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Long-term survival can be achieved in a significant fraction of older patients with core binding factor acute myeloid leukemia treated with intensive chemotherapy

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Long-term survival can be achieved in a significant fraction of older patients with core binding factor acute myeloid leukemia treated with intensive chemotherapy.

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DATA AVAILABILITY STATEMENT

Data may be available upon contact to the corresponding authors

AUTHOR CONTRIBUTIONS

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Provision of study materials of patients: FM, EB, ML, JCB, CP, CT, FF, GM, RC, EAM, CG, HJD, DC, AC, JRG, ML, SP, FL, SG, FF, AV, MF, EA, PG, GMR, GR, EDB, GV, FA, AKE, PV, GH, GB, NK, MG
Collection and assembly of data: FM, AS
Data analysis and interpretation: FM, AS, MG
Manuscript writing: FM, AS, MG
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Core Binding Factor; elderly; Acute Myeloid Leukemia; chemotherapy; t(8;21); inv(16); real-life; long-term results

ABSTRACT

Acute Myeloid Leukemia is mainly a disease of the elderly: however, the knowledge on the outcomes of treatment in core binding factor AML (CBF-AML) in older population, is limited. We retrospectively collected data on 229 patients with CBF- AML followed long-term in the last two decades.

A 5-year overall survival (OS) of 44.2% (95%CI, 39.9 - 47.5) and a 5-year event – free survival (EFS) of 32.9% (95%CI, 25.5 - 40.1) was observed. In a subgroup of ≥70-year patients who completed intensive therapy (induction + ≥3 courses of consolidation including autologous stem cell transplant: 10 patients) the median EFS was 11.8 months (95%CI, 9.4 – 15.2) and OS was 40.0% (95%CI, 36.4 – 44.1) at 5yr. In univariate analysis, age ≥70 (hazard ratio (HR) 1.78, [95%CI, 1.15 – 2.54], p=.008), failure to achieve remission following induction (HR, 8.96 [95%CI, 5.5 – 13.8], p=<.0001), no consolidation therapy (HR, 0.75 [95%CI, 0.47 – 1.84], p=.04) and less than 3 cycles of consolidation (HR, 1.48 [95%CI, 0.75 – 3.2], p=.0004), predicted poorer EFS. Our study shows that intensive therapy, in selected older CBF-AML patients, leads to longer survival. Achieving a CR seems to be the most important first step and at least 3 cycles of consolidation, an important second one. The analysis suggests that these patients should not be excluded from studies with intensive therapies.

INTRODUCTION

Acute myeloid leukemia (AML) is mainly a disease of older patients, affecting 0.11 patients for every 100.000 inhabitants *per* year overall, but with peak age-adjusted incidence rates ranging from $0.25/10^5$ to $0.28/10^5$ in age groups 65-74 and 75-84[1-3]. Even though intensive chemotherapy (CHT) proves successful in many cases, most patients who enjoy long-term survival are younger. In contrast, historically, treatment outcome in the elderly has been dismal, with less than 20% of patients >65 years old surviving beyond 5 years from diagnosis[4-5].

Older patients do poorly due to both patient and disease associated factors, including a higher incidence of adverse disease features as well as a higher risk of early death because of comorbidities and worse performance status (PS) at diagnosis, a higher incidence of treatment-related toxicities and of infectious complications[6-10]. This historical information can discourage use of intensive treatments in older patients[1,11]. However, this might be changing with advances in the prophylaxis and treatment of infections and in supportive care with several reports yielding increasingly better results in older patients[12-13]. Better understanding of and more objective assessment of patient fitness (frailty assessments, PS and comorbidities) can allow to use more intensive chemotherapy in older patients[14].

More recently, newer drugs with lower toxicity and less infectious risk, such as targeted therapy, hypomethylating agents (HMA) alone or in combination with the Bcl-2 antagonist venetoclax and low-dose chemotherapy in combination

with smoothened (SMO)- and sonic hedgehog (Shh)-signaling inhibitor, have further broadened the treatment choices for patients considered unfit for intensive conventional CHT[15-18]. CBF-AML is defined by the presence of t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22), and consists of 7-15% of AML occurring in younger patients[19-21].

CBF-AML is generally considered relatively favorable, as compared to other types of AML, due to its sensitivity to CHT, particularly at higher doses[22 -26]. These characteristics have resulted in long-term survival in approximately 60-70% of younger (<60 years) patients with CBF-AML, even without allogeneic hematopoietic stem cell transplantation (alloHSCT) in first CR (CR1)[22-23]. The incidence of CBF-AML decreases with age: in fact, it represents less than 5% of AML cases with an age >60yr[27]. Their survival is poorer as, not receiving intensive therapy, relapse is much higher compared to younger CBF-AML: 40% relapse rate in younger *vs* 70-80% in older ones[19,28-32]. Actual data in older patients with CBF-AML are rare and consequently these patients are underrepresented in large-scale studies.[1-19]

In this retrospective, multicenter study, the outcomes of older patients with CBF-AML were evaluated to reflect a real-life setting in the recent era.

METHODS

We retrospectively reviewed 251 patients (≥60 years old), diagnosed with either t(8;21)(q22;q22) or inv(16)(p13q22)/t(16;16)(p13;q22) between January 2000 to June 2019, who received intensive induction chemotherapy. Minimal

required follow-up was 6 months in surviving patients. Twenty-two patients were excluded due to incomplete data regarding therapy or follow-up: therefore, the final analysis included 229 patients (**Figure 1**).

The Italian group conceived the concept of study, collected the data from participating centers unanimously and homogeneously with pre-determined variables. Consent to collect and use the medical records was obtained by local human research boards and the study was approved by Institutional Review Board of Veneto Institute of Oncology (IOV).

AML was defined by the 2008 World Health Organization (WHO) diagnostic criteria[33] and updated versions. Fitness criteria for treatment with curative intent differed significantly among centers and years of treatment, as no universal standard was available[10]. All patients underwent a thorough physical and laboratory evaluation of comorbidities and organ functions per each institution's clinical practice.

Primary CBF designation was identified in all patients by means of either karyotype, FISH or molecular genetic testing. In molecular analysis, other common mutations in *KIT*, *FLT3*, *NPM1,* and *RAS* at diagnosis were collected when available.

Definitions: 1) **Complex karyotype**: $\geq$3 additional cytogenetic abnormalities; 2) **Renal dysfunction**: serum creatinine $\geq$2x institutional ULN and/or creatinine clearance ≤ 50 mL/min; 3) **Complete remission**: bone marrow blasts <5%; absence of circulating blasts; absence of extramedullary disease; 4) **High risk patient**: high risk of relapse because of adverse clinical or

laboratory findings at diagnosis (hyperleukocytosis: $\geq 80x10^6$/L white blood cells (WBC); extensive hepatic and/or splenic infiltration); failing to achieve CR after the first course of CHT; persistence of molecular transcripts at the end of planned consolidation.

First line treatment

The induction regimens were highly heterogeneous but could be categorized in 3 major groups according to drug types and schedule: 1) “3+7 regimens” = “anthracycline plus cytosine arabinoside”; 2) “3+7 like regimens” = “3+7 plus other drugs”; 3) “No anthracycline based regimen”. These regimens are described in detail in the *Supplementary Data* section.

Following induction therapy, patients achieving CR received consolidation (at least 1 cycle: range 1-5) or maintenance treatment. Administered consolidation was also heterogeneous but mainly consisted of cytarabine at $9g/m^2$ (IDAC) or $18g/m^2$ (HiDAC) based CHT. Autologous stem cell transplantation (autoHSCT) performed in first CR (7 patients) was considered part of consolidation treatment. Of note, no patient underwent autoHSCT after 2009.

All but 4 patients were treatment naïve before intensive induction CHT: these 4 patients received only 1 cycle of HMA with no response. We purposefully omitted patients treated with multiple courses of HMA.

Hematopoietic Stem Cell Transplantation

After induction and consolidation, allogeneic HSCT (alloHSCT) (15 patients) was implemented (as above mentioned): in patients considered at high risk of

relapse (adverse clinical or laboratory findings at diagnosis) and in patients failing to achieve CR after the first course of CHT (**Figure 1**). A reduced-intensity conditioning (RIC) was used in all cases.

STATISTICAL ANALYSIS (all revised)

Study End Points

Primary end-points: 1) objective response rate (ORR) defined as the percentage of patients achieving a CR following induction therapy; 2) overall and event free survival.

Secondary end-points: analysis of 1) biological and clinical baseline factors potentially impacting primary end-points; 2) the impact on survival of therapeutic choices (induction and consolidation) made by the physicians during treatment; 3) role of age in defining differences among the patient series using 70 years old as cutoff.

Statistics

According to the nature of the variables, Fisher exact and Pearson χ^2 tests were used to test the differences in proportions, the Mann-Whitney and two-way Student *t* tests to compare nonparametric and parametric variables, respectively. The hypothesis of normal distribution was tested by the Shapiro-Wilk. Differences were considered statistically significant for $p \leq .05$.

The impact of age and type of induction therapy on the achievement of ORR and on 30 and 60 - day mortality was tested by means of the Pearson χ^2 tests

on actual results. Odds ratio (OR) and 95%CI were estimated with a logistic regression model.

In survival analysis, overall survival (OS) was defined as the time from diagnosis to death or last follow-up; disease-free survival (DFS) was defined as the time from achievement of CR until relapse of leukemia, death or last follow-up; event free survival (EFS) was defined as the time from diagnosis to any adverse event or last follow-up. Survival functions were calculated according to the Kaplan&Meier method, and differences tested by the log-rank test. The impact of single baseline covariates was tested in univariate analysis by Cox proportional hazard modeling and results confronted by calculating the HR and corresponding 95%CI. We then chose to carry out multivariate analysis on event free survival using factors resulting in $p \leq .05$ at univariate modeling.

Relapse mortality (RM) was defined as death due to leukemia relapse, and non-relapse mortality (NRM) as death from any other cause in the absence of leukemia. Both were used as mutually exclusive competing events in the survival analysis. Cumulative incidence functions were compared using the Grey test.

To test the effects of alloHSCT, we used a Mantel-Byar approach treating alloHSCT as a time-varying covariate.

All analyses were performed by the Stata IC v.14.2 platform by StataCorp (College Station, TX).

RESULTS

Patients

Patients' characteristics are shown in **Table 1**. The study included 229 evaluable patients, aged 60.0 - 84.4 yr (median: 66.2 yr). Twenty-two patients (9.8%) had secondary AML (of whom 14 had prior myelodysplastic syndromes (MDS), the other 8 were t-AML). Complete karyotype analysis was available at diagnosis for 115 patients (50.2%) (**Tables 1 and 2**): 34/115 (29.6%) had additional cytogenetic abnormalities other than the characteristic CBF translocations, and 7/115 (6.1%) presented a complex karyotype. Patient characteristics were similar between t(8;21) and inv(16) AML patients, except for a higher WBC count at diagnosis in the case of inv(16) patients (39.5 x 10^6/L vs. 20.6 x 10^6/L, p= .002) (**Table 1**). For the study, we divided the whole series in 2 groups according to increasing age, using 70 yrs as cut-off. There were 59 patients above the age of 70 who were treated with intensive induction CHT and multiple consolidation cycles or rescue CHT (**Table 2**). No statistically significant differences in the main biological and clinical variables among age groups were observed (**Table 2**).

Induction treatment

Induction treatment is summarized in **Table 3**. Induction including anthracycline was the most frequently used regimens (201 patients – 87.8%) (**Table 3**). A statistically significant difference in the use of anthracycline during induction was observed among age groups: more than 90.0% of patients <70 years (155 – 91.1%) were treated with anthracycline compared to

less than 80.0% in the older group (46 – 77.8%) (OR, 0.75 [95%CI 0.47 – 1.84], p=.04) (**Table 3**). The more common use of induction without anthracycline in older patients (22.2% vs 8.9%) (OR, 0.75 [95%CI 0.47 – 1.84], p=.04) was likely due to fear of increased cardiac toxicity. Impressively, 193 patients achieved CR (84.3%) with no difference in CR rate was observed between younger and older patients (146 – 85.8% vs 46 – 78.0%) (OR, 1.34 [95%CI 0.87 – 2.84], p=.204), (**Table 3**). The type of induction treatment affected CR rates in the whole series (from 86.0% to 67.9%) with a statistically significant difference in favor of anthracycline based regimens (OR, 0.94 [95%CI 0.87 – 1.85], p=.03) **(Table 4a).** The trend in favor of anthracyclines use was maintained for older patients (OR, 1.4 [95%CI 0.47 – 1.84, p=.04) (**Table 4c**). Overall, 30 and 60-day mortality were 3.5% (n= 8) and 6.6% (n= 15) respectively, with no statistical difference between the younger and older group: 30-day mortality: 5 (2.9%) vs 3 (5.1%) (OR, 0.55 [95%CI 0.47 – 1.14], p=.428) and 60-day mortality: 9 (5.3%) vs 6 (10.2%) (OR, 1.55 [95%CI 0.75 – 3.14], p=.223) (**Table 3**).

Consolidation and transplantation

One hundred sixty – nine (87.5%) patients who achieved CR received consolidation (**Figure 1 and Table 3**), without a statistically significant difference between age groups: 132 (89.7%) patients <70 yrs vs 37 (80.4%) of older ones (OR, 1.8 [95%CI 1.2-2.8], p=.7). While no significant difference in number of patients consolidated with 1-2 cycles was observed between age groups (45.7% vs 58.7% younger vs older patients respectively), as expected,

only 19.6% of older patients were treated with multiple (≥3) courses as compared to 32.0% of patients <70yrs yrs, though not statistically significantly different (OR, 0.8 [95%CI 0.4 – 1.8], p=.076). Consolidation therapy in CR included alloHSCT in 12 patients. All patients who underwent transplant (except 3) were age ≤70yrs. Reasons to proceed to transplant are listed in the ***Patients and Methods*** **section**. Individual characteristics and clinical history of patients undergoing transplant as part of first-line treatment is depicted in the **Supplementary Table 1 and 2**. Twenty-four patients (12.4%) who achieved CR but did not receive intensive consolidation (CHT and/or transplant) received maintenance right after induction with various agents, including low-dose cytarabine (LDAC, n= 20), azacytidine (n= 3) and NK-cell infusions (n= 1) (**Figure 1**).

Overall – Disease and Event Free Survival

As of December 2019, 103 patients (45%) are still alive, with a median follow-up of 53.5 months (range: 6-181 months). One hundred and twenty-six patients (55.0%) have died, in most cases from leukemia relapse/progression (87 patients - 69.0%).

Survival analysis was performed excluding patients with complex karyotype (7 patients), as it was already shown these patients do not benefit from the better survival associated to CBF-AML and therefore do not represent CBF-AML prognosis.

The OS was 50.2% (95%CI, 44.2 – 56.5) at 3yr, 45.0% (95%CI, 41.3 – 49.2) at 5yr and 36.7% (95%CI, 30.2 – 42.4) at 10yr (**Figure 2A**). As expected, no difference in OS was observed between patients treated in US and European Centers: 5yr OS 51.5% (95%CI, 46.7-59.1) vs 43.2% (95%CI, 37.3 - 49.2), (HR, 0.67 [95%CI, 1.4- 2.0], p=.53). We also analyzed survival according to years of treatment, observing a statistically significantly difference: treatment years 2000 – 2009 vs 2010 – 2019: 3yrOS 47.1% (95%CI, 42.3-51.4) vs 54.7% (95%CI, 49.9 – 58.4) (HR, 1.28 [95%CI, 1.01- 1.63], p=.042). No difference in survival was observed for secondary CBF-AML: 3yrOS 50.0% (95%CI, 45.6 – 53.4) vs 48.0% (95%CI, 41.7-55.1), (HR, 1.3 [95%CI, 0.8-2.81], p=.2), de novo vs secondary CBF-AML, respectively.

The older patients experienced worse OS ranging from 5yrOS of 48.5% (95%CI, 41.9-52.1) for patients <70 years versus 33.2% (95%CI, 28.1-36.4) for >70 years (HR, 1.6 [95%CI 1.2-1.9], p= .01) (**Figure 2b**). NRM also did not significantly differ among age groups: 25.1% (95%CI, 21.4-29.3) for patients <70yr vs 27.2% (95%CI, 22.5-31.4) for older ones (HR, 0.7 [95%CI, 0.3-1.7], p=.472). However, higher leukemia-specific mortality with increasing age emerged from the analysis: 37.5% (95%CI, 31.4- 43.1) younger patients vs 51.5% (95%CI, 47.8 – 56.4) for patients >70yrs (HR, 1.1 [0.6-2.1], with a p= .053). There was no significant difference in OS between CBF subtypes: t(8;21) AML experienced a 3yr OS of 49.1% (95%CI, 45.4 – 53.5) and inv(16) 51.2% (95%CI, 48.3 – 55.4) (HR, 0.87 [95%CI, 1.1- 2.1], p= .427) (**Figure 2c**).

For the entire series, a 3yr and 5yr EFS of 36.9% (95%CI, 32.1 – 41.1) and 33.3% (95%CI, 29.2 – 37.1) respectively was observed (data not shown), with no differences between t(8;21) and inv(16) AML (HR, 0.77 [95%CI, 0.4- 1.7], p= .64) (**Figure 2c**).

Achieving CR after induction therapy was the main factor affecting survival. Overall, a high CR rate following induction (84.3% - **Table 3**) emerged from the analysis, regardless of age (OR 1.34 [95%CI 0.87 – 2.84], p=.204) (**Table 3**) and CBF subtypes (*data not shown*).

Overall, for the entire series, we observe a 3yr, 5yr and 10yr EFS of 37.5% (95%CI, 34.2 – 41.5), 34.7% (95%CI, 30.1 – 39.2) and 26.9% (95%CI, 20.2 – 31.4) respectively, again with no differences between t(8;21) and inv(16) AML (**Figure 2c**).

Interesting, an improving EFS emerged when analyzing patients according to overall dose intensity (excluding patients submitted to allo-HSCT) with a 3yr and 5yr EFS of 23.5% (95%CI, 19.9 – 27.1) and 19.5% (95%CI, 15.7 – 21.1) (without consolidation) to 29.6% (95%CI 26.1 – 33.4) and 26.5% (95%CI, 22.4 – 29.9) (1-2 consolidation courses) to 55.0% (95%CI, 52.3 – 59.1) and 52.5% (95%CI, 47.8 – 56.4) ($\geq$3 consolidation courses) (OR 9.34 [95%CI 5.87 – 14.84], p=<.00001) (**Figure 3a**).

The same trend could be observed in both younger and older patients: patients <70-year experienced 5yr EFS ranging from 19.0% (95%CI, 13.1 – 23.4) to 49.0% (95%CI, 44.9 – 53.5) from no consolidation compared to $\geq$3

consolidation cycles (OR 7.64 [95%CI 3.85 – 13.64], p=.00001) (**Figure 3b**). Patients >70 year had slightly inferior 5yr EFS, ranging from 10.8% (95%CI, 7.8 – 13.6) (no consolidation) to 40.0% (95%CI, 34.8 – 44.1) (≥3 cycles), the latter with a median EFS of 11.8 months: 10 patients (**Figure 3b**). Concerning patients in CR after induction, analyzing the intensity of consolidation treatment among age groups (omitting allo-HSCT), a nonstatistical but interesting trend (OR 0.8 [95%CI 0.4 – 1.8], p=.076) was still observed towards lower number of cycles for older patients: 9/46 (19.6%) *vs* 47/147 (32.0%) for the group of patients receiving ≥3 consolidation courses (**Table 3**).

Rescue therapy after induction failure or relapse.

Thirty-six patients did not achieve CR after induction: 4 (1.7%) died during the first cycle. Sixteen of the remaining 32 patients (50.0%) died with progression without further therapy; 14 (43.8%) received rescue CHT and 8 of these (57.1%) achieved CR: the last 2 patients went to transplant without CHT before (**Figure 1**). Overall, 3 patients received alloHSCT: 1 after rescue CHT and in CR, 2 as rescue up-front without bridge therapy. (**Supplementary Table 2 and Figure 1**). In the whole series, 110 (48.0%) patients relapsed, with no difference between t(8;21) and inv(16) AML. Median DFS was 20.8 (95%CI, 18.1 – 23.8) vs 16.3 months (95%CI, 13.4 – 20.2) for t(8;21) and inv(16) AML, respectively (HR, 0.95 [95%CI, 0.4 – 19.], p=.754). (*Data not shown*). Overall, 78 out of 110 patients (70.1%) received rescue treatment, and 58 of them (74.3%) achieved second CR (CR2). Inv(16) AML showed a

nonstatistical trend towards a better chance to achieve CR2 (39 of 44 patients (88.6%) for inv(16) AML vs 19 of 26 (73.1%) for t(8;21) AML (p=.095). Second line alloHSCT was the treatment of choice in all patients deemed fit for transplant. All the 25 transplanted patients in this group (25 out of 78 patients, 32.0%) were rescued with CHT before transplant. An impressive advantage in survival was observed for transplant *vs* conventional CHT: 3yr and 5yrOS of 62.7% (95%CI, 58.4 – 65.3) and 57.9% (95%CI, 54.4 – 60.2) *vs* 29.3% (95%CI, 26.4 – 32.5) and 19.3% (95%CI, 16.7 – 22.4) (HR, 1.9 [95%CI, 1.4 – 2.1], p=.001) (**Supplementary Figure 1**), transplant vs no transplant, respectively**.** Three (12.0%) transplanted patients died of progression; all the others (8 – 32.0%) from toxicity. The median age of transplanted patients was 64.1yr (range: 60.7 – 74.8), with 3 patients over the age of 70 yr and 12 over the age of 65 yr.

Univariate and Multivariate analysis

In the univariate analysis for EFS (**Table 5**), age (≥70 yrs), (HR, 1.78 [95%CI, 1.15 - 2.54], p= .008), failure to achieve CR following induction (HR, 8.96 [95%CI, 5.40-13.18], p =<.0001), absence of consolidation therapy (HR, 0.75 [95%CI, 0.47-1.84], p =.04) and <3 consolidation courses (including autoHSCT) (HR, 9.57 [95%CI, 5.6 – 13.4], p =.<.001) identified patients at higher risk for poor survival. Approaching statistical significance for a better EFS was also anthracycline included in the induction course (HR, 1.06 [95%CI 0.14-2.08], p =.05).

In multivariate analysis failure to achieve CR following induction (HR, 8.99 [95%CI, 3.78-16.66], p =<.001) and less than 3 consolidation courses (HR, 7.99 [95%CI, 3.18-15.7], p =<.001) remained independent predictors of poorer EFS (**Table 5**).

Univariate analysis for EFS according to cytogenetic and molecular biology (mutated FLT3-ITD and TKD; NPM1, N-RAS and K-RAS, KIT - D816V) was performed only for patients with available data: only the presence of mutated KIT – D816V at diagnosis identified patients with poor EFS (HR, 6.85 [95%CI, 3.42-11.71], p=.04) (**Supplementary Table 3** - Supplementary Material).

DISCUSSION

In our study with 229 patients with long-term follow-up, intensive induction and consolidation, provided long-term EFS with low TRM. During induction, we observed that older patients with CBF-AML respond favorably to intensive treatment with anthracyclines resulting in high CR rates. Moreover, achievement of CR was one of the most important factors in providing long-term EFS in univariate as well as multivariate analysis.

In terms of consolidations, our study showed that 3 or more consolidation courses (in 7 cases including autoHSCT) were associated with longer EFS. Previously published studies have shown that approximately 60% of older patients could not get 3 or more cycles of consolidations[28, 34-40] but, when received, were associated with a longer survival.[40] These outcomes may be due to general improvements in the treatment options for elderly AML patients[16-18, 34-42] and to a reduction of TRM following induction CHT.[28,35] It can also represent the biological features of CBF-AML, where the high sensitivity of blasts and of leukemic stem cells (LSC) to CHT provides better results[36]. Some other studies also demonstrated that CBF-AML patients can achieve long-term remission and functional cure after a fixed-term CHT also at advanced age. [2, 40-41]

Hypomethylating agents (HMA) with Bcl2-antagonists (venetoclax – VEN), have been increasingly used in older patients with AML, or in patients with medical conditions that prevent the use of standard intensive CHT. These agents are considered less toxic than conventional CHT, present lower 28-day TRM, are very active in favorable-risk AML and have provided a survival

advantage as compared to other treatments in registration trials as well as real-life experience[16-17]. Nevertheless, they have been tested only in a population of older patients defined as unfit for intensive CHT when applying currently available fitness scores. Furthermore, even though results in this unfit setting have been consistently better than low-to-intermediate CHT[16], especially in the setting of upfront treatment[17,37-38] and adverse cytogenetics[37,39], they still appear as a non-curative approach, requiring continuous treatment until disease progression, while long-lasting remissions are rare. In the setting of CBF-AML there are limited and conflicting data about the efficacy of HMA plus VEN since CBF-AML have been excluded from most VEN-based studies. Zhang K et al reported the largest series (30 patients) concerning HMA + VEN in CBF-AML. The median duration of follow-up for the entire cohort was 11.6 months and the 2-year probability of OS was 92.2%, with a trend toward a better 2-year OS for patients with inv(16)/t(16;16) vs t(8;21) (100% vs 81.5% - p=.09)[43]. Hang Y et al reported 58 consecutive patients with CBF-AML treated with intensive CHT (49 patients) or HMA + VEN (9 patients) showing a superior outcome for patients treated with intensive CHT (CR rate 25.0% vs 91.3% respectively- p=.007)[44]. Results confirmed by Zhou Y et al: 106 CBF-AML treated with various regimen of intensive CHT (97 patients) or HMA + VEN (9 patients) with a median EFS ranging from 7.9 months to 10.9 months in the intensive CHT groups vs 3.5 months in the HMA+VEN group[45].

In our analysis, we find that the presence of high tumor burden (high WBC count or hepatosplenic involvement) had no impact on CR achievement and survival (*data not shown*), as has been reported by others[19,25-26,40-41,46].

Relapses have been reported to occur in up to 35.0% of any aged CBF-AML patients[49]. In our series, including only an older population, 110 (48.0%) patients relapsed, with no difference between t(8;21) and inv(16). Overall, 78/110 relapsed patients (70.1%) received rescue treatment, with a high rate of second CR (CR2= 74.3%) achieved. Inv(16) AML showed a nonstatistical trend towards a better chance to achieve CR2: 88.6% (inv16) vs 73.1% for t(8;21) AML (p=.095).

There is no established salvage treatment for relapsed CBF – AML patients. In a retrospective analysis from the French AML Intergroup concerning 145 patients in first relapse, patients who received Gemtuzumab Ozogamycin (GO)-based CHT followed by alloHSCT had a significant higher 5yrDFS (83.0%) than those who received conventional CHT (44.0%) p=.01. It was concluded that GO combined with CHT and transplant is safe and efficient[48]. This study has some limitations due to inadequate number of patients to assess the efficacy and safety of GO upfront or in relapsed setting.

In our series only 4 patients received GO induction based-CHT: they were all treated after 2017, when GO received FDA reapproval for the first time in newly diagnosed AML patients. A meta-analysis of five randomized trials showed that the addition of GO to remission induction therapy improved survival in CBF-AML, with an absolute survival benefit of 20.7% (OR 0.47,

0.31-0.73; p=.0006)[49]. Borthakur et al reported 3-year OS and RFS rates of 85.0% and 78.0% respectively in a study of FLAG-GO in CBF – AML[50], concluding that the incorporation of GO into the remission induction should be considered standard for CBF-AML[51]. Our series included patients treated from 2000 to 2019 and this could explain why only 4 patients were treated with GO: from 2000 to 2010 GO had first FDA approval for relapsed AML patients not eligible for intensive CHT; only in the second FDA approval (2017) newly diagnosed CD33 positive AML patients were included. Furthermore, the use of GO in elderly patients is limited due to fear of drug-related toxicity. In older patients, this could probably be modulated by reducing the drug dose and/or the number of GO administrations.

We observed an impressive advantage in survival for patients in second CR (78 patients) who underwent alloHSCT (25 patients) *vs* those rescued only with conventional CHT (53 patients). These data regarding transplant in second line confirm the feasibility and efficacy of the procedure also in older patients. Despite that, transplant in older patients is not widely used: worldwide, fewer than one-third of patients aged 60-70 ever undergo a transplant and only 7.0% of those age 70-75 are transplanted. Considering the constant progress in transplant technologies over the last two decades a more careful evaluation of patient's fitness is needed to not exclude patients for a transplant procedure solely based on an age above 65 years.

We acknowledge several limitations of our study: first regarding molecular data that were incomplete, as the range of depth of analysis now allowed by

Next-Generation Genomics was unavailable at the time of treatment, and in the case of established markers such as KIT, FLT3, NPM1 and RAS, data were related to more recent years. Second, MRD monitoring, a cornerstone in the current treatment of CBF-AML, was available only in a minority of the patients of our series, not allowing conclusion regarding its predictivity in term of OS, as reported in literature.

Nevertheless, it is unlikely that a prospective study will ever be made on this topic, and the consistency of the chemotherapeutic regimens applied over the last two decades in the treatment of AML, as well as the size of our final database and the very long follow-up at our disposal, still enabled us to draw consistent long-term conclusions from the results.

In conclusion, due to the “biological fitness” related to the high chemosensitivity of CBF blast and LSC, data emerging from our analysis indicates that patients with CBF-AML can be intensively treated, with a significant chance of cure, up to a very advanced age, also including, in selected cases, alloHSCT when indicated, without a high rate of toxicity. Although HMA + VEN combinations appear to be very effective and safe treatments, currently it is unknown what is the optimal therapy for a patient, given the lack of prospective comparisons in CBF-AML. The risk and benefit of each approach should be tailored for an individual patient; however, our study clearly shows that intensive therapies should not be excluded from patients with CBF-AML, based solely on age.

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Table 1. Characteristics according to Core Binding Factor translocation.

	All patients ***n= 229***	**t(8;21)** ***n= 92***	**inv(16)** ***n= 137***	***P***
Age (years) *median (range)*	**66.9 (60-84.4)**	67.0 (60-84.4)	66.8 (60-79.6)	*.467*
Patients > 70years *n (%)*	**61 (26.6)**	23 (25)	38 (27.7)	*.698*
Male – Female *number*	**115 – 114**	49 – 43	66 – 71	*.329*
AML type	**Number (%)**			
De novo	**184 (81.8)**	74 (80.5)	110 (80.3)	
Secondary **Therapy-related**	**22 (9.8)** **19 (8.4)**	10 (10.8) 8 (8.7)	12 (8.7) 11 (8.0)	*.892*
Granulocytic sarcoma	**2/102 (1.9)**	1 (1.1)	1 (0.7)	*NA*
WBCx10^9/l *(range)*	**31.9 (0.5-417.0)**	20.6 (1.2-417.0)	39.5 (0.5-270.0)	*.002*
PB Blasts > 30%	**19/126 (15.1)**	3/53 (5.7)	16/73 (21.9)	*.006*
PB Blasts > 80%	**7/126 (5.6)**	1/53 (1.9)	6/73 (8.2)	*.080*
PLT x 10^9/l *(range)*	**56.1 (2.0-419.0)**	50.7 (5.0-331.0)	59.7 (2.0-419.0)	*.501*
PLT< 20x10^9/l	**36/175 (20.6)**	15/69 (21.7)	21/106 (19.8)	*.967*
Hb g/dL *(range)*	**8.6 (3.1-13.3)**	8.7 (4.9-12.8)	8.4 (3.1-13.3)	*.349*
Blast in marrow>80%	**29/116 (25)**	9/46 (19.6)	20/70 (28.6)	*.401*
Elevated LDH	**73/100 (73)**	26/40 (65.0)	47/60 (78.3)	*.170*
Additional cytogenetic abnormalities	**34/115 (29.6)**	10/46 (21.7)	24/69 (34.8)	*.239*
Complex karyotype	**7/115 (6.1)**	2/46 (4.3)	5/69 (7.2)	*.704*
FLT3 pos	**19/150 (12.7)**	4/57 (7.0)	15/93 (16.1)	*.224*
NPM1 pos	**3/118 (2.5)**	1/47 (2.1)	2/71 (2.8)	*.645*
cKIT pos	**18/142 (12.7)**	12/62 (19.4)	6/80 (7.5)	*.046*
RAS pos	**16/39 (41)**	3/18 (16.7)	13/21 (61.9)	*.009*

AML= acute myeloid leukemia; **WBC**= white cell count; **PB**= peripheral blood; **PLT**= platelet; **Hb**= haemoglobin

Table 2. Characteristics according to age group.

	All patients n= 229 (%)	***Aged 60-69 yrs n= 170***	***Aged 70-85 yrs n= 59***	***P***
Male – Female *n*	**115 – 114**	88 – 82	27 – 32	*.427*
AML type *(n=225)* ***De novo*** *n (%)* ***Secondary*** *n (%)* ***Therapy-related*** *n (%)*	**184 (81.8)** **22 (9.8)** **19 (8.4)**	137 (82.0) 18 (10.8) 12 (7.2)	47 (81.0) 4 (6.9) 7 (12.1)	*.390*
Hb g/dl (range)	**8.6 (3.1-13.3)**	8.4 (3.1-12.8)	8.9 (5.4-13.3)	*.225*
WBC x 10^9/l *(range)*	**31.9 (0.5-417)**	30.6 (0.5-270)	35.7 (1.6-417)	*.519*
PLT x 10^9/l *(range)*	**56.1 (2-419)**	54.2 (5-331)	61.2 (2-419)	*.458*
PB blasts x 10^9/l *(range)*	**18.5 (0.0-243)**	21.8 (0.0-243)	10.9 (0.0-61)	*.134*
PB Blasts > 30%	**19/126 (15.1)**	15/87 (17.2)	4/39 (10.3)	*.311*
PB Blasts > 80%	**7/126 (5.6)**	7/87 (8.0)	0/39 (0.0)	*.068*
Blasts in marrow >80%	**29/116 (25.0)**	22/85 (25.9)	7/31 (22.6)	*.716*
Elevated LDH	**73/100 (73.0)**	52/76 (68.4)	21/24 (87.5)	*.066*
Renal insufficiency	**23/89 (25.8)**	16/65 (24.6)	7/24 (29.2)	*.663*
Additional cytogenetic abnormalities	**34/115 (29.6)**	24/89 (27.0)	10/26 (38.5)	*.258*
Complex karyotype	**7/115 (6.1)**	4/89 (4.5)	3/26 (11.5)	*.186*

AML= acute myeloid leukemia; **WBC**= white cell count; **PB**= peripheral blood; **PLT**= platelet; **Hb**= haemoglobin

Table 3. Administered treatment according to age group.

	All ***n= 229 (%)***	*Aged 60-69* *n= 170 (%)*	*70-85* *n= 59 (%)*	*P*
INDUCTION THERAPY				
«3+7»	100 (43.6)	76 (44.7)	24 (40.6)	
«3-+7 + other drugs»	101 (44.2)	79 (46.4)	22 (37.2)	***.04***
«No anthracyclines"	28 (12.2)	15 (8.9)	13 (22.2)	
Complete remission after induction	**193 (84.3)**	147 (86.5)	46 (78.0)	*.204*
30-day mortality	**8/229 (3.5)**	5/170 (2.9)	3/59 (5.1)	*.428*
60-day mortality	**15/229 (6.6)**	9/170 (5.3)	6/59 (10.2)	.223
Patients in remission:	**193**	147	46	
Not consolidated / only maintenance therapy after remission	**24 (12.4)**	15 (10.2)	9 (19.6)	-
Consolidated with 1-2 cycles	**94 (48.8)**	67 (45.7)	27 (58.7)	-
Consolidated with ≥3 cycle (but no transplant)	**56 (29.0)**	47 (32.0)	9 (19.6)	*.076*
Consolidated also with auto-transplant	**7 (3.6)**	6 (4.0)	1 (2.1)	*NA*
Consolidated also with allo-transplant	**12 (6.2)**	12 (8.1)	0 (0.0)	*NA*

Table 4. Achievement of Complete Remission according to type of induction therapy and age.

a) All patients

Induction therapy	N. patients	CR	PR+NR+PD	Deaths	p
"3+7"	100 (43.6%)	86 (86.0%)	11 (11.0%)	3 (3.0%)	
"3+7+other drugs"	101 (44.2%)	88 (87.1%)	12 (11.9%)	1 (1.0%)	
No anthracycline	28 (12.2%)	19 (67.9%)	9 (32.1%)	0	
Overall	**229**	**193 (83.9%)**	**32 (14.4)**	**4 (1.7)**	**.03**

b) Age 60 – 69.9 years

Induction therapy	N. patients	CR (%)	PR+NR+PD (%)	Deaths (%)	P
"3+7"	76 (44.7%)	65 (85.6%)	9 (11.8%)	2 (2.6%)	
"3+7+other drugs"	79 (46.4%)	70 (88.6%)	9 (11.4%)	0	
No anthracycline	15 (8.9%)	12 (80.0%)	3 (20.0%)	0	
Overall	**170**	**147 (85.9%)**	**21 (12.3%)**	**2 (1.8%)**	**.4**

c) Age 70 – 84.4 years

Induction therapy	N. patients	CR	PR+NR+PD	Deaths	P
"3+7"	24 (40.6%)	21 (87.6%)	2 (8.3%)	1 (4.1%)	
"3+7+other drugs	22 (37.2%)	18 (81.9%)	3 (13.6%)	1 (4.5%)	
No anthracycline	13 (22.2%)	7 (53.8%)	6 (45.2%)	0	
Overall	**59**	**46 (78.0%)**	**11 (18.7%)**	**2 (3.3%)**	**.04**

Table 5. Univariate and multivariate analysis for Event Free Survival.

	HR	C.I. 95%	P	HR	C.I. 95%	P
Male sex	1.31	0.81-2.84	.29			
Secondary / Therapy-related AML	0.93	0.66-1.06	.4			
Age ≥70 years	1.78	1.15-2.54	.008	1.68	0.85-2.84	.137
CBF type	0.74	0.50-1.21	.437			
WBC at diagnosis ≥20x10^9/l	0.8	0.45 – 1.4.	.45			
WBC at diagnosis t(8;21) ≥20x10^9/l	0.9	0.55 – 1.5	.6			
WBC at diagnosis in(16)≥30x10^9/l	0.55	0.25 – 1.1	.4			
Failure to achieve CR after induction therapy	8.96	5.5-13.8	< .0001	8.99	3.78-16.66	<.001
Allo-HSCT during first-line	0.92	0.38-1.85	.700			
>1 cycle needed to achieve CR	1.59	0.73-3.50	.246			
Absence of consolidation therapy	0.75	0.47-1.84	.04	0.35	0.13-2.96	.4
1-2 cycles for consolidation	1.48	0.75-3.20	.276			
≥3 cycles for consolidation including auto-HSCT	9.57	5.6- 13.4	<.0001	7.99	3.18-15.7	<.001
No anthracycline for induction	1.06	0.14-2.08	.05	0.45	0.23 – 1.96	.26

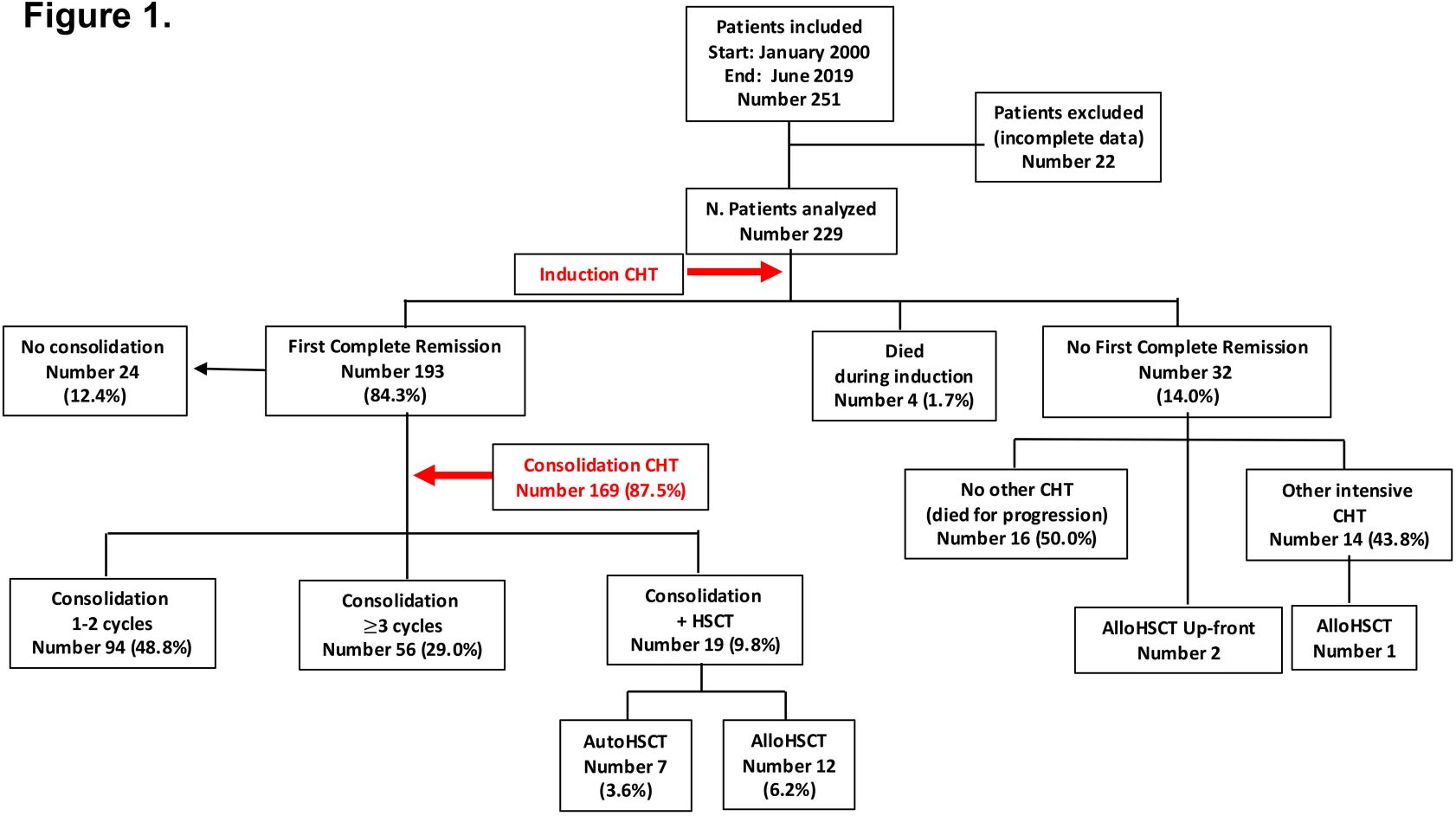
AML= acute myeloid leukemia; **WBC**= white cell count; **CR**= complete remission; **allo-HSCT**= allogeneic hematopoietic stem cell transplant; **auto-HSCT**= autologous hematopoietic stem cell transplant;

Figure 1. Flow – chart of the study

Figure 2. Overall survival and event free survival: A. entire population; B. according to age (<70 years vs >70 years); C. according to t(8;21) vs inv(16)

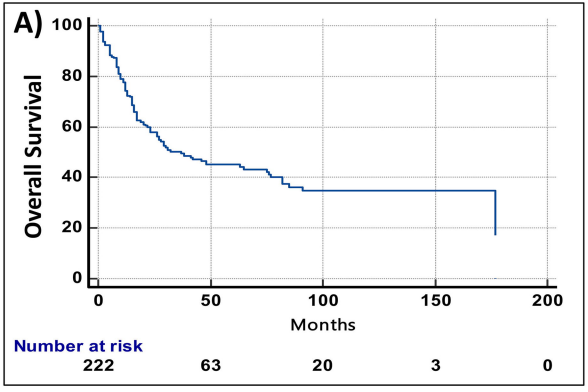
Figure 3. Event Free Survival: A. according to front line treatment; B according to front line treatment and age (<70 years vs >70 years);.

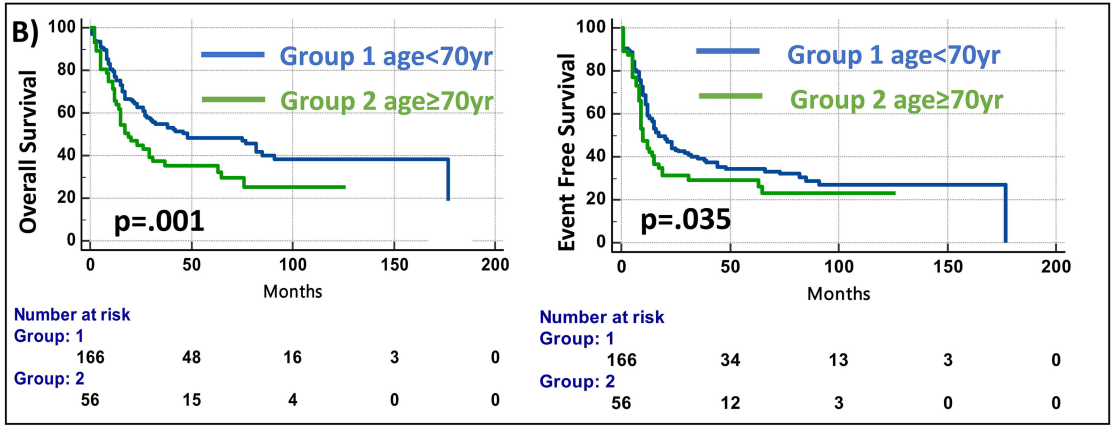
Figure 1.



Legend:
AutoHSCT= autologous stem cell transplant; alloHSCT= allogeneic stem cell transplant; CHT= chemotherapy

Figure 2. Overall and Event Free survival according to age and CBF





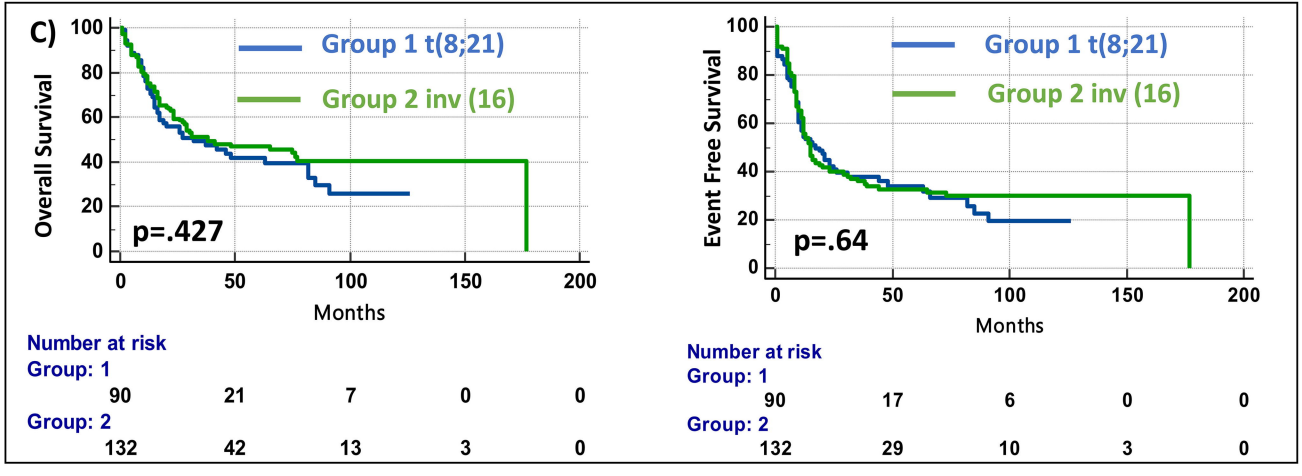
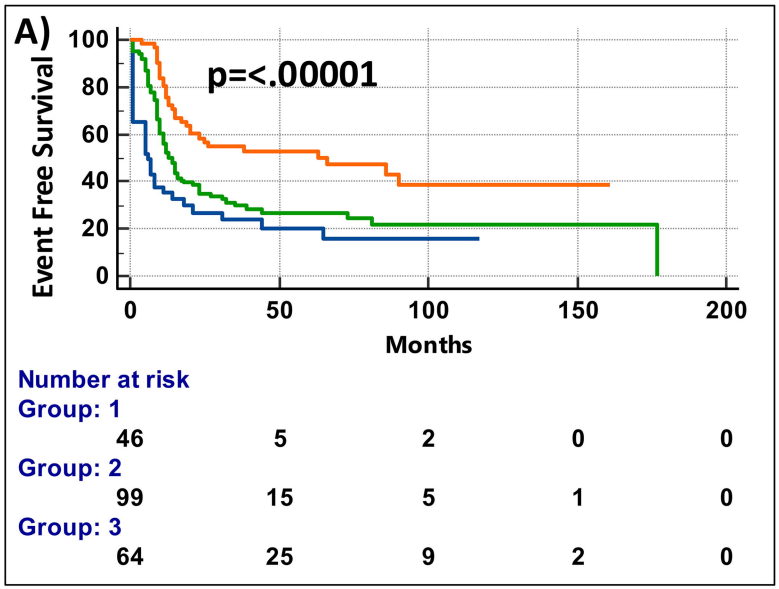


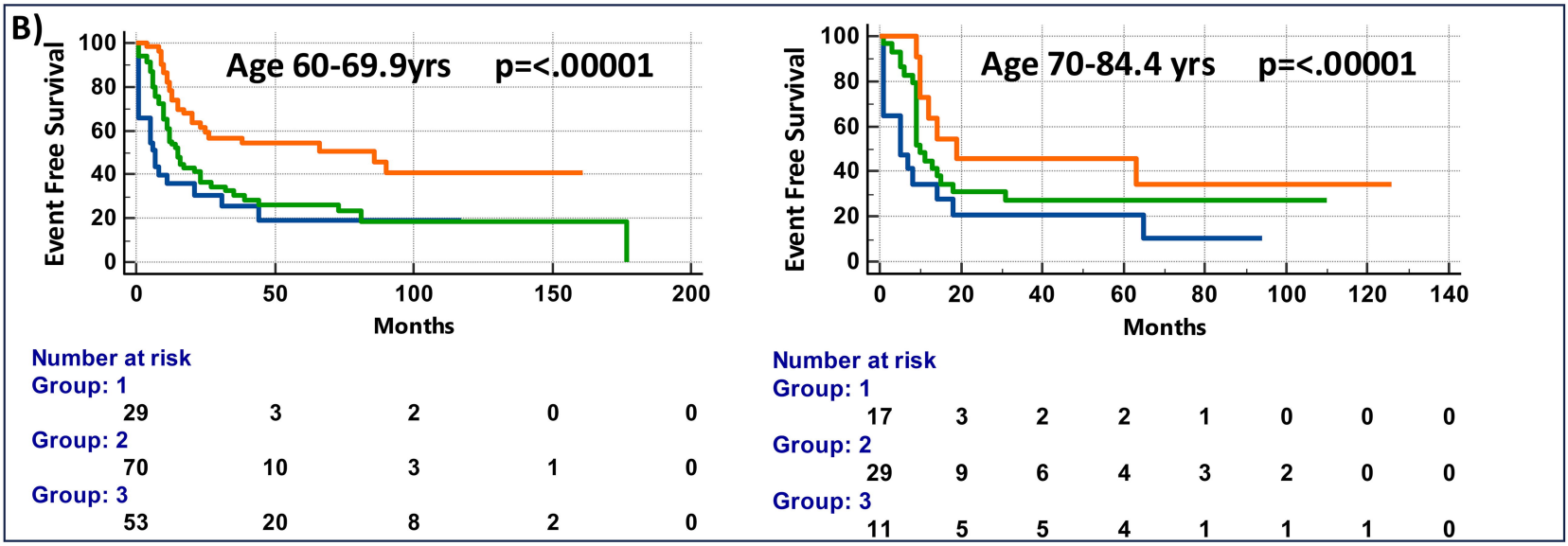
Figure 3.



GROUP 1: ≥3 consolidation (±autoHSCT)

GROUP 2: 1-2 consolidation

GROUP 3: no consolidation



SUPPLEMENTARY METHODS

Chemotherapy

Induction regimens used for the patients in this study were categorized as follows:

1. **"3+7"** regimens (n= 100 patients), consisting of Daunorubicin 45-60 mg/m^2 days 1-3 + intravenous Cytarabine continuous infusion 100-200 mg/m^2 days 1-7 (i.e. D3A7), or other similar 2-drug regimens consisting of minimal two daily doses of anthracycline (e.g. Idarubicin, 12 mg/m^2 days 1-2 or 1-3, Mitoxantrone, 7-10 mg/m^2 days 1-2 or 1-3) plus 7-days intravenous continuous-infusion 100-200 mg/m^2 Cytarabine; this group was considered together with "lower intensity regimens", based on the same drugs and scheme as "3+7", but administered for shorter periods (eg D1A5, D1A7, D2A5, overall n= 8)

2. **"3+7+ other drugs"** (n= 101 patients), incorporating experimental drugs to the D3A7 skeleton in specific trials (e.g. Midostaurin – 1 patient, G3139 – 9 patients, Bortezomib – 4 patients, Dasatinib 17 patients, Avastine 1 patient, Gemtuzumab Ozogamicin 4 patients and others), considered together with "IDAC/HiDAC-based" 2-drug regimens including intermediate-to-high dose Cytarabine (IDAC: 1-1.5 g/m^2 bid days 1-5, or HiDAC 3 g/m^2 bid days 1-5) plus an anthracycline (e.g., HAM, HiDAC + Idarubicin); Etoposide 50 mg/m^2 days 1-5 or other drugs (e.g. Thioguanine 200 mg/m^2 days 1-5 or 1-7), with the exception of purine nucleoside analogues, to anthracyclines and Cytarabine (eg, ICE, MICE, DAV/IAV/DAE/DCE, MEC, BARTS, ETI, AAT); FLA-Ida regimens and similar (e.g., FLAG-Ida, FLAI5, FLAIRG, FLAN) consisting of Fludarabine 25-30mg/mq days 1-5 together with IDAC/HiDAC and an anthracycline;

3. **"NO anthracycline - based"** regimens (n= 28 patients), consisting of 2-drug Fludarabine/Clofarabine plus Cytarabine regimens, with no anthracycline; or on other low-dose chemotherapy without anthracyclines (AraC/VP16, Clofarabine alone)

Supplementary Table 1. Characteristics of patients consolidated by autologous Hematopoietic Stem Cell Transplantation as part of frontline treatment.

CBF type	Age (yrs)	Time of therapy	Induction therapy	Response	Consolidation therapy	MRD post-consolidation	Reason for auto-HSCT	Status at follow-up
t(8;21)	70.1	2004	FLAN	CR1 (FISH pos)	MTN+AraC + IDAC	pos	MRD pos	Deceased in CR1 (heart failure)
inv(16)	62	2007	D3A7	CR1	Ida+AraC (IC) + IDAC (A8)	pos	MRD pos	Deceased (relapse)
inv(16)	60.7	2004	FLA	CR1	FLA	pos	MRD pos	Alive CR2 (after rescue and allo-HSCT)
inv(16)	67.6	2006	My-AIG	CR1	My-AIG + Ida-HiDAC	pos	MRD pos	Alive CR1
inv(16)	63.3	2009	IAV	CR1	IAV	neg	Clinical decision	Alive CR1
t(8;21)	66	2004	FLAI-5	CR1	HiDAC x 2	ND	Clinical study	Alive CR1
t(8;21)	65.6	2009	D3A7	CR1	HiDAC x 3	Neg	Clinical decision	Alive CR1

CR= complete remission; **ND**= not done; **allo-HSCT**: allogeneic hematopoietic stem cell transplant;

Supplementary Table 2. Characteristics of patients consolidated by allogeneic Hemtopoietic Stem Cell Transplantation as part of frontline treatment.

CBF type	Age (yrs)	Time of therapy	Induction therapy	Response	Consolidation therapy	MRD post-consol.	Reason for allo-HSCT	Status at follow-up
inv(16)	62.7	2017	MICE	CR1 (2 cycles)	MICE	ND	Late CR	Alive CR1
inv(16)	66	2013	I3A7	CR1	Ida+HiDAC + HiDAC	pos	MRD pos	Alive CR1
inv(16)	61.4	2009	I1A7	CR1	I1A7 + HiDAC	pos	MRD pos	Alive CR1
inv(16)	62.6	2009	I3A7	CR1	IC + A8 + A8	pos	MRD pos	Alive CR1
inv(16)	69.5	2016	MICE	CR1	HiDAC x 2	pos	MRD pos	Deceased (CR1)
inv(16), secondary AML	63.6	2016	MICE	CR1	HiDAC x 3	pos	MRD pos + sAML	Alive CR1
t(8;21), therapy-rel. AML	63.9	2011	D3A7 + Dasatinib	CR1	HiDAC	ND	tAML	Deceased (CR)
t(8;21)	69	2013	D3A7	CR1	HiDAC	ND	Clinical study	Alive CR1
t(8;21)	60.5	2009	MEC	CR1	HiDAC x 2	ND	Clinical Decision	Deceased (relapse)
t(8;21), secondary AML	61	2006	D3A7	NR	Direct to allo-HSCT	Active disease	NR after induction	Deceased (relapse)
t(8;21)	60	2009	D3A7	CR1	IDAC x 3	ND	Clinical Decision	Deceased (relapse)
t(8;21)	68.7	2014	D3A7	CR1	IDAC	ND	Clinical study	Alive CR1
inv(16)	62.3	2008	D3A7	CR1	HiDAC	ND	Clinical Study	Deceased (CR)
inv(16)	61	2013	D3A7	NR	rescue MEC + HiDAC	ND	NR after induction	Alive CR1
t(8;21), secondary AML	70	2012	Clofarabine - AraC	PD	direct to allo-HSCT (FLAMSA)	Active disease	PD after induction	Deceased (relapse)

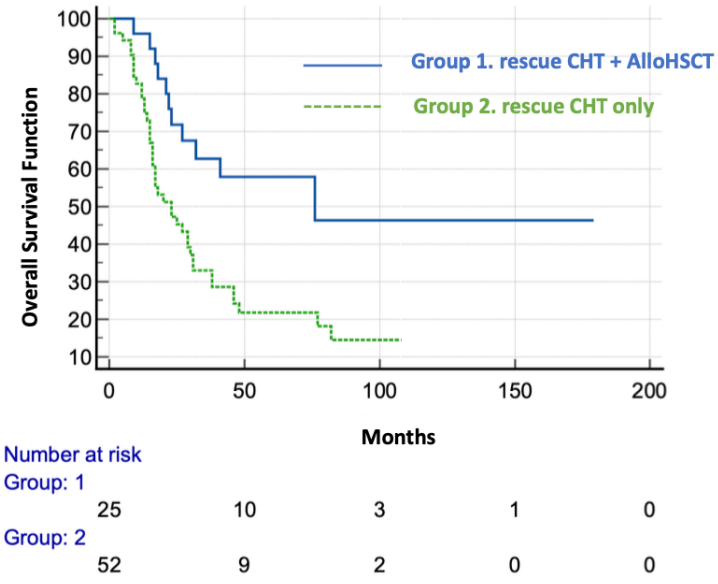
CR= complete remission; **NR**= not response; **ND**= not done; **allo-HSCT**: allogeneic hematopoietic stem cell transplant;

Table 3. Univariate analysis for Event Free Survival – cytogenetic and molecular data*

	HR	C.I. 95%	P
Complex karyotype	1.16	0.47-2.90	.3
Mutated FLT3	0.26	0.03-1.44	.2
Mutated NPM1	0.93	0.23-3.82	.8
Mutated KIT	6.85	3.42-11.71	.04

- ***Only for patients (115) with available data**

Supplementary Figure 1. Overall Survival of relapsing patients according to rescue treatment



CHT= chemotherapy; **alloHSCT**: allogeneic hematopoietic stem cell transplant