,10,18-20] This is particularly true when patients are treated with standard induction therapy followed by multiple cycles of high-dose cytarabine (HiDAC) as a consolidation regimen.[19-22] Thus, patients affected by CBF AML are not usually considered candidates for allogeneic hematopoietic stem cell transplant (HSCT) when first complete

remission (CR) is achieved.[23,24] However, as with other types of acute leukemia, relapse remains the main cause of treatment failure, with relapse rates up to 40% to 50%.[2,25] Patients with the t(8;21) translocation seem to have a worse outcome than those with inv(16).[26] The reason for such disparity could be explained by the growing evidence of the genetic heterogeneity of CBF AML.[19] In fact, in addition to the known secondary chromosome aberrations, gene mutations affecting tyrosine kinases commonly involved in the cellular cycle, such as *KIT,* fms-like tyrosine kinase 3 (*FLT3*), and *RAS*, have been found to be frequently mutated in CBF AML.[17,27-30] In particular, the *KIT* D816 mutation, detected in approximately one-third of patients with CBF AML, has been associated with unfavorable outcome, mostly in patients with the t(8;21) translocation; however, its prognostic impact in patients with inv(16) is less clear.[17,31-33] It is likely that additional molecular alterations with prognostic and therapeutic implications will be uncovered by genomic sequencing technology (eg, next-generation sequencing); nevertheless, at present, this technology is not available in daily clinical practice, and its use is limited to a few hematology institutions mainly for research purposes. Minimal residual disease (MRD) monitoring by quantitative reverse transcriptase polymerase chain reaction (RT-PCR) is currently under investigation as a tool to identify patients at high risk of relapse.[25,34] Consequently, up-front risk stratification based on feasible clinical and biological markers remains the most useful and accessible tool to date 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 2 decades. We focused our attention on identifying heterogeneity among patients with t(8;21) and inv(16) AML in terms of clinical presentation, genetic features and response to therapy. We also focused on defining the potential prognostic role of additional cytogenetic abnormalities at diagnosis, as well as molecular, clinical, and laboratory data. We then assessed the role of overall dose intensity of first-line treatment in determining final outcome, exploiting the long available follow-up. Based on our results, we identified several characteristics

commonly assessed at diagnosis in daily clinical practice that may have a useful role in helping physicians to make evidence-based decisions.

PATIENTS AND METHODS

Patients

We retrospectively reviewed the medical records of patients with CBF AML from 11 Italian hematology institutions in Treviso (n = 14), Bologna (n = 51), Vicenza (n = 34), Verona (n = 22), Udine (n = 18), Ancona (n = 17), Bolzano (n = 15), Modena (n = 9), Ascoli Piceno (n = 7), Pesaro (n = 3), and Padova (n = 2). Minimal required follow-up was 6 months for surviving patients. For July 1987 to August 2012, we collected data on 202 patients diagnosed and intensively treated with curative intent; of these, 192 (95%) had adequate clinical, laboratory, cytogenetic, and survival data to be considered eligible for the present study. The main reasons for exclusion were follow-up < 6 months or inadequate karyotype analysis at diagnosis (mainly due to failure to detect a sufficient number of mitoses).

Consent to use the medical records anonymously for research purposes was obtained from all patients as part of the informed consent for therapy, as revised and approved by the local institutional review boards of the participating centers and according to the existing regulations at the time of diagnosis and initial therapy. Part of the present clinical series (n = 46 [24%]), mainly those patients diagnosed and treated less recently, was already presented in 2 other multicenter studies.[28,35]

Clinical, laboratory, cytogenetic, and molecular data

Clinical and laboratory data were collected from medical records. In all cases, a complete clinical examination and laboratory profile were obtained at diagnosis, including full hemochromocytometric analysis confirmed by microscopic examination of peripheral blood smears; evaluation of common laboratory markers of renal and hepatic function; and levels of serum lactate dehydrogenase (LDH), coagulation markers, serum urates, and lysozyme.

Morphology and immunophenotype of leukemic blasts were evaluated in bone marrow samples in all cases.

All 192 patients had complete cytogenetic information at diagnosis. Chromosome banding analysis was performed on bone marrow cells after short-term culture (24-48 hours). A total of 20 metaphase cells were analyzed according to the International System for Human Cytogenetic Nomenclature.[36] All participating centers provided complete descriptions of CBF translocations and additional cytogenetic abnormalities and subclone analysis, when different pathologic clones coexisted, or of the CBF AML clone together with cytogenetically normal hematopoiesis. Fluorescence in situ hybridization was conducted as to confirm cytogenetic information in 39 cases (20%).

Molecular analysis in more recent years included data for mutations in *KIT*, *FLT3*, and nucleophosmin (*NPM1*) in all newly diagnosed patients. Mutation analysis of the *KIT* gene was performed by PCR using specific primers for exons 8 and 17, followed by sequencing.

The presence of internal tandem duplication and D835 mutations of the *FLT3* gene was determined by multiplex PCR, followed by restriction endonuclease digestion (for the D835 mutation) and separation by capillary electrophoresis, per the manufacturer's instructions.[37] *NPM1* exon 12 A, B, and D mutations were initially characterized by real-time PCR.[38] Given the retrospective nature of the current study, complete data were only available for a subgroup of the whole series. Overall, *KIT* analysis was performed in 59 patients (30.7%), *FLT3* in 101 patients (52.6%), and *NPM1* in 79 patients (41.1%).

For the detection of chimeric genes *RUNX1*/*RUNX1T1* and *CBFB*/*MYH11*, a standardized RT-PCR protocol was used, as described previously.[39] These transcripts were systematically used for molecular follow-up at 4 hematology institutions participating in this study (Bologna, Modena, Bolzano, and Ascoli Piceno). Detection of MRD was performed on bone marrow samples at diagnosis and at regular time points during treatment (end of induction, end of consolidation, and post-HSCT [autologous or allogeneic HSCT]). Overall,

60 patients had complete molecular follow-up data that were used for subsequent statistical analysis.

Chemotherapy and conditioning regimens

Intensive chemotherapy induction regimens used for curative purpose in patients aged 18-60 years with AML were categorized into groups: (1) “standard” D3A7 regimen, consisting of daunorubicin 45 mg/m^2 days 1-3 + intravenous cytarabine continuous infusion 100 mg/m^2 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/m^2 twice daily [bid] days 1-5 or HiDAC 3 g/m^2 bid days 1-5 plus an anthracycline (eg, HAM, IDAC/HiDAC, HiDAC + idarubicin); (3) 3-drug regimens, adding etoposide 50 mg/m^2 days 1-5 or other drugs (eg, thioguanine 200 mg/m^2 days 1-5 in the ETI and days 1-7 in the AAT regimens), excluding purine nucleoside analogues to anthracycline and cytarabine (eg, ICE, MICE, DAV/DAE/DCE, MEC, BARTS, ETI, AAT); (4) 3-drug fludarabine-based regimens, with fludarabine 25-30 mg/m^2 days 1-5 or, in a few cases, other purine analogues as the third drug together with IDAC/HiDAC and an anthracycline (eg, FLAI5, FLAIRG, FLAN, FLAIE); (5) 3-drug fludarabine-based regimens with the addition of anti-CD33 gemtuzumab ozogamicin, mainly as part of other collaborative trials in more recent years (eg, AML M7 protocol using My-FLAI5 as an induction regimen).

Following 1 cycle of intensive induction (cycle 2 in the ETI regimen [n = 2]), patients achieving complete hematologic response were consolidated with ≥ 1 (range, 1-4) heterogeneous consolidation courses depending on the time and hematology institution in which they were treated. Three patients only were consolidated with > 4 courses. All consolidation courses included IDAC/HiDAC given alone or together with other chemotherapeutic drugs. After 2-3 courses of consolidation, first-line autologous HSCT (ASCT) was implemented in (1) patients considered at high risk of relapse because of the presence of prognostically adverse clinical or laboratory findings at diagnosis (eg, hyperleukocytosis with > 10^8/L white blood cells [WBC]; extensive bone marrow, hepatic,

and splenic infiltration; granulocytic sarcoma); (2) patients failing to achieve complete hematologic remission after induction and rescued with reinduction therapy; (3) slow-responding patients who achieved hematologic but not cytogenetic remission after induction therapy; (4) patients with persisting or relapsing molecular transcripts, when the patient had regular molecular follow-up visits. Similarly, a small group of patients with available HLA-matched donors (n = 29) was treated with first-line allogeneic HSCT 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 were performed after myeloablative conditioning.

In relapsing patients, rescue therapy mainly included 3-drug fludarabine-based regimens, and responding patients were consolidated with another course of the same regimen, whenever possible. After that, second-line allogeneic HSCT was the treatment of choice in all patients capable of undergoing HSCT and 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 for older patients, when available.

Descriptive statistics, group comparison, and survival analysis

For analysis of the differences in proportions, the Fisher exact (for cell n < 5 in a contingency table) and Pearson χ^2 ($n \geq 5$) tests were used. The Mann-Whitney and Kruskal-Wallis tests were used to compare nonparametric variables between 2 or multiple groups, whereas 1-way analysis of variance and Holm-Šidák test for multiple comparison were used to compare parametric variables among multiple groups. Two-way Student *t* test was used to compare parametric variables between 2 groups. The Shapiro-Wilk test was used to test normal distribution. Differences were considered statistically significant for $P \leq .05$.

Survival data from February 2014 were retrospectively analyzed. At this time point, 134 patients (69.8%) were alive, with a median follow-up of 73.4 months (range, 6-294).

Overall survival (OS) was defined as the time from diagnosis to death from all causes or last follow-up. Disease-free survival (DFS) was defined only in patients achieving complete hematologic remission as the time from assessment of complete remission 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 of leukemia, and treatment-related death occurring during induction or consolidation therapy.

With a competing risk survival approach, relapse mortality (RM) was defined as death due to leukemia relapse and nonrelapse mortality (NRM) was defined as death from any cause in the absence of overt leukemia. When the role of allogeneic HSCT on survival was assessed, a Mantel-Byar approach was used (ie, allogeneic HSCT was treated as a time-varying covariate, and patients eventually transplanted were switched from the regular group to the group of transplanted patients only at the time of HSCT). In our opinion, as detailed in more recent literature, this enabled us to avoid the possible bias of considering the effects of allogeneic transplant (eg, graft-vs-leukemia effect) from the date of diagnosis to last follow-up, rather than the time from transplant to last follow-up.

OS curves were calculated according to the Kaplan-Meier method, and differences between patient groups were tested using the log-rank test. $P \leq .05$ was considered statistically significant. We then applied Cox proportional hazard modeling to evaluate potential prognostic factors, defining death from all causes as the event in the case of OS. This enabled us to obtain hazard ratios (HRs) and their 95% CI in both univariate (unadjusted) and multivariate (adjusted) modes. Multivariate analysis was carried out for those factors resulting in statistically significant differences (ie, $P \leq 0.05$) among patients at the univariate analysis. In 1 case (ie, t[8;21] patients), *KIT* evaluation was excluded from the multivariate analysis because of incomplete molecular data at diagnosis, which limits the multivariate analysis to approximately one-third of the whole series.

To evaluate 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. We also considered allogeneic HSCT in this setting as a time-dependent covariate when assessing its role on survival. We compared cumulative incidence functions between groups identified by the presence or absence of putative risk factors used as covariates by means of the Pepe and Mori test.[40]

Statistical analyses were performed using the Stata IC v.10.1 platform for Microsoft Windows, by StataCorp (College Station, TX).

RESULTS

Patient characteristics

Patient characteristics are summarized in Table 1. Overall, 80 patients (41.7%) presented with t(8;21) and 112 (58.3%) with inv(16) AML. Age and sex ratio were equally distributed, with 9 (11.3%) and 17 (15.2%) patients diagnosed with CBF AML at an older age (≥ 61 years). We observed 11 patients (5.7%) with secondary AML, following a previous history of hematologic or neoplastic disease. In 8 patients, the diagnosis of AML followed a previous cancer treated with either chemotherapy including alkylating agents or chemotherapy and radiotherapy (4 non-Hodgkin lymphomas, 2 Hodgkin lymphomas, 1 colon cancer, and 1 breast cancer); in 2 more patients, a previous history of myelodysplastic syndrome, as documented by hematologic anomalies lasting > 6 months, was present; and in 1 patient, AML emerged in the context of chronic myeloid leukemia but from a Philadelphia-negative clone. At diagnosis, hepatic involvement did not differ between the 2 CBF AML subtypes, whereas splenomegaly (24 vs 6; $P = .008$) and lymphadenomegaly (28 vs 7, respectively; $P = .005$) were more common with patients with inv(16) than t(8;21). Patients with inv(16) AML had higher WBC ($P < .001$) and lower platelet counts ($P = .04$), and a higher degree of blastic substitution at the initial bone marrow analysis (ie, packed marrow; $P = .02$). Hemoglobin level was lower in patients with t(8;21) AML ($P = .002$). Diffuse extramedullary leukemia not meeting the requirements of granulocytic sarcoma (eg, skin localizations, diffuse pulmonary infiltrates) was slightly more frequent in patients with inv(16) vs t(8;21) AML (12 vs 3 patients, respectively; P = nonsignificant [NS]).

Survival

Overall, OS of our entire series was 67.0% at 5 years and 63.9% at 10 years, confirming the favorable outcome of this type of AML; 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) (5-year DFS, 63.7% vs 50.5%; 10-year DFS, 61.7% vs 45.2%, respectively; *P* = .04; Figure 1). Although this trend was maintained when OS was considered, the difference between the 2 subtypes was not significant (Figure 1); in fact, patients with t(8;21) experienced 5-year and 10-year OS that was only slightly lower than that of patients with inv(16) (64.7% and 57.2% vs 68.5% for both time points, respectively; *P* = NS; Figure 1).

Both t(8;21) and inv(16) AML presented high CR (CR1) rates after intensive induction chemotherapy (92.5% vs 93.8%), with 29 of 74 patients (39.2%) relapsing after 8.9 months (range, 0.9-42) in t(8;21) and 31 of 105 patients (29.5%) relapsing after 13.9 months (range, 1-70) for inv(16). Age did not impact CR1 after induction therapy: overall, 23 older patients achieved CR1 after induction (88.5%) vs 156 younger patients (95.1%; *P* = .18). Thereafter, patients were consolidated with multiple courses of therapy and possibly first-line ASCT or allogeneic HSCT as described in the *Patients and Methods* section. Three patients died during the aplastic phase following induction therapy and 1 more died during consolidation therapy after the achievement of CR1, accounting for an overall treatment-related mortality of 2.1% in the series.

Regarding relapse, patients with inv(16) had a slightly better chance to achieve a second CR (CR2) following rescue chemotherapy (n = 21 of 25 [84.0%] vs n = 16 of 24 [66.7%], respectively; *P* = NS), resulting in a nonstatistical trend toward better final OS (Figure 1).

Eleven patients not achieving CR1 after induction therapy experienced poor survival despite administration of rescue therapy in most cases (n = 8 [72.7%]) and the later achievement of CR in 5 of 8 patients (62.5%). We could not find any correlation between

clinical, laboratory, cytogenetic, or molecular features and the chance to achieve CR1. Eventually, 7 patients of this group died early from leukemia progression, with a median survival of 2.2 months from diagnosis; 1 died later following allogeneic HSCT, due to infectious complications while still in CR. Only 3 patients survived long term: of these, 1 was treated with allogeneic HSCT following successful rescue chemotherapy, the other 2 were treated with consolidation chemotherapy alone.

Prognostic role of additional cytogenetic abnormalities

We then analyzed whether additional cytogenetic abnormalities predicted different prognoses. We detected additional cytogenetic abnormalities in 83 patients (42 patients with t[8;21] [52.5%] and 41 patients with inv[16] [36.6%; *P* = NS]). These are listed in Supplementary Table 1. We observed both quantitative (eg, aneuploidy, hyperdiploidy, trisomy) and qualitative (eg, deletions, additions, isochromosome formation) abnormalities and considered them separately in survival analysis. We also considered whether the presence of multiple subclones, as identified by the coexistence of different populations with different cytogenetic abnormalities or by the leukemic clone together with hematopoietic cells with normal karyotype, had any impact on the final outcome.

Single additional cytogenetic abnormalities were present in 43 patients and are listed in Table 2. Most anomalies were not homogenously distributed in either t(8;21) or inv(16), with anomalies involving sex chromosomes and chromosome 9 more common in patients with t(8;21) and others, such as trisomy 22, trisomy 8, and anomalies of chromosome 7, almost exclusively in patients with inv(16). We found a nonsignificant trend toward better OS and DFS for patients with inv(16) and trisomy 22 and trisomy 8 (data not shown), but proper survival analysis could not be performed for these patients, mainly due to the small numbers in each group.

We then considered the whole series according to the number of cytogenetic abnormalities besides t(8;21) and inv(16). Overall, 43 patients presented with 1 additional cytogenetic abnormality, 31 with 2, and 9 with ≥ 3; the latter were considered “complex

karyotypes" (Table 2). As presented in Figure 2, although the survival rates of patients with 1 or 2 additional cytogenetic abnormalities were not statistically different than those of patients with CBF AML with just t(8;21) or inv(16), patients with ≥ 3 additional cytogenetic abnormalities fared significantly worse than all other groups (Figure 2) in terms of DFS and EFS. Shorter OS was evident only as a trend approaching statistical significance, due to the small group number. This was confirmed by Cox proportional hazard modeling; also in this setting, the presence of ≥ 3 additional cytogenetic abnormalities identified a small subgroup of patients with dismal prognosis (HR, 2.58; 95% CI, 1.02-6.49; *P* = .044). This subgroup consisted of 9 patients (4.7% of the whole series), 5 presenting with t(8;21) and 4 with inv(16); 3 patients were aged ≥ 61 years and notably, only 2 presented with secondary AML. Eight achieved CR following induction and 4 relapsed, with a median DFS of 15.4 months. Three relapsing patients were then treated with second-line therapy, including allogeneic HSCT in 2 of the patients. When we evaluated the prognostic significance of complex karyotypes separately in patients with t(8;21) and inv(16), the presence of ≥ 3 additional cytogenetic abnormalities still identified (at the limit of statistical significance) a subgroup with dismal prognosis in t(8;21), despite the low number in the group (HR, 2.85; 95% CI, 0.98-8.29; *P* = .055; n = 5). The difference in survival became statistically nonsignificant in the analysis of patients with inv(16) (Supplementary Tables 4 and 5).

Paradoxically, as shown in Figure 2, we observed a nonstatistical trend toward better survival in patients with 2 additional cytogenetic abnormalities compared with patients without any additional abnormalities; notably, there were more patients with inv(16) in the group with 2 additional cytogenetic abnormalities compared with the groups with either 1 or ≥ 3 additional abnormalities (19 of 31 [61.3%] vs 18 of 43 [41.9%] and 4 of 9 [44.4%], respectively). Trisomy 22 and trisomy 8 were both common among patients with 2 additional abnormalities, being present in 14 patients of this group overall (45.2%), either separately or combined together. If we excluded these patients from the group with 2 additional cytogenetic abnormalities, the advantage in survival shown by this group remained (still as a trend) but diminished considerably (data not shown).

Finally, we did not detect any prognostic role for the presence of subclones as detected by classic karyotyping or by the presence of additional abnormalities listed as qualitative or quantitative.

Prognostic role of molecular data

Details on molecular data are listed in Supplementary Table 2. In the small group for whom data had been consistently collected, we observed the presence of mutated *FLT3* or *NPM1* in rare cases (10 of 101 [9.9%] and 2 of 79 [2.5%], respectively), whereas *KIT* was mutated in 7 of 59 patients (11.8%). Despite the low numbers, *KIT* mutations proved to be an indicator of shorter OS at the univariate analysis in patients with t(8;21) (HR, 12.5; 95% CI, 1.12-139.33; *P* = .04) but not in patients with inv(16) or in the whole series (Table 3). *KIT* mutations did not indicate worse DFS in patients with either t(8;21) or inv(16) (data not shown). *FLT3* mutations did not predict worse OS or DFS, whereas *NPM1* mutations could not be analyzed due to the presence of just 2 patients with these mutations in the whole series.

Postinduction consolidation

We first used D3A7 as a reference for induction therapy against more recent and intensive regimens. Overall, 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); the characteristics of the 2 cohorts are summarized in Supplementary Table 3. Interestingly, there was no temporal bias toward the D3A7 regimen in the first decade covered by our study (1987-2000) compared with the more recent one (19 vs 132 compared with 6 vs 35; *P* = NS), indicating that D3A7 was still used as a potential induction regimen in more recent years. Furthermore, patients treated with more intensive schemes did not statistically differ in age, presence of secondary leukemia, blood cell counts, LDH levels, or degree of marrow involvement, whereas the presence of granulocytic sarcoma and liver and splenic involvement appeared to be more frequent in the group treated with D3A7. With

these differences, we observed a more favorable outcome after 3-drug or fludarabine-based regimens than after D3A7 (Supplementary Figure 1). Although this appeared only as a statistical trend toward better OS and DFS for intensively treated patients (*P* = NS for both), the difference was statistically significant for EFS (*P* = .043).

We further investigated this issue by dividing patients with CR1 into groups according to the dose intensity of first-line therapy. This resulted in 4 different groups: (1) patients treated with induction + 1-2 consolidation courses (n = 60 [33.5%]); (2) patients treated with induction + ≥ 3 intensive consolidation courses (n = 57 [31.8%]); (3) patients treated with 2-3 consolidation courses and first-line ASCT (n = 33 [18.4%]); and (4) patients treated with first-line allogeneic HSCT (n = 27 [15.1%]). Results are shown in Figure 3. We recognized a distinctive trend toward better outcomes as the dose intensity during first-line treatment increased. Differences were statistically significant for DFS (*P* < .001) and OS (*P* = .005). Although limited consolidation ultimately resulted in poor DFS and OS at 5 years (29.7% and 52.7%, respectively), outcome significantly improved with more intensive consolidation (61.8% and 73.0%) and especially with first-line ASCT (71.3% and 80.3%) or allogeneic HSCT (83.7% and 91.3%). In a small group (n = 27) of patients who were treated with allogeneic HSCT, we observed the best DFS and OS. Notably, only 3 deaths occurred (due to leukemia relapse in one case and to HSCT complications in the other two) in this group.

Given the importance of age in predicting the outcome of patients with AML, we performed a subanalysis of elderly (≥ 61 years) patients (n = 26 [13.5%]). The CR rate was high in this group, with 23 of 26 patients (88.4%) achieving CR1 after induction. Fourteen patients of this group (53.8%) were further consolidated by 1-2 courses and 4 by ≥ 3 courses (15.3%), whereas 4 more patients (15.3%) received ASCT as additional first-line treatment after 2 previous consolidation courses. The sole remaining patient with CR, aged 61 years, was treated with first-line allogeneic reduced-induction conditioning HSCT from a haploidentical related donor. 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% (for both DFS and OS) in those who were treated

with more intensive first-line consolidation (P = .002 and P = .019, respectively). Notably, there were no deaths during induction and consolidation in the elderly patients of our series.

Molecular MRD

In those patients for whom 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 (Supplementary Figure 2). This was true for OS (log-rank P < 0.001; HR, 8.73; 95% CI, 3.30-23.12; Cox univariate analysis P < .001), DFS (P < .001; HR, 5.02; 95% CI, 2.22-11.34; P < .001), and EFS (P < .001; HR, 4.90; 95% CI, 2.24-10.71; P < .001; data not shown). Twenty-three patients (38.3%) never achieved molecular remission and had a median OS of 16.7 months regardless of the treatment they received (Supplementary Figure 2), including first-line ASCT and allogeneic HSCT in 4 and 3 patients, respectively.

Due to the small number of patients, when we stratified patients according to first-line treatment (ie, chemotherapy only, chemotherapy + ASCT, chemotherapy + allogeneic HSCT), we could not find a consistent relationship between time point of achievement of MRD negativity and final outcome.

Cox HR survival modeling

We systematically checked the putative prognostic factors listed in Table 3, chosen on the basis of the literature on AML. In the univariate analysis for OS, age (> 60 years; HR, 3.05; 95% CI, 1.69-5.51; P < .001), severe thrombocytopenia at diagnosis (platelet count, < 20 × $10^3/mm^3$; HR, 2.24; 95% CI, 1.29-3.91; P = .004), increased LDH levels (HR, 3.6; 95% CI, 1.12-11.57; P = .032), presence of ≥ 3 additional cytogenetic abnormalities (other than t[8;21] or inv[16]/t[16;16]; HR, 2.58; 95% CI, 1.02-6.49; P = .044), and failure to achieve CR following induction (HR, 6.21; 95% CI, 2.92-13.22; P < .001) identified patients at higher risk. Of these, only age (HR, 4.08; 95% CI, 2.03-8.21; P < .001), severe thrombocytopenia (HR, 2.1; 95% CI, 1.15-3.83; P = .016), increased LDH levels (HR, 3.46; 95% CI, 1.06-11.35;

P = .041), and failure to achieve CR after induction (HR, 5.33; 95% CI, 2.27-12.48) proved to be independent prognostic factors in the multivariate analysis. When we analyzed OS for patients with t(8;21) or inv(16) AML separately (Supplementary Tables 4 and 5), we found that age, presence of ≥ 3 additional cytogenetic abnormalities, and failure to achieve CR after induction were independent prognostic factors for patients with t(8;21), whereas only age and severe thrombocytopenia remained independent prognostic factors for OS in patients with inv(16). Notably, in our series, the *KIT* D816 mutation identified patients with worse prognosis only in those with t(8;21) AML and only in univariate analysis (we excluded this term from the multivariate analysis because of narrow data coverage [30.7% of the whole series]; Table 3 and Supplementary Table 4). Conversely, failure to achieve CR after induction did indicate adverse prognosis in univariate analysis for patients with inv(16) but did not remain an independent marker for OS in the multivariate analysis, thus highlighting the possibility to rescue failing patients with more intensive second-line therapies (Table 3 and Supplementary Table 5).

Role of allogeneic HSCT

As detailed in the *Patients and Methods* section, to assess the role of allogeneic HSCT, we used a Mantel-Byar approach and a competing-risk analysis to distinguish death due to leukemia persistence or recurrence (ie, RM) and death from all other causes (ie, NRM) as mutually exclusive, competing events. First-line allogeneic HSCT was performed in CR achieved after initial induction (CR1) in 27 patients and in CR achieved after reinduction following failure of initial chemotherapy (CR2) in 2 more. In this small series of 29 patients, we found that first-line allogeneic HSCT deeply reduced RM (*P* < .001) without significantly increasing NRM (P = NS), thus determining a good final outcome, with 5-year OS at 88.5% and 5-year DFS at 83.7% (Figure 4). This resulted in a strong statistical trend (*P* = .059) toward longer OS of the first-line transplanted group compared with patients who did not receive transplants. As previously shown, patients receiving allogeneic HSCT and ASCT as part of first-line treatment experienced the best outcome compared with those who received

consolidation by chemotherapy only (Figure 3). Twenty-two patients treated with allogeneic HSCT as part of rescue or second-line therapy were considered separately.

Relapsing patients and second-line therapy

Overall, 60 patients (31.2%) relapsed after achieving CR, with similar rates in those with t(8;21) and inv(16) AML (n = 29 [39.2%] vs n = 31 [29.5%]; *P* = NS), resulting in only a slight advantage of patients with inv(16) compared with t(8;21) for DFS (*P* = .04; Figure 1). When the intensity of treatment given as first line was tested specifically, we did not find any significant difference between patients with t(8;21) vs inv(16) (data not shown). In both groups, moreover, the chance of achieving CR2 following rescue therapy was good, with 49 patients (81.7%) actually receiving second-line treatment and 37 of these (75.5%) achieving CR2. The chance of achieving CR2 was slightly better for patients with inv(16) vs t(8;21) (n = 16 [66.7%] vs n = 21 [84.0%]; *P* = NS).

The type of induction therapy did not impact the chance of achieving second remission following relapse: 5 of 8 relapsing patients after D3A7 achieved CR2 vs 32 of 41 relapsing after more intensive induction regimens (*P* = NS). Similarly, the overall intensity of first-line treatment did not influence the possibility to rescue patients after relapse: the chance of achieving CR2 was similar for reduced-intensity consolidation (1-2 courses), intensive consolidation (> 3 courses), or ASCT (17 of 22 vs 14 of 19 vs 5 of 7; *P* = NS).

Twenty-two of the 37 patients achieving CR2 were consolidated by allogeneic HSCT. Patients only received second-line allogeneic HSCT while in hematologic remission; this group did not include first-line refractory patients. We analyzed the role of second-line allogeneic HSCT by applying the same time-dependent, competing-risk approach we used for first-line allogeneic HSCT, as previously described. We found better relapse survival for the allotransplanted group compared with the one consolidated with second-line therapy not including HSCT (*P* = .044; Supplementary Figure 3); this was explained by a strong effect of allogeneic transplantation to diminish RM (*P* < .001; Supplementary Figure 3), which was ultimately balanced by an equally powerful NRM in second-line transplanted patients

(*P* = .011; Supplementary Figure 3). NRM accounted for 7 deaths (n = 11 [31.8% of all]) in patients transplanted as part of their second-line therapy.

DISCUSSION

We critically reviewed the clinical experience of 11 Italian hematology institutions, trying to identify predictors of poor prognosis among patients with CBF AML.

Overall, age proved to be a pivotal independent factor in multivariate analysis. In most forms of AML, poor outcome in elderly patients reflects the presence of more aggressive biological features of AML blasts, making them resistant to cytotoxic drugs, as well as the effect of comorbidities that mostly prevent the administration of an adequate treatment intensity.[41-43] Using a cutoff of 60 years, we observed a CR rate comparable for younger and older patients but a higher relapse rate in older patients, ultimately leading to poorer OS. This is similar to what was reported in a recent study,[44] in which these results were linked to the clinical decision not to administer consolidation therapy to most older patients because of high induction-related toxicity.[44] Similarly, only one-third of the older patients in our cohort received intensive postremission therapy (data not shown). It proved impossible to assess whether this reflected a poorer tolerance to chemotherapy or a specific attitude by physicians. Nevertheless, long-term DFS and OS could still be achieved in a significant proportion of elderly patients with CBF AML when intensive consolidation was provided, an achievement not usually observed in other forms of AML. We believe that this is derived from the preserved chemosensitivity of CBF AML blasts also in elderly patients[11,44,45]; we therefore believe that less toxic induction regimens should be given to older patients with CBF AML and later intensive postremission treatment provided to a larger proportion of responding patients.

Besides age, elevated LDH levels (*P* = .041) and low platelet count at diagnosis (*P* = .016) proved to be independent predictors of shorter OS in multivariate modeling, as in other previously published studies[25,46,47]; the precise biological mechanisms highlighted by these statistics are still largely unknown.

We also tested whether other clinical and laboratory data, such as the presence of high tumor burden (ie, high WBC count, deeper bone marrow substitution, or hepatic and splenic involvement), might impact CR rate and survival. Surprisingly, we found no correlation between clinical and laboratory data and CR rate after induction or final outcome. This is similar to what was reported by Appelbaum et al,[15] Marcucci et al,[26] and others,[25] in which cytogenetic and molecular data proved to be more powerful prognostic factors.

We therefore addressed the role of cytogenetics. In agreement with most previous studies,[14,16,26] we could not detect a prognostic value for single additional abnormalities, which showed only a nonsignificant trend toward worse DFS and EFS but no difference in OS. Seemingly a paradox, the group defined by the presence of 2 additional independent abnormalities showed a nonstatistical advantage in survival; we think this may be explained by the prevalence of patients with inv(16) in this group, as well as by the presence of cytogenetic findings such as trisomy 22 and trisomy 21, which were already linked to better prognosis in patients with inv(16) in other studies.[14,16,25]

Most significantly, though, we found that ≥ 3 additional cytogenetic abnormalities, herein defined as "complex karyotype," predicted significantly worse outcome in terms of DFS, EFS, and to a lesser degree, OS. This was proved despite the relative rarity of this subgroup and independently from the intensity of treatment these patients received. Effects were more evident for patients with t(8;21) than those with inv(16), for whom only nonsignificant trends could be observed. So far, complex karyotype AML has been defined on a functional and statistical basis from the results of clinical trials as an indicator of poor prognosis due to chemotherapy resistence.[10,48] Biological studies addressing this type of AML as a putative unique disease entity[48] have found multiple molecular changes, such as *TP53* deletion[49] and alterations in NuMA proteins[50] linked to the multiple chromosomal abnormalities used to identify patients. An overall status of "genetic instability" was determined to be a typical feature of the disease, highlighted by a specific gene expression signature that separates this subgroup from other types of AML. However, there is poor interlaboratory consistency on this issue, and the identified gene expression signatures for

complex karyotype do not overlap between different laboratories.[51,52] Therefore, the definition of complex karyotype has remained functional, and the cutoff of independent chromosomal aberrations has ranged from ≥ 3 to ≥ 5 in different clinical series, even by the same cooperative group.[2,7-10,24]

In CBF AML, most studies have used a cutoff of ≥ 3 independent abnormalities overall to define complex karyotype[8,14,48]; as such, opposite to our own results, most of these studies failed to detect a prognostic impact of additional chromosomal abnormalities on OS.[25] We believe this discrepancy to be the consequence of several issues: (1) the possible lack of complete karyotypic data prior to chemotherapy, due to the failure to detect a proper number of mitoses or, in more recent years, to the ever-growing use of molecular data (ie, *RUNX1*/*RUNX1T1* and *CBFB*/*MYH11*) as an alternative to cytogenetic analysis; (2) the different definition of "complex karyotype" AML that in our series, as in another large series by the Medical Research Council (MRC) group in the United Kingdom, required ≥ 4 independent cytogenetic abnormalities overall to identify patients with worse outcome[10]; (3) the relative rarity of such patients; and (4) the deeper impact on DFS and EFS than on OS, as a consequence of the relatively good chance to achieve CR2 after relapse and rescue therapy. Despite this, we speculate that complex karyotype, as a sign of intrinsic genetic instability, may present a higher chance of clonal evolution eventually leading to disease relapse and treatment failure in CBF AML. In our opinion, these patients might deserve higher first-line intensity, possibly including first-line ASCT or, in selected patients, allogeneic HSCT.

Due to the small number of patients, we could not assess the prognostic value of single specific alterations, such as trisomy 22 for patients with inv(16), as indicated by others[14,16]; we observed only a nonstatistical trend of these patients toward better survival. In contrast to what has been shown by others,[53] the presence of different pathological subclones or the coexistence of the leukemic clone with residual cytogenetically normal hematopoiesis did not yield different survival results.

With the limitation of incomplete data, we also addressed the role of genes already well known for their prognostic impact, such as *KIT*,[17,27,28] *FLT3*,[54] and *NPM1*.[38] The *KIT* D816 mutation, present in approximately one-third of patients with CBF AML,[25] has been proposed to identify intermediate-risk CBF AML[17,27,28,31]; patients with the *KIT* mutation are also considered to have intermediate risk by the ongoing cooperative Gruppo Italiano Malattie Ematologiche dell'Adulto AML1310 trial and other studies by the National Cancer Institute (ClinicalTrials.gov NCT01238211) and German Deutsch-Österreichische Studiengruppe Akute Myeloische Leukämie (AMLSG) 11-08 trial (ClinicalTrials.gov NCT00850382) that both add dasatinib to standard treatment. More mixed results have been obtained in the patients with inv(16) AML.[16,35,55] In our series, the *KIT* mutation predicted independent unfavorable OS for t(8;21) but not inv(16); this fact is particularly relevant when the low number of patients with mutations is considered. With the limitation of incomplete data, *NPM1* mutations proved mutually exclusive with *CBF* translocations, as has been previously observed,[56] whereas *FLT3* mutations did not seem to predict worse OS or EFS, similarly to what has been shown in some studies[57,58] and opposite to what has been shown in others.[59,60] It has recently been proposed that the effect of *FLT3* mutations on the prognosis of CBF AML might depend on the relative mutant level,[61] which might explain the differences among studies.

Besides cytogenetics, the overall dose-intensity of chemotherapy administered as first line proved to be more important. We observed a high CR rate with few deaths occurring during induction, thus highlighting the efficacy of modern first-line 3-drug regimens. The high CR rate was maintained even though there was hepatic and splenic involvement in the D3A7 group, as well as a larger presence of granulocytic sarcoma. A study from MD Anderson Cancer Center[62] reported similar results comparing fludarabine-based regimens with more conventional induction protocols, as did studies by the Eastern Cooperative Oncology Group[63] and Hemato-Oncologie voor Volwassenen Nederland (HOVON)/AMLSG/Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung (SAKK)[64] testing intensification of daunorubicin doses in younger and older patients. 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.[65] 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 following relapse and rescue therapy. Despite this, failure to achieve CR after induction still translated into more than 6 times higher relative risk of dying of disease ($P < .001$).

Following achievement of CR, repetitive HiDAC courses given as consolidation therapy are currently considered the standard primary treatment for CBF AML.[24] Nevertheless, the overall dose of cytarabine and the optimal number of consolidation courses are still a matter of debate.[22] A clear advantage in terms of DFS has been demonstrated for HiDAC compared with IDAC and lower doses of cytarabine[66,67] or as repetitive courses compared with 1 cycle only.[26] Despite this, not all related studies eventually demonstrate a significant prolongation in OS.[22] In addition, the HOVON/SAKK group showed similar EFS and OS for patients treated with a cumulative dose of 13.4 g/m^2 cytarabine (ie, IDAC) compared with a more intensive treatment (26 g/m^2 [ie, HiDAC]).[22]

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 outcome. We believe this is due to the high chemosensitivity of this particular form of AML. A considerable minority of selected patients also were treated with first-line allogeneic HSCT, mainly in less recent years, as a consequence of the presence at diagnosis of features usually linked to poorer prognosis or following incomplete or late response to treatment. Allogeneic HSCT was mostly chosen as an alternative to ASCT, depending on the availability of an HLA-matched donor. Although we believe the good outcome observed in patients who received first-line allotransplant in our series was mostly a consequence of patient selection and a surprisingly low NRM, we think it also may be a consequence of transplant conditioning and the graft-vs-leukemia effect. All of this considered, in line with experience with pediatric patients with CBF AML[68] and following the

current approach by the Cancer and Leukemia Group B cooperative group,[26] we believe that 3 courses of HiDAC should remain the standard consolidation treatment of patients with CBF AML in first CR. Alternatively, ASCT has been proven to be safe and beneficial for selected patients presenting with features of aggressive disease.[69-71] Despite the good results of our series, we do not suggest the use of first-line allogeneic HSCT due to the possibility to achieve comparable results with less toxic approaches.

Overall intensity of first-line treatment might be rationally modulated by the use of molecular MRD monitoring. In the minority of patients in our series for whom data had been systematically evaluated, we found a fundamental advantage in OS, EFS, and DFS for patients achieving molecular MRD negativity compared with patients never achieving it; on the other hand, we could not further refine this evidence by identifying a specific time point when molecular MRD negativity acquired significance. This further stresses the importance of first-line dose intensity in the eradication of the leukemic clone; in fact, most molecular CRs have been achieved without the use of allogeneic HSCT and any graft-vs-leukemia effect. Retrospective as well as prospective trials[72-75] have proven the importance of molecular MRD monitoring as a tool to guide first-line treatment of patients with CBF AML.

As postulated by others[14,26,76,77] and as in the 2008 WHO classification,[6] we believe t(8;21) and inv(16) to be distinct biological entities. They have different characteristics at presentation: patients with t(8;21) had more frequent additional cytogenetic abnormalities and patients with inv(16) had higher WBC and lower platelet counts and more frequent hepatic, lymphonodal, splenic and bone marrow involvement. DFS and response to second-line therapy proved to be overall in favor of better survival for patients with inv(16). In our opinion, these data address biological differences in the pathogenesis of the 2 forms: because CBF translocations are not enough to induce leukemogenesis,[78,79] we speculate that additional multistep events might be more common in patients with t(8;21) than inv(16). This fact could also be implied by the importance of the *KIT* mutation in determining more aggressive disease in patients with t(8;21) but not inv(16), as in other studies.[17,27,28,35]

The difference between patients with t(8;21) and inv(16), in our opinion, becomes clinically relevant especially when relapsing patients are analyzed. Although a high CR2 rate was achieved after rescue therapy in both cases, in our series ultimate survival following relapse was significantly poorer with t(8;21). This is in accordance with existing literature.[14,26,44] Recently, Kurosawa et al[12] reported the acquisition of additional cytogenetic abnormalities at relapse in patients with t(8;21) compared with those with inv(16). At the same time, a survival benefit from the use of second-line allogeneic HSCT was observed only for patients with t(8;21), as the result of a significantly poorer response to rescue therapy[12]. More recently, t(8;21) it has been demonstrated that AML blasts harbor alterations in the transcript levels of mitotic spindle kinases, such as checkpoint kinase 1 and aurora kinase A, which have been postulated to be responsible for an easier and more aggressive pattern of cytogenetic progression in t(8;21) than in inv(16) AML, despite similar initial genetics.[33] In our cohort, allogeneic HSCT appeared to be the best approach to achieve long-term disease control in relapsing patients, despite high NRM and thanks to low RM compared with that seen for chemotherapy.

We acknowledge the limitations of our study: data were retrospectively collected over a long time period, molecular data and MRD monitoring were incomplete, and treatment was heterogeneous due to the collection of clinical records from different Institutions. Despite this, the fundamental criteria on which modern AML therapeutic regimens are based have not changed much in the last 2 decades, and we could benefit from a long available follow-up.

In conclusion, we believe that our study contributes to the knowledge about this form of AML by highlighting the presence of a small group of patients with CBF AML, especially those with t(8;21), characterized by the presence of ≥ 3 additional cytogenetic abnormalities, who ultimately have a poor outcome despite intensive chemotherapy, including allogeneic HSCT in some cases. Furthermore, we found significant differences in the pathogenesis and outcome of patients with t(8;21) and inv(16). Finally, we 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 to improve final outcome. We found evidence indicating conserved chemosensitivity in elderly patients, a setting in which results, in our opinion, could be improved: future studies are warranted to refine more precise exclusion criteria for unfit elderly patients.

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TABLES AND FIGURES

Table 1. 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 (13-73)	NS
Patients ≥ 61 years, n (%)	26 (13.5)	9 (11.3)	17 (15.2)	NS
Male:female ratio	111:81	43:37	68:44	NS
AML type, n (%)				
De novo	181 (94.3)	73 (91.3)	108 (96.4)	NS
Secondary	11 (5.7)	7 (8.7)	4 (3.6)	NS
Splenomegaly, n (%)	30 (15.6)	6 (7.5)	24 (21.4)	0.008
Hepatomegaly, n (%)	41 (21.4)	13 (16.3)	28 (25.0)	NS
Lymphadenomegaly, n (%)	35 (18.2)	7 (8.8)	28 (25.0)	0.005
Extramedullary disease, n (%)	15 (7.8)	3 (3.8)	12 (10.7)	NS
Granulocytic sarcoma, n (%)	6 (3.1)	4 (5.0)	2 (1.8)	NS
WBC (range), $\times 10^3/mm^3$	18.9 (1.2-656.0)	10.5 (1.2-289.4)	32.2 (1.7-656.0)	<0.001
WBC ≥ $30 \times 10^3/mm^3$, n (%)	67 (34.9)	11 (13.8)	56 (50.0)	<0.001
WBC ≥ $100 \times 10^3/mm^3$, n (%)	15 (7.8)	2 (2.5)	13 (11.6)	0.017
Platelets (range), $\times 10^3/mm^3$	38.0 (4.0-586.0)	31 (4-586)	41.5 (6-331)	0.04
Platelets ≤ $20 \times 10^3/mm^3$, 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)	NS

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

Table 2. Additional cytogenetic abnormalities.

	All (n = 83)	t(8;21) (n = 42 [50.6%])	inv(16) (n = 41 [49.4%])
Single additional abnormality	**43 (51.8)**	**25**	**18**
Trisomy 22	5 (11.6)	—	5
Chromosome 7	2 (4.7)	—	2
Chromosome 9	6 (13.9)	6	—
Trisomy 8	4 (9.3)	1	3
Chromosome 21	1 (2.4)	—	1
Chromosomes X or Y	20 (46.5)	18	2
Mixed	5 (11.6)	—	5
Two additional abnormalities	**31 (37.4)**	**12**	**19**
Three (or more) additional abnormalities	**9 (10.8)**	**5**	**4**

Data are presented as n (%).

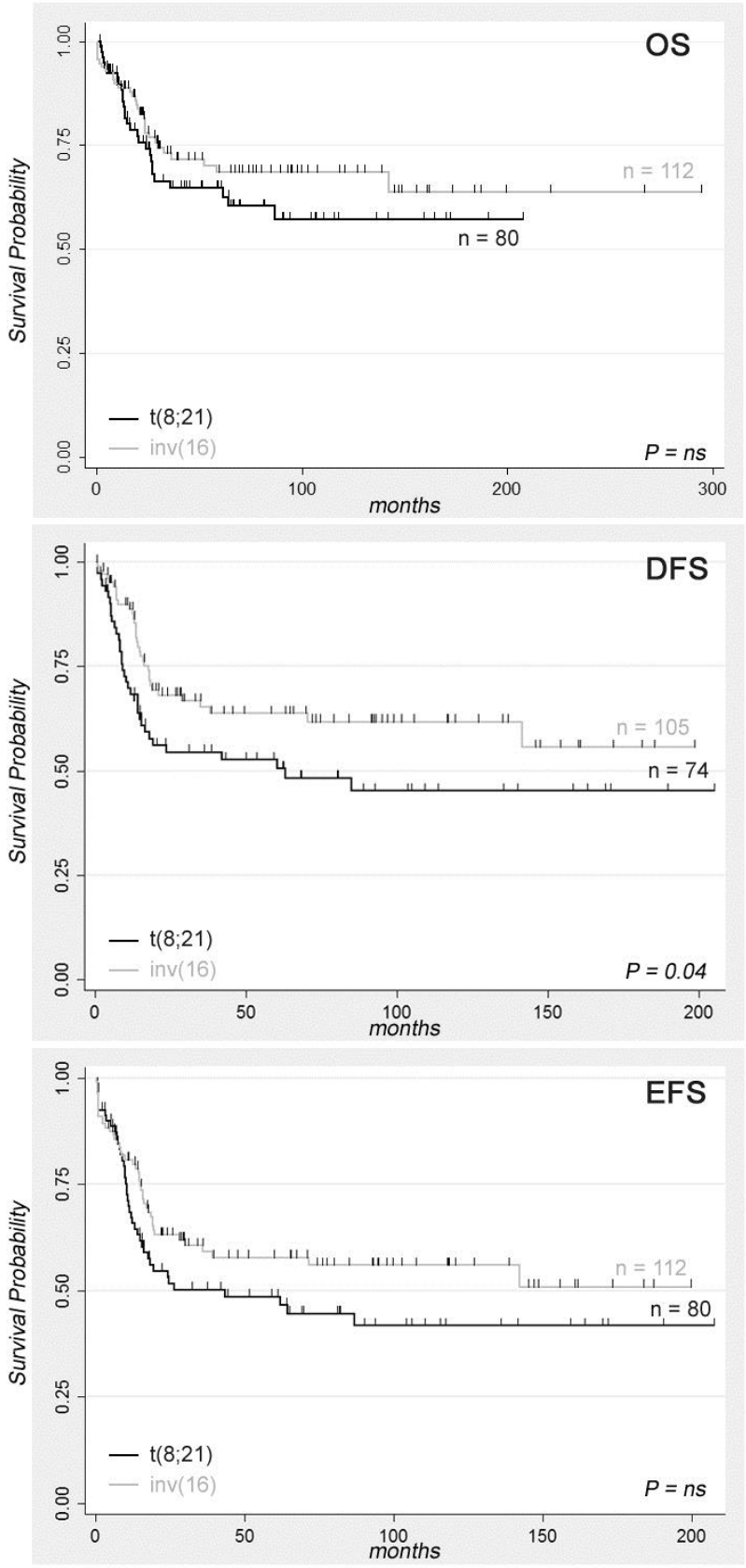
Table 3. Univariate and multivariate proportional hazard modeling for potential factors impacting overall survival.

	RR (95% CI)	*P*	RR (95% CI)	*P*
Age > 60 years	3.05 (1.69-5.51)	< .001	4.52 (2.24-9.12)	< .001
Secondary AML	2.30 (0.98-5.39)	.056		
Male	0.98 (0.58-1.66)	NS		
Splenomegaly	1.02 (0.50-2.08)	NS		
Hepatomegaly	1.13 (0.62-2.07)	NS		
≥ 2 lymph nodes	0.41 (0.15-1.13)	.084		
Extramedullary disease	1.44 (0.68-3.04)	NS		
Granulocytic sarcoma	1.50 (0.47-4.80)	NS		
WBC ≥ $30 \times 10^3/mm^3$	1.07 (0.62-1.84)	NS		
Platelets ≤ $20 \times 10^3/mm^3$	2.24 (1.29-3.91)	.004	1.99 (1.08-3.66)	.027
Elevated LDH	3.60 (1.12-11.57)	.032	3.52 (1.07-11.60)	.038
DIC	0.70 (0.33-1.48)	NS		
t(8;21) vs inv(16)	0.75 (0.45-1.26)	NS		
≥ 3 additional cytogenetic abnormalities	2.58 (1.02-6.49)	.044	1.47 (0.48-4.48)	NS
Presence of subclones	1.15 (0.66-1.98)	NS		
Mutated *KIT*	2.33 (0.61-8.8)	NS		
Mutated *FLT3*	0.95 (0.28-3.17)	NS		
Packed marrow	1.37 (0.79-2.38)	NS		
Failure to achieve CR after induction therapy	6.21 (2.92-13.22)	< .001	5.43 (2.33-12.68)	< .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).

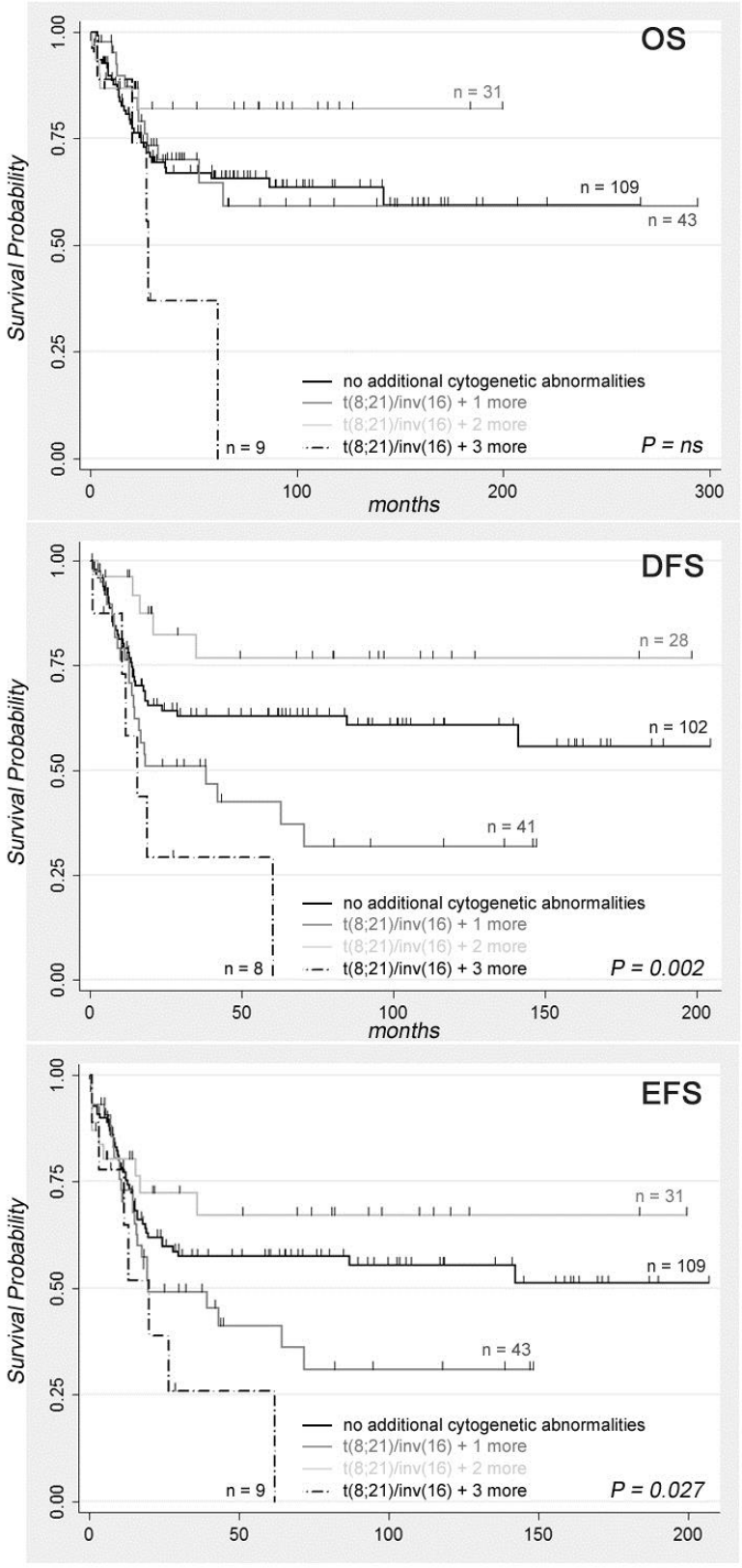
AML, acute myeloid leukemia; CR, complete remission; DIC, disseminated intravascular coagulation; FLT3, fms-like tyrosine kinase 3; LDH, lactate dehydrogenase; NS, nonsignificant; RR, relative risk; WBC, white blood cells.

Figure 1. Survival of patients with t(8;21) and inv(16) AML.



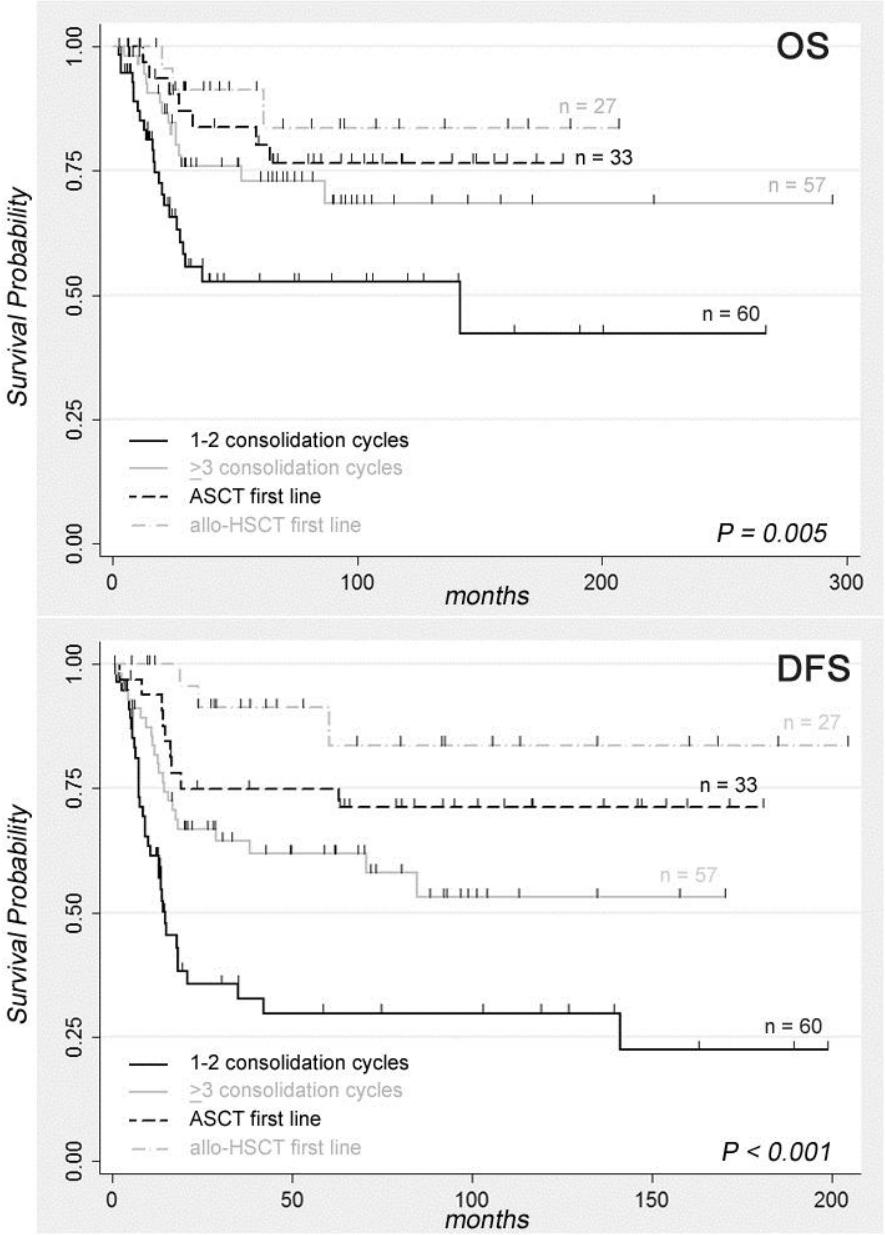
AML, acute myeloid leukemia; DFS, disease-free survival; EFS, event-free survival; ns, nonsignificant; OS, overall survival.

Figure 2. Survival according to additional cytogenetic abnormalities.



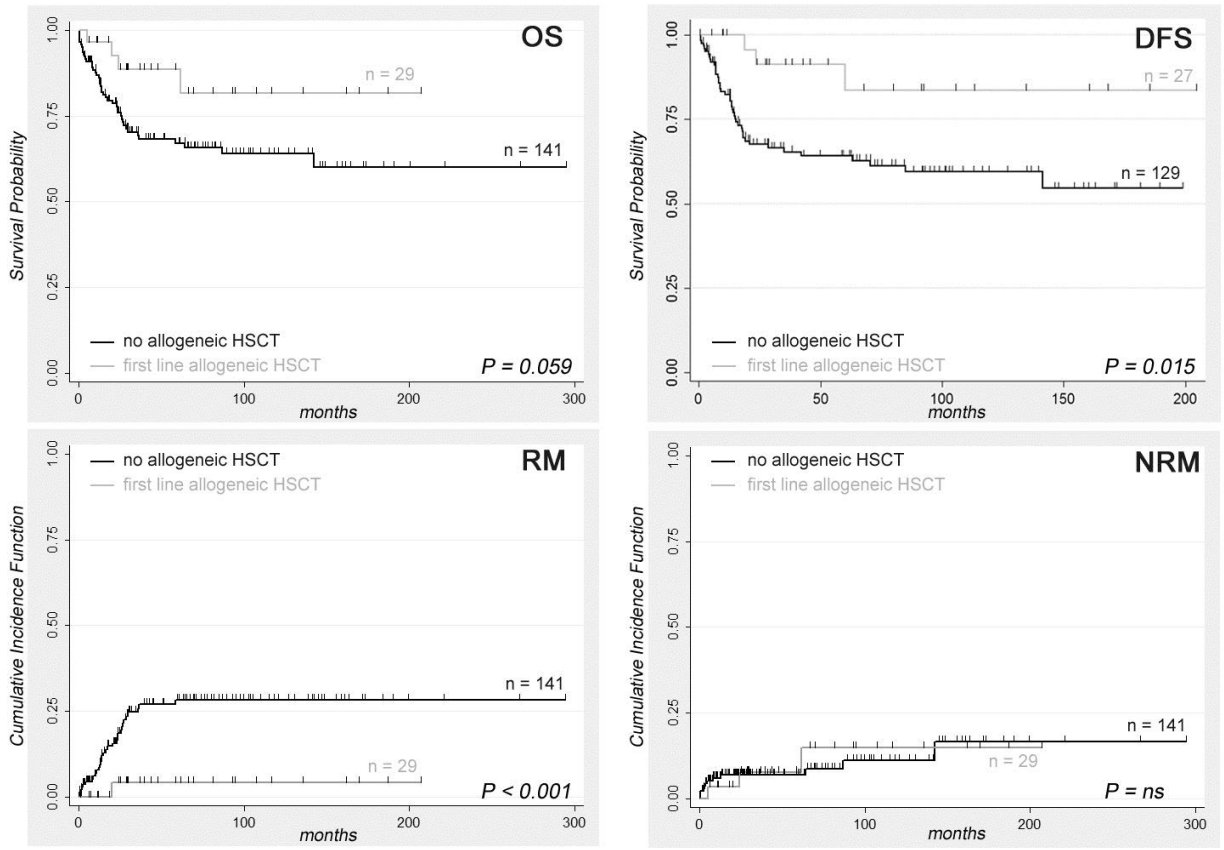
AML, acute myeloid leukemia; DFS, disease-free survival; EFS, event-free survival; ns, nonsignificant; OS, overall survival.

Figure 3. OS and DFS according to dose intensity of first-line treatment.



allo-HSCT, allogeneic hematopoietic stem cell transplant; ASCT, autologous stem cell transplant; DFS, disease-free survival; OS, overall survival.

Figure 4. Survival according to first-line allogeneic HSCT.



DFS, disease-free survival; HSCT, hematopoietic stem cell transplant; NRM, nonrelapse mortality; ns, nonsignificant; OS, overall survival; RM: relapse mortality.

SUPPLEMENTARY TABLES AND FIGURES

Supplementary Table 1. List of additional cytogenetic abnormalities.

Patient #	One additional cytogenetic abnormality
1	46,XY [6]; 46,XY,del(7)(q21q22),inv(16)(p13q22) [14]
2	46,XX [1]; 46,XX,t(8;21)(q22;q22) [2]; 46,t(8;21)(q22q22),del(9)(q?12q?21) [17]
3	45,X, −Y,t(8;21)(q22;q22) [20]
4	46,XY,inv(16)(p13q22) [13]; 47,XY,inv(16)(p13q22),+22 [7]
5	46,XY [1]; 45,X, −Y,t(8;21)(q22;q22) [19]
6	46,XY [1]; 46,XY,del(7)(q32),inv(16)(p13q22) [19]
7	45,X, −Y,t(8;21)(q22q22) [20]
8	47,XX,t(16;16)(p13;q22),+22 [20]
9	46,XY,t(8;21)(q22q22),del(9)(q11) [20]
10	45,X, −Y,t(8;21)(q22q22) [20]
11	45,X, −Y,t(8;21)(q22q22) [20]
12	45,X, −Y,t(8;21)(q22;q22) [20]
13	45,X, −X,t(8;21)(q22q22) [20]
14	46,XY,inv(16)(p13q22) [10]; 47,XY,+8,inv(16)(p13q22) [10]
15	46,XX,inv(11)(p12p15),inv(16)(p13q22) [20]
16	46,XX [4]; 46,XX,inv(16)(p13q22) [12]; 47,XX,inv(16)(p13q22),+22 [4]
17	46,XY,inv(16)(p13q22),del(17q23) [20]
18	45,X, −X,inv(16)(p13q22) [20]
19	45,X, −Y,t(8;21)(q22q22) [18]; 46,XY [2]
20	46,XY [12]; 45,X, −Y,t(8;21)(q22q22) [8]
21	46,XY [8]; 46,XY,t(8;21)(q22;q22) [3]; 46,XY,t(8;21)(q22;q22),del(9)(q13q22) [9]
22	46,XY [1]; 45,X, −Y,t(8;21)(q22;q22) [19]
23	45,X, −X,t(8;21)(q22q22) [19]; 46,XX [1]
24	46,XX [7]; 45,X, −X,t(8;21)(q22q22) [13]
25	46,XX,t(8;21)(q22q22) [19]; 47,XX,+8 [1]
26	46,XX,t(8;21)(q22q22) [19]; 46,XX,t(8;21)(q22q22),add(9)(q34) [1]
27	47,XY,inv(16)(p13,q22),+22 [20]
28	46,XY,inv(16)(p13;q22),t(7;15) [20]
29	46,XX[8]; 47,XX,inv(16)(p13;q22),+22 [12]
30	46,XY,t(8;21)q(22),−del(9)(q24) [20]
31	45,X, −Y,t(8;21)(q22q22) [20]
32	45,X, −Y,t(8;21)(q22q22) [20]
33	46,XY,t(8;21)(q22q22),del(9) [20]
34	46,XX [2]; 45,X, −X,t(8;21)(q22q22) [18]
35	45,X, −X,t(8;21)(q22q22) [20]
36	46,XY,inv(16)(p13q22) [15]; 46,XY,inv(16)(p13q22),+22 [5]

37	45,X, −Y,t(8;21)(q22q22) [20]
38	45,X, −Y,t(8;21)(q22q22) [20]
39	46,XX,inv(16)(p13q22),del16q [20]
40	46,XY [1]; 46,XY,inv(16)(p13q22),+8 [19]
41	46,XY,inv(16)(p13q22) [16]; 46,XY,inv(16)(p13q22), −21 [4]
42	46,XY,inv(16)(p13q22),del(11) [20]
43	46,XX,inv(16)(p13q22),+8 [20]
	Two additional cytogenetic abnormalities
44	46,XX,inv(16)(p13q22) [11]; 47,XX,inv(16)(p13q22),+22[7]; 48,XX,+8,inv(16)(p13q22),+22 [2]
45	45,X, −X,inv(7)(q22q36),t(8;21)(q22;q22) [20]
46	46,XY[3]; 46,XY,inv(16)(p13q22) [15]; 46,XY,inv(16)(p13q22),+19,+22 [5]
47	46,XY[1]; 46,XY,t(8;21)(q22;q22) [11]; 46,XY,del(2)(p21),t(8;21)(q22;q22) [6]; 46,XY,t(8,21)(q22;q22),del(11)(q22;q32) [3]
48	46,XX,t(8;21)(q22q22) [18]; 46,XX, −21,+der(21),t(8;21)(q22q22) [2]
49	46,X, −Y,del(1)(q42),t(8;21)(q22;q22) [20]
50	46,XX,del(X)(q22),t(8;21)(q22q22) [4]; 45,XX, −9,del(X)(q22),t(8;21)(q22q22) [16]
51	46,XY,inv(16)(p13q22),1q+,10q− [20]
52	46XX,t(16;16)(p13q22);add(15)(p13),add(21)(p13) [20]
53	46XY,del(7)(q32),del(16)(q22),t(16;16)(p13q22) [20]
54	45,X,t(8;21)(q22q22),del(Y),+8 [20]
55	46,XY,inv(16)(p13q22),del(16)(q22),t(9;11) [20]
56	45,X,add(7q),t(8;21)(q22q22) [20]
57	46,XX [2]; 46,XX,inv(16)(p13q22) [10]; 46,XX,inv(16)(p13q22),+8,+21 [8]
58	46,XX,inv(16)(p13q22) [2]; 46,XX,+14,inv(16)(p13q22),+21 [18]
59	46,XY,inv(16)(p13q22),+22 [12]; 47,XY,inv(16)(p13q22),+22,t(9;19) [8]
60	46,XX [4]; 45,X,t(8;21)(q22q22),del(9)(q22q34) [16]
61	48,XY,+13,inv(16)(p13q22),+22 [20]
62	46,XY,t(11;12)(q11;11.2),inv(16)(p13q22) [10]; 47,XY,t(11;12)(q11;11.2),inv(16)(p13q22),+22 [10]
63	46,XY [4]; 45X, −Y,t(8;10;21)(q22;p12;q22) [16]
64	45,X, −X,t(8;21)(q22q22),del(9q?) [20]
65	44,X, −X,t(8;21)(q22q22),del(13;14) [20]
66	45,X, −Y,t(8;21)(q22q22),del(9)(q22) [20]
67	46,XX,inv(16)(p13q22),del(7q)(q22q34),amp(11)(q23) [20]
68	46,XX,inv(16)(p13q22) [9]; 47,XX,inv(16)(p13q22),+22 [10]; 48,XX,inv(16)(p13q22),+8,+22 [1]
69	46,XX [4]; 46,XX, −7,inv(16)(p13q22), −22 [16]
70	46,XX,inv(16)(p13q22) [7]; 46,XX,inv(16)(p13q22),+8,t(5;20) [13]
71	46,XX,inv(16)(p13q22) [3]; 46,XX,inv(16)(p13q22),+22 [13]; 46,XX,inv(16)(p13q22),+22,del(7q) [4]
72	48,XX,+8,inv(16)(p13q22),+2 [20]

73	inv(16)(p13q22),+8,+21 [20]
74	46,XY,inv(16)p13q22) [3]; 48,XY,+8,inv(16)p13q22),+21 [17]
	Three additional cytogenetic abnormalities
75	
76	46,XY,t(8;21)(q22q22) [2]; 47,XY,t(8;21)(q22q22),+4 [10]; 49,XY,t(8;21)(q22q22),+4,+6,+19 [8]
77	45,X,t(8;21)(q22q22), −9,+8, −X [20]
78	47,XY,+8,inv(16)p13q22) [9]; 47,XY,+8,t(9;17)(q34q21),inv(16)(p13q22) [8]; 47,XY,+8,add(8)(q24),t(9;17)(q34q21),inv(16)(p13q22) [3]
79	46,XY [3]; 47,XY,del(7)(q32q36),t(16;16)(p13q22),+22 [15]; 47,XY,del(7)(q32q36),t(16;16)(p13q22),+21,+22 [2]
80	46,XX,inv(16)(p13q22) [9]; 47,XX,+8,inv(16)(p13q22) [7]; 47,XX,+8,+11,inv(16)(p13q22) [2]; 47,XX,+3,+8,+11,inv(16)(p13q22) [2]
81	46,XX,t(8;21)(q22q22),del(9)(q22q34),t(10;18)(q22q23) [18]; 47,XX,+X,t(8;21)(q22q22),del(9)(q22q34),t(10;18)(q22q23) [2]
82	46,XX,t(8;21)(q22q22) [16]; 46,XX,t(8;21)(q22q22), −3,add(16)(q23),+21 [4]
83	46,XY [1]; 46,XY,del(7),16−,+22(?) [19]

Detailed karyotype at diagnosis of each patient presenting with additional cytogenetic abnormalities besides t(8;21)(q22q22) or inv(16)(p13q22)/t(16;16)(p13q22). The numbers in square brackets represent the number of observed mitoses bearing the detailed karyotype.

Supplementary Table 2. Molecular data regarding *KIT*, *FLT3*, and *NPM1* status.

	All ***KIT* (n = 59)** ***FLT3* (n = 101)** ***NPM1* (n = 79)**	**t(8;21)** ***KIT* (n = 20)** ***FLT3* (n = 35)** ***NPM1* (n = 32)**	**inv(16)** ***KIT* (n = 39)** ***FLT3* (n = 66)** ***NPM1* (n = 47)**
Mutated *KIT* (D816)	7 (11.8)	3 (15.0)	4 (10.2)
Mutated *FLT3* TKD (D835)	4 (3.9)	2 (5.7)	2 (3.0)
mutated *FLT3* ITD	6 (5.9)	4 (11.4)	2 (3.0)
mutated *NPM1*	2 (2.5)	—	2 (4.2)

Data are presented as n (%).

FLT3, fms-like tyrosine kinase 3; ITD, internal tandem duplication; NPM1, nucleophosmin; TKD, tyrosine kinase domain.

Supplementary Table 3. Patient characteristics according to type of induction course.

	DA37 (n = 25)	More intensive induction therapy (n = 167)	*P*
Age (range), years	39.5 (15-68)	44.3 (15-79)	NS
Patients ≥ 61 years, n (%)	2 (8.0)	24 (14.4)	NS
Male:female ratio	15:10	96:71	
AML type, n (%)			
De novo	23 (92.0)	158 (94.6)	NS
Secondary	2 (8.0)	9 (5.4)	NS
Splenomegaly, n (%)	8 (32.0)	22 (13.2)	.017
Hepatomegaly n (%)	10 (40.0)	31 (18.6)	.016
Lymph nodes n (%)	4 (16.0)	31 (18.6)	NS
Extramedullary disease, n (%)	4 (16.0)	17 (10.2)	NS
Granulocytic sarcoma, n (%)	3 (12.0)	3 (1.8)	.037
WBC (range), × 10^3/mm^3	12.9 (2.2-235.0)	21.4 (1.3-656.0)	NS
WBC ≥ 30 × 10^3/mm^3, n (%)	5 (20.0)	62 (37.1)	NS
WBC ≥ 100 × 10^3/mm^3, n (%)	1 (4.0)	14 (8.3)	
Platelets (range), × 10^3/mm^3	29.0 (7.0-180.0)	38.0 (4.0-586.0)	NS
Platelets ≤ 20 × 10^3/mm^3, n (%)	7 (28.0)	43 (25.7)	NS
Hemoglobin (range), g/dL	8.8 (3.7-12.8)	8.9 (3.1-15.0)	NS
Packed marrow, n (%)	9 (36.0)	79 (47.3)	NS
Elevated LDH, n (%)	19 (76.0)	119 (71.2)	NS
t(8;21):inv(16) ratio	13:12	67:100	NS

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

Supplementary Table 4. Univariate and multivariate proportional hazard modeling for potential factors impacting overall survival—patients with t(8;21) only.

	RR (95% CI)	*P*	RR (95% CI)	*P*
Age > 60 years	4.26 (1.87-9.70)	.001	5.87 (2.31-14.93)	< .001
Secondary AML	2.82 (1.06-7.55)	.039	1.92 (0.66-5.55)	NS
Male	0.72 (0.34-1.52)	NS		
Splenomegaly	0.83 (0.20-3.51)	NS		
Hepatomegaly	0.93 (0.35-2.46)	NS		
≥ 2 lymph nodes	1.04 (0.25-4.43)	NS		
Extramedullary disease	2.21 (0.76-6.41)	NS		
Granulocytic sarcoma	2.38 (0.71-7.90)	NS		
WBC ≥ 30 × 10^3/mm^3	0.95 (0.33-2.76)	NS		
Platelets ≤ 20 × 10^3/mm^3	1.38 (0.60-3.14)	NS		
Elevated LDH	4.94 (0.66-36.82)	NS		
DIC	0.63 (0.19-2.11)	NS		
t(8;21) vs inv(16)	NA	—		
≥ 3 additional cytogenetic abnormalities	2.85 (0.98-8.29)	.055	4.67 (1.43-15.18)	.011
Subclones	1.92 (0.89-4.10)	.092		
Mutated *KIT*	12.52 (1.12-139.33)	.04	Not considered for multivariate analysis	
Mutated *FLT3*	1.51 (0.40-5.71)	NS		
Packed marrow	1.16 (0.53-2.53)	NS		
Failure to achieve CR after induction therapy	5.33 (2.01-14.17)	< .001	9.58 (3.31-27.75)	< .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; NA, not applicable; NS, nonsignificant; RR, relative risk; WBC, white blood cells.

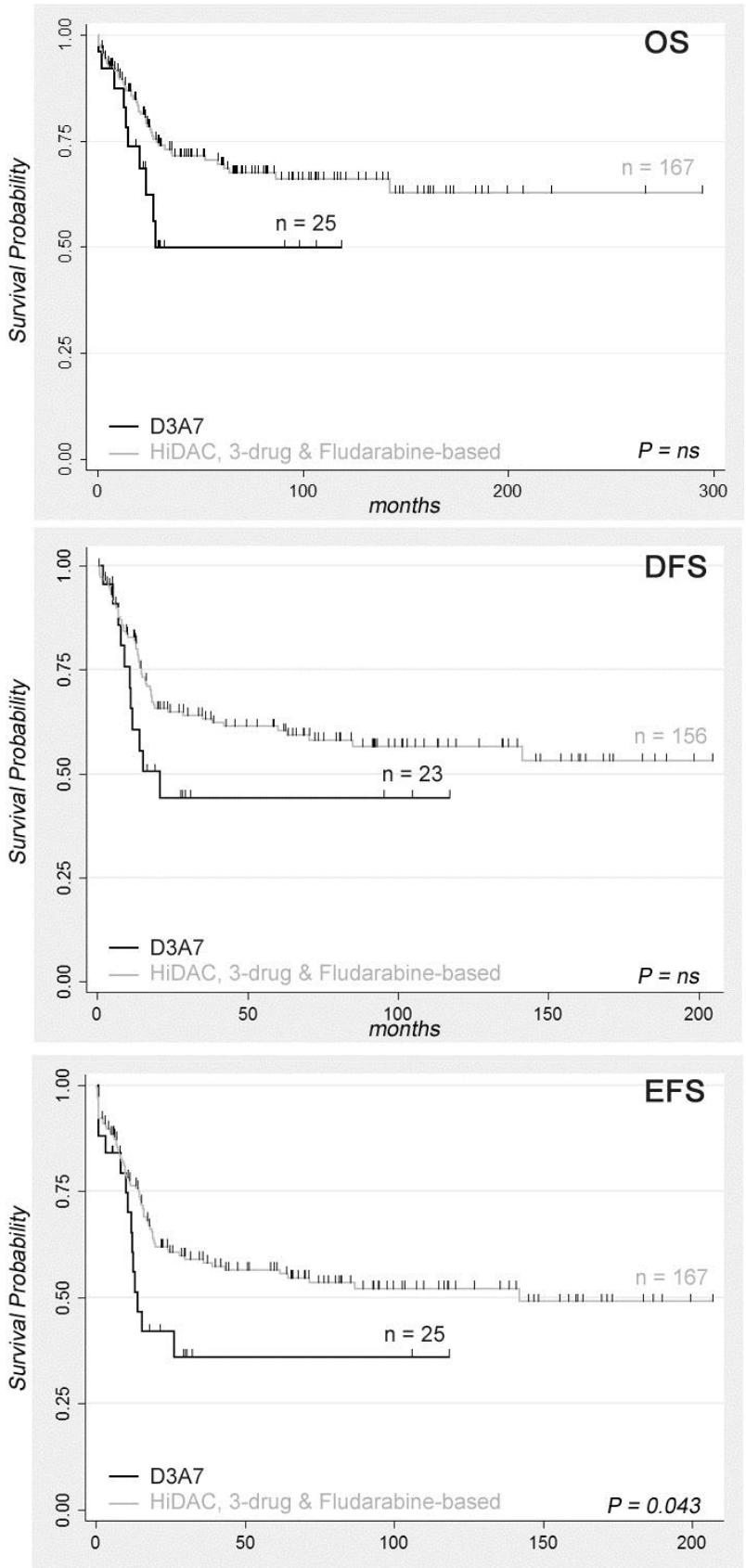
Supplementary Table 5. Univariate and multivariate proportional hazard modeling for potential factors impacting overall survival—patients with inv(16) only.

	RR (95% CI)	*P*	RR (95% CI)	*P*
Age > 60 years	2.32 (0.98-5.47)	.054	3.32 (1.34-8.22)	.009
Secondary AML	1.16 (0.16-8.59)	NS		
Male sex	1.31 (0.61-2.81)	NS		
Splenomegaly	1.21 (0.52-2.84)	NS		
Hepatomegaly	1.33 (0.61-2.93)	NS		
≥ 2 lymph nodes	0.26 (0.06-1.11)	.069		
Extramedullary disease	1.13 (0.39-3.27)	NS		
Granulocytic sarcoma	NA	—		
WBC ≥ 30 × 10^3/mm^3	1.41 (0.67-2.95)	NS		
Platelets ≤ 20 × 10^3/mm^3	3.26 (1.54-6.90)	.002	2.91 (1.28-6.63)	.011
Elevated LDH	2.86 (0.68-12.07)	NS		
DIC	0.77 (0.30-2.03)	NS		
t(8;21) vs inv(16)	NA	—		
≥ 3 additional cytogenetic abnormalities	1.60 (0.21-11.93)	NS		
Subclones	0.73 (0.32-1.64)	NS		
Mutated *KIT*	0.68 (0.08-5.59)	NS		
Mutated *FLT3*	—	—		
Packed marrow	2.01 (0.85-4.79)	NS		
Failure to achieve CR after induction therapy	7.03 (2.09-23.64)	.002	2.46 (0.54-11.12)	NS

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).

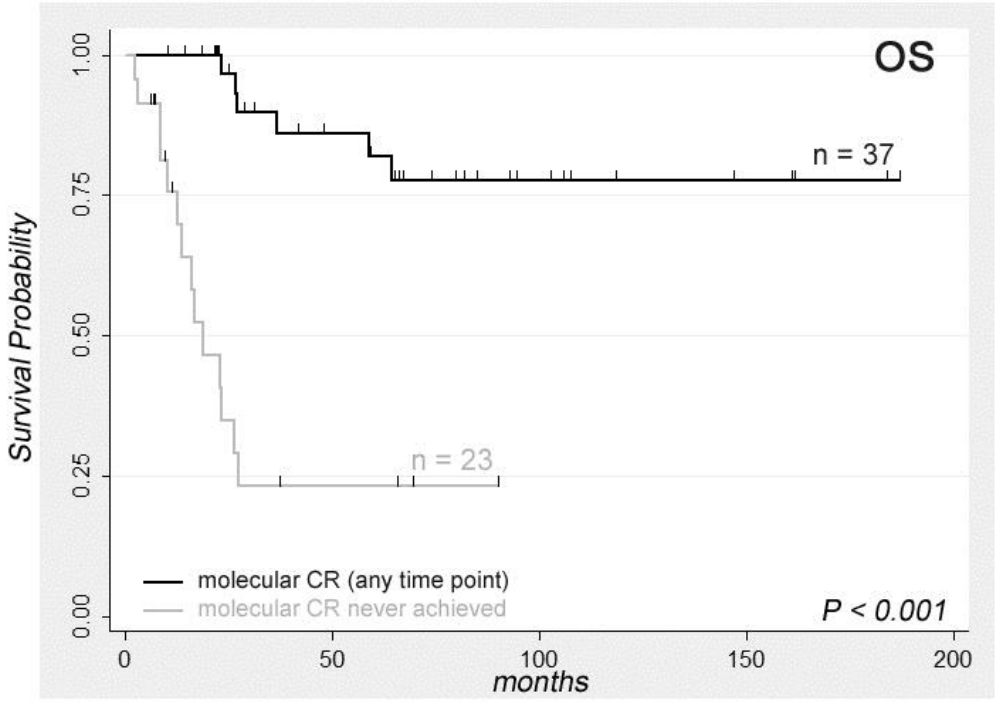
AML, acute myeloid leukemia; CR, complete remission; DIC, disseminated intravascular coagulation; FLT3, fms-like tyrosine kinase 3; LDH, lactate dehydrogenase; NA, not applicable; NS, nonsignificant; RR, relative risk; WBC, white blood cells.

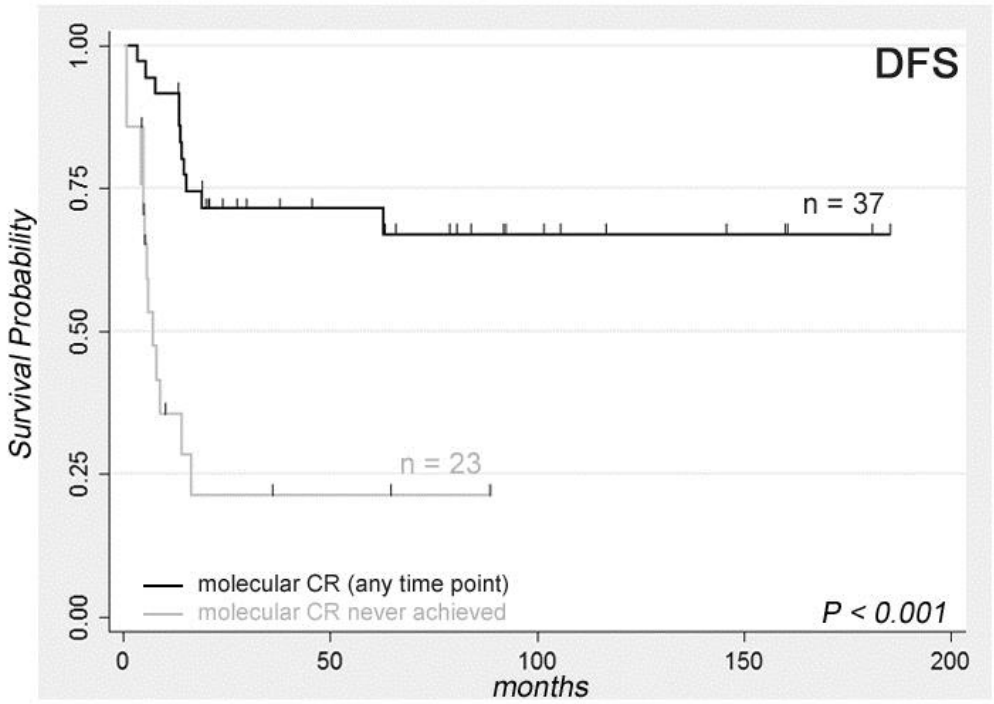
Supplementary Figure 1. Survival according to the type of induction treatment.



DFS, disease-free survival; EFS, event-free survival; HiDAC, high-dose cytarabine; OS, overall survival.

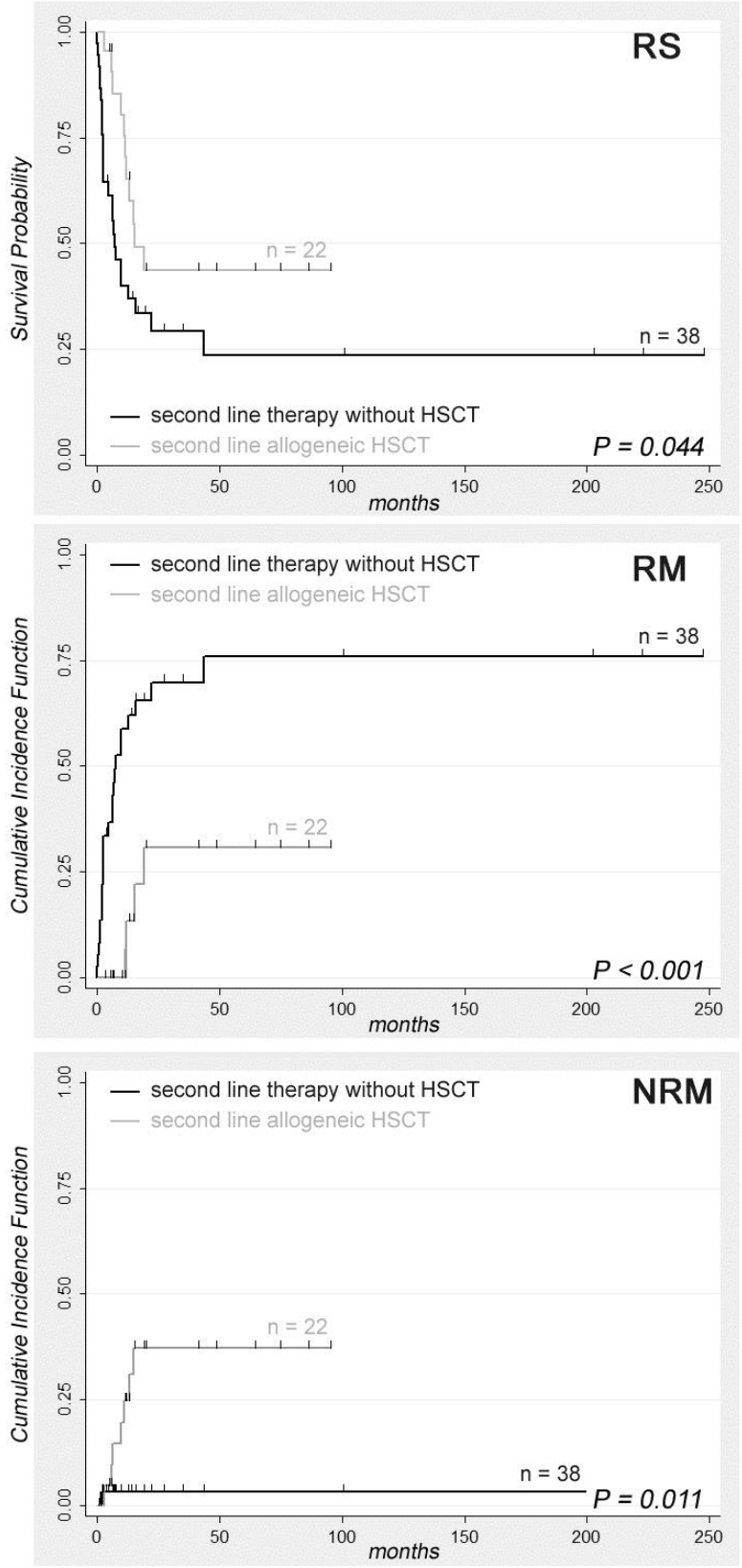
Supplementary Figure 2. OS and DFS according to the achievement of molecular complete remission.





CR, complete remission; DFS, disease-free survival; OS, overall survival.

Supplementary Figure 3. Survival of relapsing patients according to the type of second line treatment.



HSCT, hematopoietic stem cell transplant; NRM, nonrelapse mortality; RM, relapse mortality; RS, relapse survival.