M4 acute myeloid leukemia: the role of eosinophilia and cytogenetics in treatment response and survival. The GIMEMA experience

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ABSTRACT

Background
Myelomonocytic acute myeloid leukemia (M4-AML) is frequently associated with the cytogenetic marker inv(16) and/or the presence of eosinophilia. The aim of this study was to analyze the incidence and prognostic role of these factors in a large series of patients.

Design and Methods
Adult patients with acute myeloid leukemia consecutively enrolled in the GIMEMA trials AML10 and LAM99p were retrospectively analyzed.

Results
Among 1686 patients, 400 cases of M4-AML were identified; of these, 78% had neither eosinophilia nor inv(16), 6% had eosinophilia only, 8% had inv(16) only and 8% had both. Univariate analysis showed that both eosinophilia and inv(16) were correlated with a higher probability of complete remission, lower resistance to chemotherapy and increased overall survival. Multivariate analysis showed that the simultaneous presence of the two factors significantly increased the probabilities of both complete remission and overall survival. The presence of only one of the two factors also increased the probabilities of complete remission and overall survival, but not to a statistically significant extent. The relapse-free survival of the responding patients was not influenced by the two factors.

Conclusions
In a large series of patients with M4-AML we confirmed the favorable role of inv(16), but the weight of this factor among the whole M4 population was of limited relevance. Eosinophilia, which affects a small proportion of cases, also emerged as a favorable prognostic factor. Based on the results of this large case population, overall and relapse-free survival rates of patients with M4-AML are not significantly better than those of patients with non-M4 AML, while the concomitant presence of both inv(16) and eosinophilia was associated with a significantly improved prognosis.

Key words: myelo-monocytic acute leukemia, M4-AML, eosinophilia, inv(16).

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Introduction

Myelomonocytic acute myeloid leukemia (M4-AML) is frequently associated with inv (16) (p13q22) or the variant t(16;16)(p13;q22).[1-5] These result in the fusion of two genes, *CBFB* at 16q22, which encodes the β subunit of the core binding factor (CBFβ), and the *MYH11* gene at 16p13, which encodes the smooth muscle myosin heavy chain (SMMHC). The chimeric gene *CBFB*, in frame with the 3' portion of *MYH11*, results in the production of the chimeric protein CBFβ-SMMHC, whose biological effect is a block of the differentiation process of myeloid leukemic cells.[6-13] Large studies have shown that the presence of inv (16) or t(16;16) is a favorable prognostic factor and these cytogenetic findings are currently considered an important guide to therapy.[14-18] Nevertheless, the treatment results of patients carrying these cytogenetics markers are frequently evaluated together with other cytogenetic abnormalities, such as t(8;21) and only few studies have analyzed the role of inv (16) or t(16;16) alone. The use of high dose cytosine arabinoside has been suggested to be a key factor for a good prognosis in these patients.[14,15] M4-AML with inv(16) is commonly associated with eosinophilia; the abnormal eosinophils are part of the leukemic clone, as demonstrated by fluorescence *in situ* hybridization (FISH).[5] Nevertheless, not all AML cases carrying the cytogenetic marker inv (16) or t(16;16) have eosinophilia and not all cases with eosinophilia have an M4 FAB subtype, nor are they all characterized by the presence of inv(16) or t(16;16).

The aim of this study was to analyze, in a large series of patients from GIMEMA AML trials, the proportion of M4-AML cases carrying inv(16) or t(16;16), the proportion of cases with eosinophilia and the prognostic significance of these factors, considered both alone and in combination.

Design and Methods

Between 17/11/1993 and 03/12/2002, 1702 consecutive adult patients with AML were enrolled in two prospective clinical trials: AML10 (1166 patients), and LAM99p (536 patients). Patients had to be over 15 and under 61 year old for recruitment into the two trials. The median age of patients enrolled in the first study was 44.5 years (range, 15.2-60.99) while in the second it was 46.6 years (range, 15.7-60.95). The AML10 was a randomized phase III study carried out by the European Organization for Research and Treatment of Cancer (EORTC) leukemia group and the *Gruppo Italiano Malattie Ematologiche dell'Adulto* (GIMEMA) in 80 European centers between 1993 and 1999. The main objective of the study was to evaluate the relative efficacy and toxicity of an intensive remission induction and consolidation chemotherapy incorporating one of three intercalating agents, daunorubicin, mitoxantrone or idarubicin, in combination with cytosine arabinoside 25 mg/m^2, as an intravenous bolus followed immediately by 100 mg/m^2 given as a continuous infusion daily for 10 days (days 1-10), and etoposide in patients with newly diagnosed AML. Two induction courses of this schedule were followed by a consolidation course including intermediate dose cytosine arabinoside: 500 mg/m^2 12-hourly in 2-hour intravenous infusions on days 1-6 (12 doses), and the same anthracycline employed in the induction. An amendment to the protocol was adopted in 1994, introducing a second randomization to compare the feasibility and results of peripheral blood *vs.* bone marrow autologous stem cell transplantation as rescue from myeloablative therapy following remission consolidation in patients without an available HLA-identical sibling donor. The primary end-point of the first randomization was overall survival, while secondary end-points were the complete remission rate after induction, relapse-free survival and survival from complete remission, type and grade of toxicity related to different treatment steps, time to recovery, feasibility of stem cell harvest after the consolidation course and the rate of completion of autologous and allogeneic stem cell transplantation. The primary end-point of the second randomization was disease-free survival, whereas the secondary end-point was survival after the second randomization.[21] The GIMEMA LAM99p protocol included 5 days of pre-treatment with hydroxyurea at a dose of 2 g/m^2/day from days –4 to 0 and induction treatment with a three-drug regimen: daunorubicin 50 mg/m^2/day on days 1, 3 and 5, cytosine arabinoside 100 mg/m^2/day on days 1 to 10, and etoposide 100 mg/m^2/day on days 1 to 5. The course was repeated in the case of partial remission. Patients who achieved a complete remission after either the first or the second cycle of induction were given consolidation therapy with daunorubicin (50 mg/m^2/day on days 4 to 6) and intermediate dose cytosine arabinoside (500 mg/m^2/12 h on days 1 to 6). Post-consolidation treatment consisted of allogeneic stem cell transplantation for patients with an HLA-identical sibling, and a peripheral blood stem cell autograft for patients without a donor.[22] Five patients in the first and 11 in the second study were lost to follow-up just after inclusion in the study; overall 1686 evaluable cases were therefore, considered. Of these, 400 (23.7%) were diagnosed as having acute myelomonocytic leukemia (M4-AML) according to the FAB classification;[23] in 45 of them (11.2%), typical eosinophilia was observed (M4-Eo). Peripheral blood and bone marrow smears of all cases were reviewed centrally by a commission composed of three experienced morphologists; specific cytochemical stainings were performed (CAE, toluidine blue). According to the criteria established by the FAB classification M4-Eo AML is characterized by the presence of eosinophils in a proportion ≥5% of non-erythroid bone marrow cells. The eosinophils are described as morphologically abnormal, showing cytological abnormalities such as nuclear hyperlobulation or hypolobulation and/or the presence of large pro-eosinophilic granules, and cytochemical abnormalities.[23] No cases with basophilia were observed.

The equivalence of patients enrolled in the AML10 and AML99p trials with respect to prognostic factors at diagnosis was assessed before combining the two groups. The median age of the entire AML-M4 population was 44.6 years (range, 15.2-60.9), with 49% males and 51% females. Cytogenetic data were available for only 240 patients; cytogenetic analysis was not done in 128 cases and in 32 (20%) failed. When compared to the overall series, this subset of 240 patients with available cytogenetic data resulted comparable in terms of prognostic factors and clinical outcome, thus guaranteeing the absence of a bias when restricting the analysis to the cases with available cytogenetics. The cytogenetic analysis was performed by conventional cytogenetic and banding techniques in peripheral centers in the AML10 study and in a central laboratory in the other study (AML99p); FISH analysis was not, therefore, performed in all cases.

Statistics

The populations enrolled into the two consecutive trials, AML10 and AML99p, were grouped together after assessment of their homogeneity with respect to the main stratification and prognostic factors, and with respect to outcome; to take into account the obvious difference in follow-up between the two protocols, time-to-event outcomes were stopped at 5 years. The subpopulation of patients for whom cytogenetic information was available (n=240) was representative of the whole population (n=400) in terms of both characteristics and outcomes, thus guaranteeing absence of bias for the results of the analysis restricted to the former population.

Differences with respect to categorical covariates were evaluated using the χ^2 test or Fisher's exact test on appropriate cross-tabulations. Differences with respect to continuous covariates were evaluated using the non-parametric Wilcoxon or Kruskal-Wallis test. Complete remission rates were estimated as the number of responders over the total population and compared in univariate analysis by the χ^2 test and in multivariable analysis by logistic regression. Overall survival was defined as time from diagnosis to death, censoring patients alive at last follow-up. Relapse-free survival was defined as the time since assessment of complete remission to either relapse or death in first complete remission, censoring patients alive and relapse-free at last follow-up. Overall and relapse-free survival probabilities were estimated according to the Kaplan-Meier product limit method and compared in univariate analysis by the log-rank test, while effects of factors on hazard rates were estimated in multivariate analysis using the Cox proportional hazards model. The analysis of relapse rate was carried out estimating the cumulative incidence curve considering death in first remission as a competing risk and the differences were tested using the Gray test. In the multivariate models, linear hypotheses tests allowed pair-wise comparisons of the four groups defined by presence/absence of eosinophilia and inv(16), as well as tests for the marginal effects. All results were similar after adjustment for age (*data not shown*). The role of white blood cell count above 50×10^9/L was also analyzed in the multivariate setting as a possible adverse prognostic factor in patients with M4 AML, but it did not result as an independently significant prognostic factor.

Results

The probability of complete remission and the relapse-free and overall survival rates of patients with M4-AML were compared to those of the whole non-M4 AML population. The probability of complete remission in the 400 patients with M4-AML considered as a single group, irrespectively of eosinophilia and cytogenetic profile, was significantly higher (76.0%) than that of the 1270 non-M4 AML patients (67.2%, p=0.0009). As concerns relapse-free and overall survival rates, only non-significant advantages were seen in the former group (Figure 1).

The prognostic significance of the presence of eosinophilia and/or the cytogenetic profile was then analyzed in univariate and multivariate models.

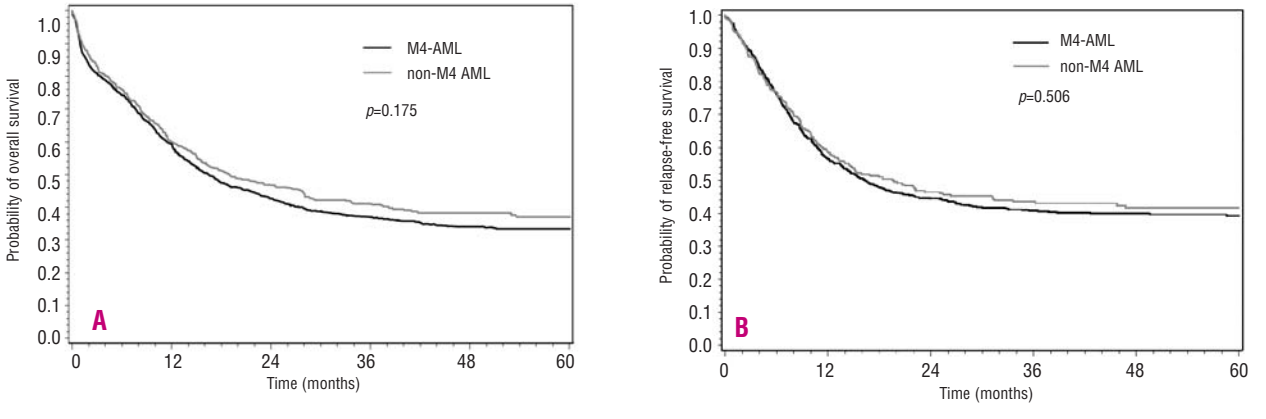

Figure 1. (A) Overall survival and (B) relapse-free survival of the 400 patients with M4-AML and the entire population of non-M4-AML enrolled in the two consecutive GIMEMA studies, AML10 (869 patients) and LAM 99p (433 patients).

Univariate analysis

Role of eosinophilia

The main prognostic factors in the two groups of 355 patients with M4-AML without eosinophilia (M4-Eos$^-$) and 45 with eosinophilia (M4-Eos$^+$) were compared. With respect to the clinical trial and to the assigned treatment, the proportions of patients were similar (10% of the M4-AML patients in the AML10 study and 13% of those in the AML99p trial had eosinophilia, p=0.383).

Patients with M4-Eos$^+$ were younger than the M4-Eos$^-$ patients. The age distribution of the MA-Eos$^-$ patients resembled that of the entire AML population, with an increased frequency in older age groups, while the age distribution of patients with M4-Eos$^+$ was rather uniform with an isolated peak in the age range between 40 and 50 years old (Figure 2). The presence of the cytogenetic marker inv(16) was correlated with eosinophilia: it was found in 57.1% of M4-Eos$^+$ patients and in only 9.8% of the M4-Eos$^-$ patients (p<0.0001).

Univariate analysis showed a trend for an association between the presence of eosinophilia and the probability of achieving complete remission (86.7% *vs.* 74.7% p=0.075); the probability of induction death was identical, while the proportion of M4-AML patients with resistant disease was higher among those without eosinophilia than among those with eosinophilia (Table 1). Overall and relapse-free survival rates were also significantly higher in patients with eosinophilia. The overall survival rate of the group with eosinophilia was 64% at 36 months (95% CI: 56-74) (median, 23.3 months) while in patients without eosinophilia it was 38% (95% CI: 36-40) (median 15.8 months) (Figure 3A). The relapse-free survival rate of the patients with eosinophilia was 55% at 36 months (95% CI: 46-65) (median never achieved) and 42% (95% CI: 39-45) (median 15.6 months) in the other group (Figure 3B).

The incidence of relapse appeared to be higher in patients with M4-Eos$^-$ than in those with M4$^-$Eos$^+$ only in the first year, but on the whole it was equivalent (p=0.326). There was also a trend to a significant advantage in terms of non-relapse mortality among the M4-AML patients with eosinophilia (p=0.090).

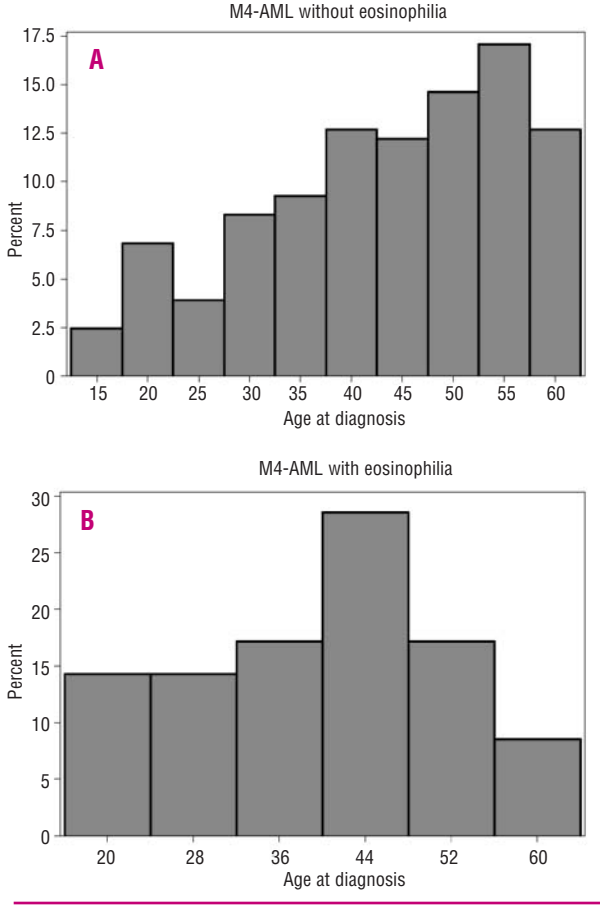

Figure 2. Age distribution of M4-AML patients without eosinophilia (A) and (B) with eosinophilia. The age distribution of patients without eosinophilia was similar to that of the global population with acute myeloid leukemia, the frequency increasing with age. The frequency in patients with eosinophilia was, in contrast, is homogeneous in the various age groups, with an isolated peak (40-50 years).

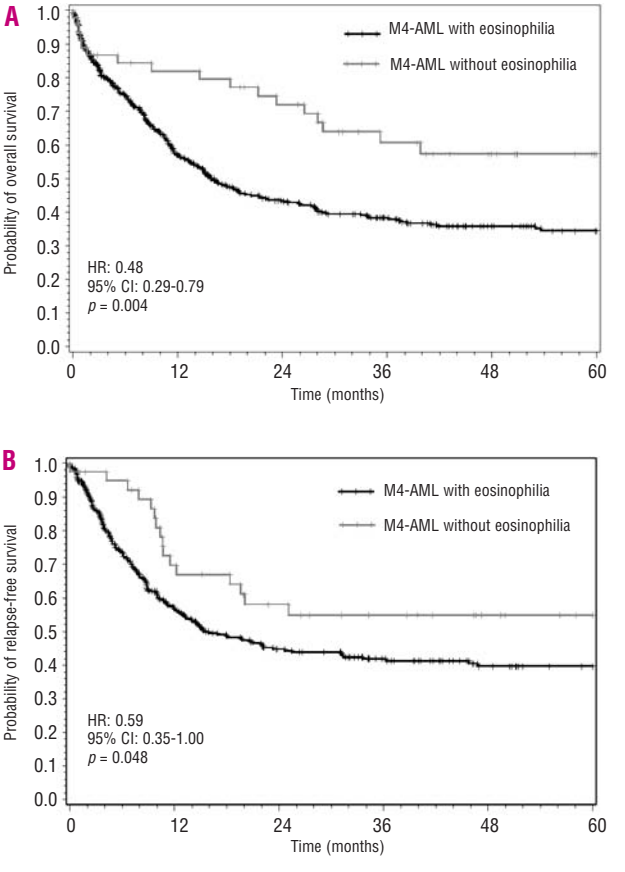

Figure 3. (A) Overall survival and (B) relapse-free survival of patients with acute myeloid leukemia with eosinophilia and without eosinophilia. The univariate analysis showed significant advantages associated with the presence of eosinophilia.

Role of inv(16)

Not surprisingly, patients with inv(16) were younger than those without: 65% versus 48% were below 45 years of age (p=0.049). However, this association seems to be due to the correlation between inv(16) and eosinophilia. No other significant association was found with other factors. In the univariate analysis also the presence of inv(16) was associated with a higher probability of complete remission (90% *vs*. 72.5%, p=0.025) and lower probabilities of induction death and resistance (Table 1). Figure 4A shows the effect of inv(16) on overall survival, which was significantly superior in patients carrying the inv(16), (HR=0.50; 95% CI: 0.30-0.85; p=0.010). The overall survival rate at 36 months of patients with inv(16) was 60% (95% CI: 51-70) (median never achieved) whereas it was 39% (95% CI: 36-42) (median 15.6 months) in patients without inv(16). Figure 4B shows the relapse-free survival in responding patients (HR=0.79, 95% CI: 0.48-1.31; p=0.366), which, at 36 months was 44% (95% CI: 37-52) (median 22.7 months) in patients with inv(16); and 39% (95% CI: 36-42) (median 15.6 months) in patients with inv(16). As *intention-to-treat* criteria, all patients enrolled in the studies who obtained a complete remission should have had consolidation therapy with autologous or allogeneic stem cell transplantation. Of the 400 patients with M4 AML, 304 obtained a complete remission and, of them, 167 were transplanted (109 with an autologous graft, 58 with an allogeneic graft). The overall survival of these patients was compared to that of the non-M4-AML patients enrolled in the same studies, showing no difference. The prognostic role of eosinophilia and cytogenetics among transplanted patients could not be analyzed because the groups were too small.

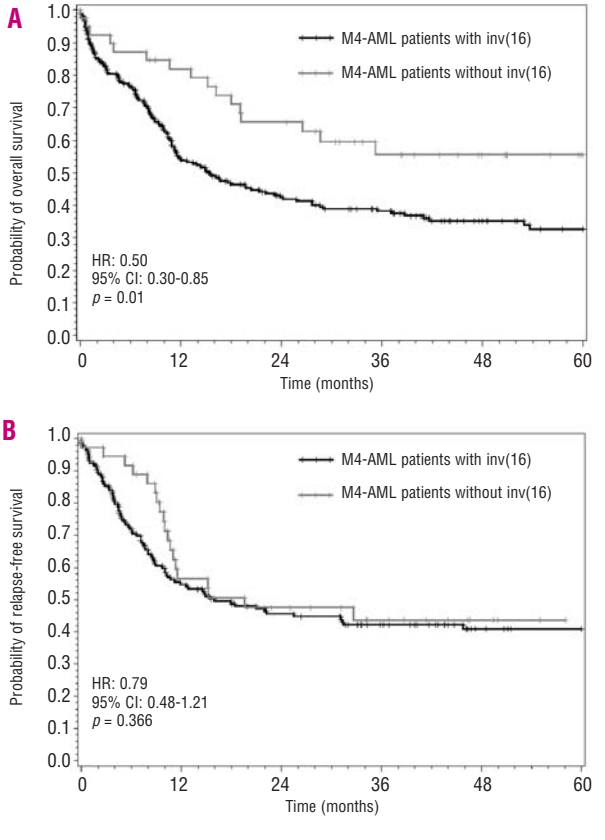

Figure 4. (A) Overall survival and (B) relapse-free survival of M4-AML patients with inv(16) and without inv(16).

Table 1. Probability of response to induction treatment in patients with M4(Eos$^-$) compared to those with M4(Eos$^+$) acute myeloid leukemia and in patients with inv(16) compared to those with normal cytogenetics (univariate analysis).

	ID (%)	*RES (%)*	*CR (%)*	*CR vs. no CR p*
Morphology				
Eos$^-$	42 (11.8)	48 (13.5)	265 (74.6)	0.075
Eos$^+$	5 (11.1)	1 (2.2)	39 (86.7)	
Cytogenetics				
Inv(16)$^-$	23 (11.5)	32 (16)	145 (72.5)	0.025
Inv(16)$^+$	2 (5)	2 (5)	36 (90)	

ID: induction death; RES: resistance to chemotherapy; CR: complete remission.

Multivariate analysis

In order to assess the independent effect of each of the two correlated factors, eosinophilia and inv(16) on achieving complete remission, multivariate analysis took into account their combination, assessing pair-

Table 2. Probabilities of complete remission (CR) and overall survival (OS) for each combination of morphological (Eos$^{+/-}$) and cytogenetic (inv(16)$^{+/-}$), profile.

	inv(16)-	*inv(16)+*
Eos$^-$	1 (baseline)	CR: 0.61-7.67 OS: 0.43-1.47
Eos$^+$	CR: 0.32-3.44 OS: 0.36-1.50	CR: 0.94-55.41 OS: 0.11-0.65

	CR p value	*OS p value*
Eos$^-$ inv(16)+ *vs.* Eos$^-$ inv(16)$^-$	0.235	0.467
Eos$^+$ inv(16)+ *vs.* Eos$^+$ inv(16)$^-$	0.102	0.076
Eos$^-$ inv(16)+ *vs.* Eos$^+$ inv(16)$^+$	0.314	0.043
Eos$^-$ inv(16)– *vs.* Eos$^+$ inv(16)$^-$	0.940	0.394
Eos$^+$ inv(16)+ *vs.* Eos$^-$ inv(16)$^-$	0.057	0.004

Odds ratios (CR) and hazard ratios (OS) with 95% confidence intervals are indicated (multivariate analysis) with p-values for the different combinations. The contemporary presence of the two factors, eosinophilia and inv(16) was associated with significantly higher probabilities of CR and OS in comparison with double negative cases; the presence of only one of the two factors was not associated with a significant advantage.

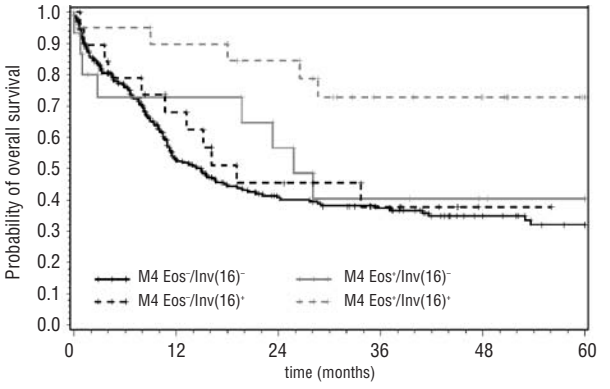

Figure 5. Overall survival of patients with M4-AML divided according to various combinations of presence/absence of eosinophilia and inv(16). Cases with contemporary presence of eosinophilia and inv(16) had a significantly better overall survival in comparison to the other groups (p=0.05).

wise differences between the four groups. Only the presence of both factors was associated with a significantly higher probability of complete remission.

Applying the same approach for overall survival, again the presence of both factors was significantly advantageous compared to cases without either factor (p=0.004), but also compared to cases with inv(16) only (p=0.043) and, less significantly, compared to cases with eosinophilia only (p=0.076). The presence of a single factor, eosinophilia or inv(16), conversely, did not offer a survival advantage (Table 2). As shown in Figure 5 the overall survival of cases with both eosinophilia and inv(16) appears to be significantly longer than that of cases with all other combinations of the two factors. In this group of patients the overall survival rate was also significantly higher than that of non-M4 AML patients enrolled in the same clinical trials.

The relapse-free survival of responding patients did not appear to be significantly influenced by eosinophilia and inv(16), even in combination, when the same model was applied (*data not shown*).

Discussion

It is well known that AML patients carrying inv(16), as well as other cytogenetic abnormalities [t(8;21)(q22;q22)] disrupting genes encoding subunits of the core-binding factor (a heterodimeric transcription factor involved in regulation of hematopoiesis), have a relatively favorable outcome, particularly if treated with consolidation regimens containing high doses of cytarabine. The favorable role of cytosine arabinoside in the treatment subtypes of AML with favorable cytogenetics was demonstrated by studies in the late 1990s[14,15] but was also confirmed more recently by a CALGB study in patients specifically bearing inv(16) or t(16;16).[24] Cytosine arabinoside was present in both treatment protocols employed in our series of patients, although not at the doses and with the schedule used in the CALGB experience. The prognosis of this AML subgroup seems to be affected by mutations occurring in the *Kit* gene structure.[25-27] However, while the relation between *Kit* mutations and prognosis seems to be established in t(8;21) AML, the prognostic impact of *Kit* mutations in inv(16) AML remains controversial. Unfortunately we could not investigate this in the present study.

M4 is the subtype of AML in which inv(16) occurs most frequently, together with eosinophilia. The aim of this study was to investigate this point and to what extent the presence of these two factors influences the features of M4-AML, as well as the overall prognosis. From our analysis it appears that only a minority of cases carries these two factors, alone or in combination: only 22% of the entire M4-AML population had eosinophilia and/or inv(16). Unfortunately, cytogenetic data were not available for all the patients and this is a major limitation of our analysis. To determine whether there was a possible bias derived from restricting the analysis to the 240 cases with available cytogenetic data (necessary when inv(16) is considered in the various computations presented) the two groups of patients – with or without cytogeneic data – were compared with respect to the different known risk factors and outcome parameters. The two groups were found to be very similar, with no significant differences, thus underlying the validity of the correlation analysis carried out.

Our data confirm the favorable prognostic role of inv(16) demonstrating that M4-AML patients with this cytogenetic abnormality have a higher probability of attaining complete remission and lower probabilities of resistance and relapse when compared to the other M4 patients in univariate analysis; however the weight of this favourable factor within the entire M4 population is limited.

The other relevant point that emerged is that the presence of eosinophilia also correlates with a better outcome: with respect to patients carrying inv(16) only, the contemporary presence of eosinophilia confers a statistically significant survival advantage. A synergistic amplification of the favorable effect is thus evident when the two factors are associated. The population of patients with eosinophilia seems to have some particular features: the patients are younger and their age distribution is different from that of patients with other forms of AML, not showing the usual progressive increase in incidence with more advanced age, but rather a homogeneous distribution in the age classes with the exception of a peak incidence around the age of 45. These data suggest the possible existence of a specific subgroup among cases of M4-AML, but a larger analysis is necessary to confirm this point. Central morphological revision revealed no cases with a malignant eosinophil-basophil morphology, described as being associated with a bad prognosis, in our study.

Among the entire M4-AML population the proportion of cases carrying inv(16) and/or eosinophilia was,

however, limited (22%) and when the outcome of the entire group of M4-AML patients was compared with that of patients with other forms of AML, only small, non-significant advantages in survival and relapse-free survival were observed.

In conclusion, this analysis on a large GIMEMA population of patients with M4-AML confirmed the favorable prognostic role of inv(16), demonstrated the good prognostic role of eosinophilia and revealed an enhancement of the effect when the two factors were both present. On the other hand, the impact of the presence of these factors is limited when the prognosis of the whole M4-AML population was compared to that of patients with non-M4 AML.

Authorship and Disclosures

AP participated in the conception of the study and wrote the paper; SI conducted the statistical analyses; MBe, MBo, AC, NC, FDR, FD, FL, VL, FM, LMa, LMe, GM, SM, GS, CGV, and AV produced the patients' data from peripheral GIMEMA centres and participated in revising the paper; AM participated in the preparation of the manuscript; PF was responsible for data collection from the centers and participated in the statistical analyses; GL, RF, FM participated in the conception of the study and revised the manuscript; LP participated in the conception of the study, writing, analysis and revision of the manuscript. The authors reported no potential conflicts of interest.

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