# 9 ANEXOS

## 9.1 Artículo Anexo 1

Adès L, Sanz MA, Chevret S, Montesinos P, Chevallier P, Raffoux E, Vellenga E, Guerci A, Pigneux A, Huguet F, Rayon C, Stoppa AM, de la Serna J, Cahn JY, Meyer-Monard S, Pabst T, Thomas X, de Botton S, Parody R, Bergua J, Lamy T, Vekhoff A, Negri S, Ifrah N, Dombret H, Ferrant A, Bron D, Degos L, Fenaux P.

Treatment of newly diagnosed acute promyelocytic leukemia (APL): a comparison of French-Belgian-Swiss and PETHEMA results.

Blood. 2008 Feb 1;111(3):1078-84.

CLINICAL TRIALS AND OBSERVATIONS

# Treatment of newly diagnosed acute promyelocytic leukemia (APL): a comparison of French-Belgian-Swiss and PETHEMA results

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**All-*trans* retinoic acid (ATRA) plus anthracycline chemotherapy is the reference treatment of newly diagnosed acute promyelocytic leukemia (APL), whereas the role of cytosine arabinoside (AraC) remains disputed. We performed a joint analysis of patients younger than 65 years included in Programa para el Estudio de la Terapéutica en Hemopatía Maligna (PETHEMA) LPA 99 trial, where patients received no AraC in addition to ATRA, high cumulative dose idarubicin, and mitoxantrone, and APL 2000 trial, where patients received AraC in addition to ATRA and lower cumulative dose daunorubicin. In patients with white blood cell (WBC) count less than $10 \times 10^9$/L, complete remission (CR) rates were similar, but 3-year cumulative incidence of relapse (CIR) was significantly lower in LPA 99 trial: 4.2% versus 14.3% ($P = .03$), although 3-year survival was similar in both trials. This suggested that AraC is not required in APL with WBC count less than $10 \times 10^9$/L, at least in trials with high-dose anthracycline and maintenance treatment. In patients with WBC of $10 \times 10^9$/L or more, however, the CR rate (95.1% vs 83.6% $P = .018$) and 3-year survival (91.5% vs 80.8%, $P = .026$) were significantly higher in APL 2000 trial, and there was a trend for lower 3-year CIR (9.9% vs 18.5%, $P = .12$), suggesting a beneficial role for AraC in those patients. (Blood. 2008;111: 1078-1084)**



## Introduction

Acute promyelocytic leukemia (APL) is a distinct subtype of acute myeloid leukemia characterized by its morphology, t(15; 17) translocation leading to *PML-RARa* fusion gene, and by a life-threatening coagulopathy.[1-5] All-*trans* retinoic acid (ATRA) combined with anthracycline-based chemotherapy yield complete remission (CR) rate in approximately 90% of newly diagnosed APLs and 25% to 30% subsequently relapse.[6] Maintenance treatment (especially combining continuous 6-mercaptopurine [6MP] and methotrexate [MTX] to ATRA) appears to further reduce the relapse risk to 10% to 15%.[6-9] Pretreatment white blood cell (WBC) count is the main factor associated with relapse in APL and a predictive model for relapse risk (Sanz score) based on pretreatment WBC and platelet counts allowing for the distinction among low-risk patients (with WBC count $< 10 \times 10^9$/L and platelet count $> 40 \times 10^9$/L), intermediate-risk patients (with WBC count $< 10 \times 10^9$/L and platelet count $< 40 \times 10^9$/L), and high-risk patients (with WBC count $\geq 10 \times 10^9$/L).[10]

Another sizable subset of patients die in CR from complications of consolidation treatment, mainly from infection due to chemotherapy-induced myelosuppression.[7,11-13] To decrease mortality in CR, the Programa para el Estudio de la Terapéutica en Hemopatía Maligna (PETHEMA) group reduced the intensity of consolidation chemotherapy by avoiding cytosine arabinoside (AraC) in the chemotherapy regimen.[8,9] They observed CR rates comparable with regimens using a combination of AraC with anthracycline, a lower rate of death in CR (2%) and a low cumulative incidence of relapse (10%). On the other hand, the French-Belgian-Swiss APL group, in a randomized trial in patients with WBC count less than $10 \times 10^9$/L that tested the role of AraC in addition to ATRA and anthracyclines, found a significantly higher cumulative incidence of relapse and lower survival in patients treated without AraC.[14]

To assess those discrepancies between PETHEMA and French-Belgian-Swiss results, particularly regarding the role of AraC, we performed a joint analysis of results of LPA 99 (PETHEMA group) and APL 2000 (French-Belgian-Swiss APL group) studies. We restricted, in APL 2000 trial, the joint analysis to patients who received AraC, excluding low-risk patients who were randomized to receive induction treatment without AraC.

Submitted July 9, 2007; accepted October 17, 2007. Prepublished online as *Blood* First Edition paper, November 1, 2007; 10.1182/blood-2007-07-099978.

L.A. and M.S. contributed equally to this work.

The online version of this article contains a data supplement.

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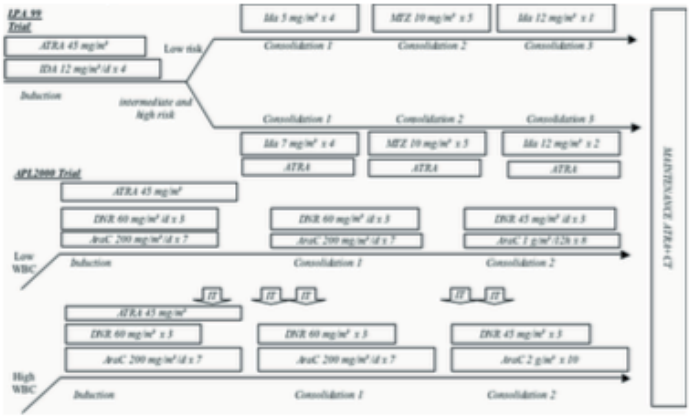
Figure 1. Design of the LPA99 and APL2000 trials.




## Methods

### Design of LPA 99 and APL 2000 trials

Eligibility criteria for both studies were a diagnosis of de novo APL with demonstration of the t(15;17) or *PML/RAR* rearrangement, no cardiac contraindication to anthracycline chemotherapy, and a signed informed consent. Informed consent was obtained in accordance with the Declaration of Helsinki, and both protocols were approved by the Research Ethics Board of each participating hospital.

***LPA 99 trial.*** The induction regimen consisted of oral ATRA (45 mg/m$^2$ per day), divided into 2 daily doses and maintained until complete hematologic remission, and idarubicin (12 mg/m$^2$ per day) given on days 2, 4, 6, and 8. After CR achievement, low-risk patients according to Sanz score received 3 monthly consolidation courses consisting of idarubicin (5 mg/m$^2$ per day) on days 1 through 4, mitoxantrone (10 mg/m$^2$ per day) on days 1 through 5, and idarubicin (12 mg/m$^2$ per day) on day. Intermediate- and high-risk patients received ATRA (45 mg/m$^2$ per day given during 15 days) combined with reinforced consolidation chemotherapy (idarubicin 7 mg/m$^2$ per day during the first course and idarubicin for 2 consecutive days instead of one in the third course). Maintenance therapy consisted of 6-mercaptopurine (50 mg/m$^2$ per day), methotrexate (15 mg/m$^2$ per week), and intermittent ATRA (45 mg/m$^2$ per day) for 15 days every 3 months. Maintenance therapy was continued for 2 years (Figure 1).[8]

***APL 2000 trial.*** Patients 60 years or younger with WBC count less than $10 \times 10^9$/L were randomized to receive the reference ATRA plus CT treatment of the previous APL 93 trial (ie, ATRA 45 mg/m$^2$ per day until hematologic CR and chemotherapy with daunorubicin (DNR) 60 mg/m$^2$ per day during 3 days and AraC 200 mg/m$^2$ per day during 7 days) or the same treatment but without AraC (AraC-negative group). After CR achievement, consolidation treatment consisted of 2 intensive chemotherapy courses with DNR 60 mg/m$^2$ per day for days 1 to 3 and AraC 200 mg/m$^2$ per day for days 1 to 7 and daunorubicin 45 mg/m$^2$ per day for days 1 to 3 and AraC 1 g/m$^2$ per 12 hours for days 1 to 4 in the AraC-positive arm, which were similar but without AraC in the AraC-negative arm.[14]

Patients 60 years or younger with a WBC count of $10 \times 10^9$/L or more received the same treatment as those 60 years or younger with WBC count less than $10 \times 10^9$/L included in AraC-positive group, but with increased dose of AraC (2 g/m$^2$ per 12 hours during 5 days in patients younger than 50 years, and 1.5 g/m$^2$ per 12 hours during 5 days in patients aged 50 to 60 years) with central nervous system (CNS) prophylaxis (consisting of 5 intrathecal injections of MTX 15 mg, AraC 50 mg, and deponedrol).

Patients older than 60 years with WBC count less than $10 \times 10^9$/L received the same treatment as patients 60 years or younger with WBC count less than $10 \times 10^9$/L included in the AraC-negative group (ie, ATRA 45 mg/m$^2$ per day until hematologic CR and chemotherapy with daunorubicin 60 mg/m$^2$ per day during 3 days) followed by consolidation treatment consisting of 2 intensive chemotherapy courses with DNR 60 mg/m$^2$ per day for days 1 to 3 and daunorubicin 45 mg/m$^2$ per day for days 1 to 3. Patients older than 60 years with initial WBC count of $10 \times 10^9$/L or more received the same treatment as patients 60 years or younger with WBC count less than $10 \times 10^9$/L included in the AraC-positive group (ie, ATRA 45 mg/m$^2$ per day until hematologic CR and chemotherapy with daunorubicin 60 mg/m$^2$ per day during 3 days and AraC 200 mg/m$^2$ per day during 7 days) followed by consolidation treatment with 2 intensive chemotherapy courses with DNR 60 mg/m$^2$ per day for days 1 to 3 and AraC 200 mg/m$^2$ per day for days 1 to 7 and daunorubicin 45 mg/m$^2$ per day for days 1 to 3 and AraC 1 g/m$^2$ per 12 hours for days 1 to 4.[14]

For all patients, maintenance was the same as in LPA 99 trial.

### Eligibility criteria for the joint study

A joint analysis of the 2 studies was made. It included all patients from LPA 99 younger than 65 years, and patients from APL 2000 trial younger than 65 years who were randomized or assigned to treatment arms with AraC.

### Study end points

Event-free survival (EFS), cumulative incidence of relapse (CIR), and overall survival (OS) were defined as the study end points. Complete remission (CR) and relapse were defined according to International Working Group criteria.[15] Early death was defined as death occurring during induction therapy or during the period of aplasia that followed chemotherapy. Total deaths included deaths irrespective of their cause (early deaths, deaths after relapse, deaths in CR).

### Statistical analysis

Statistical analysis was performed at the reference date of January 1, 2007 (in 410 patients included in LPA 99 before August 2004, and 178 patients included in APL 2000 before February 2004) dealing with the main end point and overall survival.

In APL 2000 trial, treatment was stratified on initial WBC count (more or less than $10 \times 10^9$/L) and age, whereas in PETHEMA LPA 99 trial, it was stratified according to Sanz score, where initial WBC count ($10 \times 10^9$/L) discriminated high-risk patients and low- and intermediate-risk patients. For this reason, joint analysis of the 2 trials was performed according to initial WBC count, separating low- and intermediate-risk patients (with WBC count $< 10 \times 10^9$/L), on the one hand, from high-risk patients (with WBC count $\geq 10 \times 10^9$/L) on the other hand.

Baseline characteristics and CR rates in the 2 groups were compared by nonparametric tests (exact Fisher test for qualitative variables, Kruskal-Wallis test for quantitative variables). Censored (EFS, DFS, OS)[16,17] end

**Table 1. Pretreatment characteristics and outcome of APL patients included in the joint analysis**

| Characteristics | LPA 99 trial, N = 410 | APL 2000 trial, N = 178 | P | Adjusted P* |
|---|---|---|---|---|
| Age, y, median (Q1-Q3) | 37 (24-49) | 43 (29-51) | .016 | — |
| Age younger than 60 y, no. (%) | 378 (92.2) | 167 (93.8) | .60 | — |
| Male sex, no. (%) | 200 (48.8) | 80 (44.9) | .42 | — |
| WBC count, $10^9$/L, median (Q1-Q3) | 2.5 (1.2-10.5) | 7.1 (1.5-27.5) | < .001 | — |
| Platelet count, $10^9$/L, median (Q1-Q3) | 22 (13-38) | 32 (19-58) | <.001 | — |
| Fibrinogen level, g/L, median (Q1-Q3) | 1.5 (1.0-2.3) | 1.5 (1.0-2.4) | .97 | — |
| **Sanz score, no. (%)** | | | | |
| Low risk | 73 (17.8) | 43 (24.2) | <.001 | — |
| Intermediate risk | 233 (56.8) | 53 (29.8) | — | — |
| High risk | 104 (25.4) | 82 (46.0) | — | — |
| Complete remission, no. (%) | 381 (92.9) | 173 (97.2) | .053 | .018 |
| Deaths in CR, no. (%) | 5/381 (1.3) | 5/173 (2.9) | .049 | .18 |
| Relapses, no. (3-y cumulative incidence, %) | 33/381 (7.4) | 16/173 (12.0) | .32 | .33 |
| Total events, no. (3-y EFS, %) | 66 (85.3) | 26 (86) | .97 | .81 |
| Total deaths, no. (3-y OS, %) | 43 (90.5) | 11 (93.7) | .14 | .086 |

— indicates not applicable.
*Adjusted for age, sex, WBC count, and platelet count.

points were estimated by the nonparametric Kaplan-Meier method, then compared between randomized groups by the log-rank test, after checking for proportional hazards over time. Cox models allowed estimating hazard ratio (HR) of event with 95% confidence intervals (95% CIs).[18-20]

In estimating relapses, we took into account for competing risks deaths in first CR using the cumulative incidence curves, then compared by the Gray test, while the Fine and Gray model was used to estimate subdistribution HR (SHR).[21,22]

Crude estimates were computed. Estimations were finally adjusted for potential predictors including age, sex, platelet count, and WBC count. Such adjustments were performed on the basis of multivariate regression models, which differed according to the end point: Logistic model for CR rates, Cox model for censored end points, and Fine and Gray model for the cumulative incidence of relapse.

Type I error was fixed at the 5% level. All tests were 2 tailed. Statistical analysis was performed using SAS version 9.1 software (SAS, Cary, NC) and R (R Foundation for Statistical Computing, Vienna, Austria) software packages.

## Results

### Initial patient characteristics and overall treatment results

In LPA 99 trial, 410 consecutive patients younger than 65 with newly diagnosed APL from 74 institutions from Spain, Argentina, the Netherlands, and Czech Republic were registered between September 1999 and August 2004. Median follow-up was 67 months.

In APL 2000 trial, between January 2000 and February 2004, 178 APL patients younger than 65 years from 63 centers from France, Switzerland, and Belgium, randomized or assigned to receive AraC, were selected for the present joint study. Median follow-up was 62 months.

Main clinical and biologic presenting features of patients included in each trial are summarized in Table 1. Significantly higher WBC count (median: 7.1 × $10^9$/L vs 2.5 × $10^9$/L, $P < .001$), higher platelet count (median: 32 × $10^9$/L vs 22 × $10^9$/L, $P < .001$), and older age (median: 43 vs 37 years, $P = .016$) were observed in APL 2000 trial compared with LPA 99 trial, while no significant difference was seen for other pretreatment factors.

The distribution of patients in the 3 risk groups (according to Sanz score) was as follows: low-risk group, 24.2% versus 17.8%; intermediate risk group, 29.8% versus 56.8%; and high-risk group, 46% versus 25.4% ($P < .001$) in APL 2000 and LPA 99 trials, respectively. The imbalance between the 2 trials resulted from the fact that we restricted, in APL 2000 trial, the joint analysis to patients who received AraC, excluding low-risk patients who were randomized years with WBC or assigned to receive induction treatment without AraC. For those reasons, comparisons between the 2 studies were adjusted for age, sex, and WBC and platelet counts.

Overall, in LPA 99 trial, CR was obtained in 381 (92.9%) of 410 patients, while 28 (6.8%) patients had early death and 1 (0.2%) had resistant leukemia. The cumulative incidence of relapse (CIR) at 2 and 3 years was 5.5% and 7.4%, respectively, and 5 patients (1.3%) died in CR.

In APL 2000 trial, CR was obtained in 173 (97.2%) of 178 patients, while 5 (2.8%) patients had early death and no patient had resistant leukemia. The cumulative incidence of relapse at 2 and 3 years was 4.5% and 12.0%, respectively, and 5 (2.9%) patients died in CR.

### Comparisons in the different risk groups

***Low- and intermediate-risk groups.*** Main presenting features of the 402 low- and intermediate-risk patients (306 treated in LPA 99 trial, 96 treated in APL 2000 trial) are summarized in Table 2.

The CR rates were 99% in APL 2000 trial versus 96.1% in LPA 99 trial ($P = .32$). Nine patients relapsed in APL 2000 trial compared with 16 in LPA 99 trial. No extramedullary relapse was seen in APL 2000 trial but 2 in LPA 99 trial (both involving the CNS), both in the intermediate-risk group (after 24 and 86 months). The 3-year CIR and rate of death in CR were 14.3% versus 4.2% ($P = .03$) and 2.1% versus 1.4% ($P = .38$) in APL 2000 and LPA 99 trials, respectively. The 3-year EFS and overall survival were 89.4% versus 91.4% ($P = .16$) and 95.6% versus 93.8% ($P = .53$) in APL 2000 and LPA 99 trials, respectively (Figure 2).

Median duration of hospital stay was 72 days compared with 50 days in APL 2000 and LPA 99 trials, respectively ($P < .001$).

***High-risk group.*** Their presenting characteristics (82 treated in APL 2000 trial, 104 treated in LPA 99 trial) are summarized in Table 3. The CR rate was 95.1% in APL 2000 trial and 83.6% in LPA 99 trial ($P = .018$). There were 4 induction deaths (4.9%) in APL 2000 trial (3 from sepsis and 1 from bleeding) and 17 (16.4%) in LPA 99 trial (12 from bleeding, 3 from differentiation syndrome, and 2 from infection). Seven patients relapsed in APL 2000 trial compared with 17 in LPA 99 trial. Two relapses involved the CNS

**Table 2. Pretreatment characteristics and outcome of the patients (low- and intermediate-risk groups) included in the joint analysis**

| Characteristics | LPA 99 trial, N = 306 | APL 2000 trial, N = 96 | *P* | Adjusted *P** |
|---|---|---|---|---|
| Age, y, median (Q1-Q3) | 39 (27-50) | 43 (32-51.5) | .15 | — |
| Age younger than 60 y, no. (%) | 281 (91.8) | 94 (97.9) | .036 | — |
| Male sex, no. (%) | 148 (48.4) | 41 (42.7) | .35 | — |
| WBC count, $10^9$/L, median (Q1-Q3) | 1.6 (1.0-3.2) | 1.8 (1.0-3.8) | .73 | — |
| Platelet count, $10^9$/L, median (Q1-Q3) | 23 (13-40) | 36 (20-63) | <.001 | — |
| Fibrinogen level, g/L, median (Q1-Q3) | 1.6 (1.1-2.5) | 1.8 (1.1-2.7) | .58 | — |
| **Sanz score, no. (%)** | | | | |
| Low risk | 73 (23.9) | 43 (44.8) | <.001 | — |
| Intermediate risk | 233 (76.1) | 53 (55.2) | — | — |
| Complete remission, no. (%) | 294 (96.1) | 95 (99.0) | .32 | .28 |
| Deaths in CR, no. (%) | 4/294 (1.4) | 2/95 (2.1) | .38 | .57 |
| Relapses, no. (3-y cumulative incidence, %) | 16/294 (4.2) | 9/95 (14.3) | .03 | .005 |
| Total events, no. (3-y EFS, %) | 31 (91.4) | 12 (89.4) | .16 | .12 |
| Total deaths, no. (3-y OS, %) | 20 (93.8) | 4 (96.6) | .53 | .59 |

— indicates not applicable.
*Adjusted for age, sex, WBC, and platelet count.

in LPA 99 (at 13 and 30 months from CR) and none in APL 2000. The 3-year CIR and rate of deaths in CR were 9.9% versus 18.5% ($P = .12$) and 3.8% versus 1.1% ($P = .24$) in APL 2000 and LPA 99 trials, respectively. The 3-year EFS and overall survival were 82.2% versus 67.3% ($P = .023$) and 91.5% versus 80.8% ($P = .026$) in APL 2000 and LPA 99 trials, respectively (Figure 3). Median duration of hospital stay was 71 days in APL 2000 compared with 50 days in LPA 99 trial ($P < .001$).

## Discussion

The 2 trials (LPA 99 and APL 2000) analyzed together here shared many similarities including frontline treatment with ATRA and anthracycline-based chemotherapy followed by anthracycline-based consolidation treatment and a maintenance treatment combining intermittent ATRA and continuous 6-mercaptopurine and methotrexate, which are now widely considered as references in the treatment of APL. Differences between the trials were mainly the type of anthracycline used and the addition (or not) of AraC to anthracycline (Figure 1).[8,14]

The joint analysis we performed suggests that, in patients with WBC count less than $10 \times 10^9$/L, the PETHEMA approach yields even fewer relapses than a classical ATRA plus DNR plus AraC regimen, while being less myelosuppressive, and therefore leading to significantly shorter hospital stays and less mortality in CR. The lower rate of relapse seen in PETHEMA

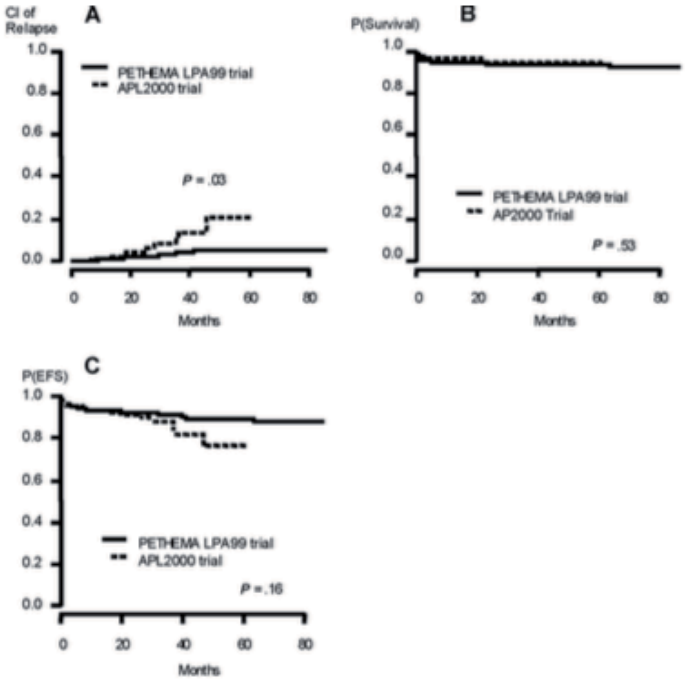



**Figure 2. Outcome of the patients (low- and intermediate-risk group) included in the joint analysis.** (A) Cumulative incidence of relapse. (B) Overall survival. (C) Event-free survival.

**Table 3. Pretreatment characteristics and outcome of the patients (high-risk group) included in the joint analysis**

| Characteristics | LPA 99 trial, N = 104 | APL 2000 trial, N = 82 | P | Adjusted P* |
|---|---|---|---|---|
| Age, y, median (Q1-Q3) | 34 (21.5-42.5) | 41 (27-51) | .007 | — |
| Age younger than 60 y, no. (%) | 97 (93.3) | 73 (89.0) | .43 | — |
| Male sex, no. (%) | 52 (50.0) | 39 (47.6) | .77 | — |
| WBC, $10^9$/L, median (Q1-Q3) | 27.3 (15.4-54.1) | 30.1 (14.3-58.5) | .89 | — |
| Platelets, $10^9$/L, median (Q1-Q3) | 19 (12-34.5) | 27 (16-54) | .001 | — |
| Fibrinogen level, g/L, median (Q1-Q3) | 1.2 (0.7-1.8) | 1.25 (0.9-1.8) | .22 | — |
| CR, no. (%) | 87 (83.6) | 78 (95.1) | .018 | .001 |
| Deaths in CR, no. (%) | 1/87 (1.1) | 3/78 (3.8) | .24 | .22 |
| Relapses, no. (3-y cumulative incidence, %) | 17/87 (18.5) | 7/78 (9.9) | .12 | .15 |
| Total events, no. (3-y EFS, %) | 35 (67.3) | 14 (82.2) | .023 | .024 |
| Total deaths, no. (3-y OS, %) | 23 (80.8) | 7 (91.5) | .026 | .022 |

— indicates not applicable.
*Adjusted for age, sex, white blood cell count, and platelet count.

regimen may result from several factors. One reason may be the anthracycline used (idarubicin and mitoxantrone instead of DNR). The superiority of idarubicin over daunorubicin has been suggested in several randomized AML trials, although the cumulative dose of daunorubicin may have been lower than that of idarubicin in some of those studies.[23-25] In addition, those studies did not specifically address APL.[23-25] A higher anthracycline cumulative dose may also have contributed to the superiority of the PETHEMA approach in patients with low WBC count. Indeed, the PETHEMA group used cumulative doses of idarubicin and mitoxantrone of 80 (100 for intermediate-risk patients) and 50 mg/m$^2$, respectively. Using a 1- to 5-dose equivalence between idarubicin (or mitoxantrone) and daunorubicin (although there is no clear consensus on this point)[26] the cumulative anthracycline dose used in the LPA 99 trial would correspond to 130% to 150% of that used in APL 2000 (495 mg/m$^2$ of DNR). Interestingly, before the ATRA era, the Italian GIMEMA group randomized idarubicin alone versus idarubicin plus AraC in the treatment of newly diagnosed APL.[27] The cumulative dose of anthracycline was higher in patients treated with idarubicin alone (60 mg/m$^2$) than in those treated with idarubicin-AraC (48 mg/m$^2$). The 8-year event-free survival rate was significantly better for the patients treated with idarubicin alone (35% vs 23%) suggesting not only that cytarabine could be avoided in the treatment of APL when high cumulative doses of an anthracycline are used, but also a dose-effect relationship with anthracycline in APL. Other retrospective analyses published before the ATRA era also suggested a benefit for high-dose anthracycline in APL.[28-30] Of note, no cardiac toxicity was observed in APL 2000 and LPA 99 trials. Long-term toxicity of high cumulative anthracycline doses is difficult to estimate after a median follow-up of only 5 years, but longer

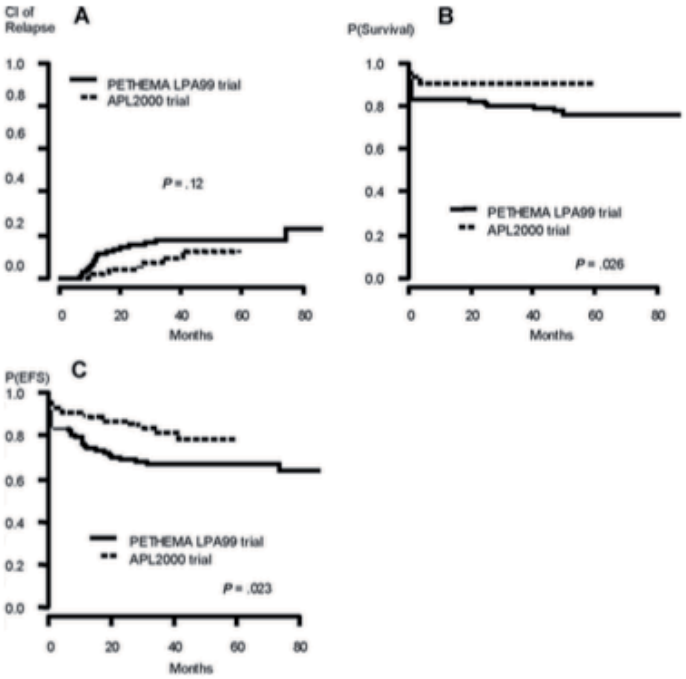



**Figure 3. Outcome of the patients (high-risk group) included in the joint analysis.** (A) Cumulative incidence of relapse. (B) Overall survival. (C) Event-free survival.

experience of the Spanish PETHEMA group over the past 12 years with high cumulative doses of idarubicin in APL has so far revealed no long-term cardiac toxicity (M.A.S, oral communication, 2006).

Finally, the difference between APL 2000 and LPA 99 trials could have been due to the addition of ATRA during consolidation courses in the LPA 99 trial in intermediate-risk patients, which may have contributed to a lower relapse risk in this group, although such benefit would, of course, have to be confirmed in a randomized trial.

The better results obtained in LPA 99 trial in low- and intermediate-risk patients suggest that, at least with high doses of idarubicin during induction and consolidation, and possibly with addition of ATRA during consolidation courses, AraC is not required for the treatment of low- and intermediate-risk APL. Furthermore, avoiding AraC reduced myelosuppression in LPA 99 trial, leading to a mortality in CR of 1% compared with 3% in APL 2000.

In patients with high WBC counts, by contrast, APL 2000 results yielded better 2-year EFS (87.7% vs 69.2%) and survival (91.5% vs 81.7%) and a trend for fewer relapses, suggesting a beneficial role for AraC in this subset of patients, possibly at high dose. Indeed, in APL 2000 trial, higher doses of AraC were used in the last consolidation course (2 g/m$^2$ per 12 hours during 5 days). A significantly higher CR rate (95.1% vs 83.6%) was also obtained in APL 2000 trial, suggesting that AraC during induction might play a role in APL patients with high initial WBC count.

The Italian GIMEMA group compared results of 2 successive treatment regimens in patients who had achieved CR with ATRA and idarubicin, one with high-dose AraC (and also etoposide and 6-thioguanine) during consolidation courses (AIDA-2000) and one with idarubicin alone (AIDA 04-93 trial).[31] Although the incidence of relapse was similar between the 2 trials for low and intermediate risk, a significantly higher incidence of relapse was observed in the AIDA-0493 versus AIDA-2000 study in high-risk patients (29% vs 2%), also suggesting a role for AraC in this group of patients. A prospective German study evaluating the role of an intensified double induction (6-thioguanine, Ara C, DNR/high-dose AraC, mitoxantrone [TAD/HAM]) regimen with AraC at conventional dose followed by high-dose (3 g/m$^2$ per 12 hours during 3 days) and maintenance therapy found a CR rate of 92% and a 2-year overall survival and relapse-free survival of 88% and 96%, respectively.[32] A higher initial leukocyte count was not identified as a risk factor for relapse, contrary to previous studies of that group with conventional dose AraC, suggesting a high antileukemic effectiveness of high-dose AraC in high-risk patients. In our study, we found only a favorable trend for a lower CIR in the APL 2000 study ($P = .15$).

In conclusion, this study suggests that ATRA combined with a high cumulative dose of idarubicin without AraC but with maintenance treatment, like the PETHEMA approach, may give excellent results with limited toxicity in low- or intermediate-risk APL (ie, with WBC count $< 10 \times 10^9$/L). Those results will have to be confirmed in randomized trials. We are currently randomizing, in our ongoing APL 2006 trial (conducted in France, Belgium, and Switzerland) in low- and intermediate-risk APL, consolidation treatment between idarubicin plus AraC and idarubicin plus ATRA (and also idarubicin + arsenic trioxide).

On the other hand, present data suggest that the addition of AraC to ATRA and anthracyclines in high-risk patients may result in a trend toward lower incidence of relapse, and to better survival. Preliminary results suggest that arsenic derivatives may also be useful in the consolidation treatment of APL with high WBC counts, in combination or perhaps instead of AraC.[33] We are also testing, in APL 2006 trial, the role of arsenic derivatives in patients with high WBC counts.

## Acknowledgments

This study was supported in part by the program Hospitalier de Recherche Clinique and the CHU of Lille-France, the Association pour la Recherche sur le Cancer and the Ligue Contre le Cancer (Comité du Nord), grant 2006/0137 from the Fundación para la Investigación Hospital Universitario La Fe-Ayudas Bancaja, and grant RD06/0020/0031from the Red Temática de Investigación Cooperativa en Cáncer.

Additional study participants are listed in Document S1, available on the *Blood* website; see the Supplemental Materials link at the top of the online article.

## Authorship

Contribution: L.A., M.A.S., P.M., H.D., P.F., and L.D. conceived the study, and collected, analyzed, and interpreted the data; L.A., M.A.S. P.M., and P.F. wrote the paper; S.C did the statistical analyses; L.A., M.A.S., P.M., P.C., E.R., E.V., A.G., A.P., F.H., C.R., A.M.S., J.S., J.-Y.C., S.M.-M., T.P., X.T., S.B., R.P., J.B., T.L., A.V., S.N., N.I., H.D., A.F., D.B., and P.F. recruited the patients and collected the data.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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## 9.2 Artículo Anexo 2

Sanz MA, Montesinos P, Rayón C, Holowiecka A, de la Serna J, Milone G, de Lisa E, Brunet S, Rubio V, Ribera JM, Rivas C, Krsnik I, Bergua J, González J, Díaz-Mediavilla J, Rojas R, Manso F, Ossenkoppele G, González JD, Lowenberg B; PETHEMA and HOVON Groups.

Risk-adapted treatment of acute promyelocytic leukemia based on all-trans retinoic acid and anthracycline with addition of cytarabine in consolidation therapy for high-risk patients: further improvements in treatment outcome.

Blood. 2010 Jun 24;115(25):5137-46.

CLINICAL TRIALS AND OBSERVATIONS

CME article

# Risk-adapted treatment of acute promyelocytic leukemia based on all-*trans* retinoic acid and anthracycline with addition of cytarabine in consolidation therapy for high-risk patients: further improvements in treatment outcome

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**A risk-adapted strategy based on all-*trans* retinoic acid (ATRA) and anthracycline monochemotherapy (PETHEMA LPA99 trial) has demonstrated a high antileukemic efficacy in acute promyelocytic leukemia. We designed a new trial (LPA2005) with the objective of achieving stepwise improvements in outcome. Between July 2005 and April 2009, low- and intermediate-risk patients (leukocytes < 10 × 10⁹/L) received a reduced dose of mitoxantrone for the second consolidation course, whereas high-risk patients younger than 60 years of age received cytarabine combined with ATRA and idarubicin in the first and third consolidation courses. Of 372 patients attaining complete remission after ATRA plus idarubicin (92.5%), 368 proceeded to consolidation therapy. For low- and intermediate-risk patients, duration of neutropenia and thrombocytopenia and hospital stay were significantly reduced without sacrificing antileukemic efficacy, compared with the previous LPA99 trial. For high-risk patients, the 3-year relapse rate was significantly lower in the LPA2005 trial (11%) than in the LPA99 (26%; $P = .03$). Overall disease-free survival was also better in the LPA2005 trial ($P = .04$). In conclusion, the lower dose of mitoxantrone resulted in a significant reduction of toxicity and hospital stay while maintaining the antileukemic activity, and the combination of ATRA, idarubicin, and cytarabine for high-risk acute promyelocytic leukemia significantly reduced the relapse rate in this setting. Registered at http://www.clinicaltrials.gov as NCT00408278. (*Blood*. 2010;115(25):5137-5146)**

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**Disclosures**

The authors and Associate Editor Martin S. Tallman declare no competing financial interests. The CME questions author Désirée Lie, University of California, Irvine, CA, served as a nonproduct speaker for "Topics in Health" for Merck Speaker Services.

**Learning objectives**

Upon completion of this activity, participants will be able to:

1. Describe the overall differences between the LPA99 (AIDA-ATRA + idarubicin-) and LPA2005 (AIDA + cytarabine in high-risk) treatment protocols for acute promyelocytic leukemia
2. Identify differences in the treatment of low-risk, intermediate-risk, and high-risk patients in the AIDA and AIDA + cytarabine in high-risk protocols
3. Compare the rate of differentiation syndrome among patients who received the AIDA and AIDA + cytarabine in high-risk protocols
4. Compare hospitalization outcomes for patients who received the AIDA versus the AIDA + cytarabine in high-risk protocols
5. Compare differences in relapse rate among patients with acute promyelocytic leukemia on the AIDA versus the AIDA + cytarabine in high-risk protocol

Submitted January 21, 2010; accepted March 30, 2010. Prepublished online as *Blood* First edition paper, April 14, 2010; DOI 10.1182/blood-2010-01-266007.

The online version of this article contains a data supplement.

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## Introduction

A risk-adapted strategy designed by the cooperative group Programa Español de Tratamientos en Hematología (PETHEMA), which was based on the combination of all-*trans* retinoic acid (ATRA) and anthracycline monochemotherapy for induction and consolidation (PETHEMA LPA99 trial), has previously demonstrated a high antileukemic efficacy and high protocol compliance coupled with moderate toxicity in patients with acute promyelocytic leukemia (APL).[1,2]

A new trial (LPA2005 trial) to improve the outcome of this strategy was designed with the primary objective of decreasing the relapse rate in high-risk patients (ie, presenting white blood cell [WBC] counts $> 10 \times 10^9$/L)[3] younger than 60 years of age. To accomplish this objective, cytarabine was added to the combination of ATRA and idarubicin in consolidation therapy. In addition, the protocol intended to reduce toxicity during consolidation therapy in low- and intermediate-risk patients (ie, presenting WBC counts $< 10 \times 10^9$/L) by a dose reduction of mitoxantrone. Although the potential benefit of the addition of cytarabine has been suggested in previous studies,[4] the specific use of this agent for high-risk patients was mainly supported by a joint study of the PETHEMA and the European APL groups[5] and a study of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA).[6] The authors of both studies have suggested a benefit in terms of reduction of relapse risk with the use of cytarabine in consolidation in patients with high-risk disease. We report here the results obtained in 402 consecutive patients with newly diagnosed APL who were enrolled in the LPA 2005 trial. The outcome was compared with that achieved in 561 patients treated with the previous LPA99 trial.

## Methods

### Eligibility

Patients enrolled in the consecutive PETHEMA LPA99 and LPA2005 trials were required to have a diagnosis of de novo APL with demonstration of the t(15;17) or *PML/RARA* rearrangements, normal hepatic and renal function, no cardiac contraindications to anthracycline chemotherapy, and an Eastern Cooperative Oncology Group (ECOG) performance status of less than 4. Informed consent was obtained from all patients. According to the Declaration of Helsinki, the protocol was approved by the Research Ethics Board of each participating hospital. The trial is registered at http://www.ClinicalTrials.gov (NCT00408278).

### Induction therapy

Induction therapy consisted of oral ATRA (45 mg/m$^2$/d) divided into 2 daily doses, which was maintained until complete hematologic remission and idarubicin (12 mg/m$^2$/d) given as an intravenous bolus on days 2, 4, 6, and 8 (ATRA and idarubicin [AIDA] regimen), except for patients older than 70 years of age who received only the 3 first doses of idarubicin. For patients 20 years of age or younger, the ATRA was adjusted to 25 mg/m$^2$/d. Induction therapy was identical for both LPA2005 and LPA99 trials.

### Postremission therapy

Following a risk-adapted strategy, patients who achieved complete remission (CR) received 3 monthly consolidation courses with ATRA and anthracycline-based chemotherapy. Figure 1 summarizes the ATRA and chemotherapy dose and schedule of consolidation therapy in both LPA2005 and LPA99 trials. In brief, compared with the LPA99 trial that has been described elsewhere,[1] the changes implemented in the LPA2005 were the following: (1) For low-risk patients, ATRA was combined to chemotherapy in the 3 consolidation courses; in addition, mitoxantrone was reduced from 5 to 3 days in the second course of consolidation. (2) For intermediate-risk patients, mitoxantrone was also reduced from 5 to 3 days in the second course of consolidation. (3) For high-risk patients, cytarabine was added together with a slight reduction of idarubicin in the first and third consolidation course. High-risk patients older than 60 years did not receive cytarabine and were treated as intermediate-risk patients. Maintenance therapy was identical in both PETHEMA LPA99 and LPA2005 trials as was described elsewhere.[1]

### Supportive measures

Platelet transfusions, fresh-frozen plasma, and cryoprecipitate for the management of coagulopathy in the LPA99 and LPA2005 trials were given as previously described.[7] In the LPA2005 trial, tranexamic acid prohylaxis was not used. The management of differentiation syndrome (DS) also was similar in both trials,[1] except for prophylaxis. In the LPA99 trial, DS prophylaxis with prednisone (0.5 mg/kg/d orally for 15 days) was given to all patients, whereas in the LPA2005 trial, only patients with WBC count greater than $5 \times 10^9$/L at presentation or achieved during the 2 first weeks of ATRA therapy received dexamethasone (2.5 mg/m$^2$/12 hours intravenously for 15 days).

### Definitions and study end points

Remission induction response was assessed according to the recently revised criteria by Cheson et al[8] For morphologic assessment of leukemia resistance, it was required that sufficient time had passed to allow for full terminal differentiation of the malignant promyelocytes (up to 40-50 days). Molecular remission was defined as the disappearance on an ethidium bromide gel of the PML/RARA-specific band visualized at diagnosis with the use of reverse-transcription polymerase chain reaction (RT-PCR) assays with a sensitivity level of one leukemic cell in $10^{-4}$ cells. Molecular persistence was defined as PCR positivity in 2 consecutive bone marrow samples collected at the end of consolidation therapy. Molecular relapse was defined as previously reported.[3] Diagnosis of the DS was made according to the presence of the following signs or symptoms described by Frankel et al[9]: unexplained fever, dyspnea, pleural and/or pericardial effusion, pulmonary infiltrates, renal failure, hypotension, and unexplained weight gain greater than 5 kg. No single sign or symptom was considered sufficient to make a diagnosis of the syndrome. Patients with alternative explanations for the clinical complex, such as pulmonary hemorrhage, septic shock, pneumonia, or cardiac failure, were considered not to have DS. As defined elsewhere,[10] patients with 4 or more of the aforementioned signs or symptoms were classified as having severe DS, whereas those with 2 or 3 signs or symptoms were considered to have moderate DS. Relapse-risk groups were defined as reported elsewhere[3] as follows: low-risk patients had a WBC count less than $10 \times 10^9$/L and a platelet count more than $40 \times 10^9$/L; intermediate-risk patients had a WBC count less than $10 \times 10^9$/L and a platelet count less than $40 \times 10^9$/L; and high-risk patients had a WBC count equal to or more than $10 \times 10^9$/L. Hematologic toxicity was graded according to the National Cancer Institute Common Toxicity Criteria, version 2.

### Statistical analysis

The $\chi^2$ test, with Yates correction if necessary, was used to analyze differences in the distribution of categorical variables between patient subsets. The *t* test and Mann-Whitney *U* test were used to detect differences in the distribution of continuous parametric and nonparametric variables, respectively. Results were analyzed on an intention-to-treat basis. Unadjusted time-to-event analyses were performed by use of the Kaplan-Meier estimate[11] and, for comparisons, log-rank tests.[12] The probability of relapse was also estimated by the cumulative incidence method (for marginal probability).[13,14] Overall survival (OS) was calculated from the start date of induction therapy, whereas cumulative incidence of relapse (CIR) and disease-free survival (DFS) were calculated from the date of CR. In the analysis of DFS, relapse, development of secondary myelodysplastic syndrome or acute leukemia, and death in CR were considered uncensored events, whichever occurred first. For cumulative incidence analysis, death

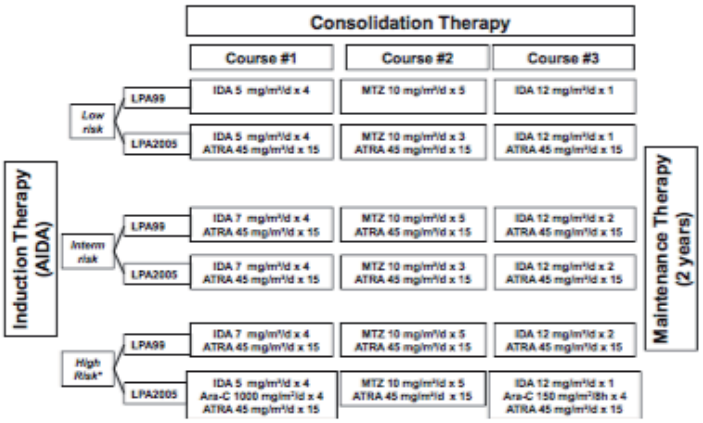



**Figure 1. Treatment schedule of the LPA99 and LPA2005 trials.** Ara-C indicates cytarabine; IDA, idarubicin; and MTZ, mitoxantrone. High-risk patients older than 60 years did not receive cytarabine and were treated as intermediate-risk patients.

in CR and development of secondary myelodysplastic syndrome or acute leukemia were considered as a competing cause of failure. For all estimates in which the event "relapse" was considered as an end point, hematologic and molecular relapse, as well as molecular persistence at the end of consolidation, were each considered as uncensored events. Patient follow-up was updated on October 15, 2009. All $P$ values reported are 2-sided. Multivariate analyses were performed by use of the Cox model for DFS and OS,[15] and Fine and Gray model for CIR.[16] Computations were performed by use of the 3D, 4F, 1L, and 2L programs from the BMDP statistical library (BMDP Statistical Software), and R 2.9.2 software package for CIR and Fine and Gray model.

## Results

### Accrual and patient characteristics

Between July 2005 and April 2009, 437 consecutive patients with genetic diagnosis of APL were registered from 81 institutions from Spain, Poland, the Netherlands, Argentina, Uruguay, and the Czech Republic (see the supplemental Appendix, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). A total of 33 patients (7.6%) were considered not eligible for the treatment because of severe clinical condition contraindicating the administration of chemotherapy. Thus, 404 patients met the previously defined entry criteria and were enrolled in the LPA2005 trial. Two additional patients were not evaluated because of protocol violations during induction therapy (addition of cytarabine in one patient and use of daunorubicin instead of idarubicin in another). The main clinical and biologic characteristics of the 402 patients evaluable for induction are shown in Table 1. For comparison, data of 561 patients treated with the previously reported LPA99 trial[2] also are included in Table 1. Information on enrollment, eligible patients, lost at follow-up, and exclusion from analysis is shown in a flow diagram (Figure 2). Patients in both trials were comparable for all baseline characteristics except for age and ECOG performance status. The proportion of patients younger than 18 years in the LPA2005 was lower than in the LPA99 trial ($P = .01$), whereas the proportion of those with ECOG 0 to 1 was greater ($P = .02$).

### Induction therapy

***Response and induction mortality.*** Three hundred seventy-two of the 402 evaluable patients achieved morphologic CR (92.5%; 95% confidence interval 90.6%-94.4%). The median time interval to CR was 39 days (range, 18-81 days). The median time to reach neutrophil counts greater than $1 \times 10^9$/L, and platelet counts greater than $50 \times 10^9$/L was 24 days (range, 6-72 days) and 19 days (range, 4-80 days), respectively. All the 30 induction failures were the result of death during induction.

Hemorrhage and infection accounted for most of the deaths during induction therapy (15 and 6 patients, respectively). Deaths caused by hemorrhage were caused by intracranial (12 patients, 80%), pulmonary (2 patients, 13%), and gastrointestinal hemorrhages (1 patient, 7%). DS and acute myocardial infarction were contributing causes of death in 4 and 2 patients, respectively. The remaining 3 deaths were attributable to massive suprahepatic thrombosis, myocarditis, and cardiac failure in 1 patient each. The response rates and causes of induction death were quite similar in both the LPA2005 and LPA99 studies (Table 2).

Patients with WBC counts greater than 10 and $50 \times 10^9$/L had a poorer response rate (83% and 73%, respectively) compared with those with low- and intermediate-risk patients (99% and 96%, respectively; $P < .001$). Patients older than 60 years had also a lower CR rate (88%) than younger patients (93%), but this difference was not statistically significant ($P = .15$),

***Differentiation syndrome.*** One hundred six of the 372 patients (28.5%) who were evaluable for this complication developed DS. Severe DS was diagnosed in 45 patients (12.1%), 4 of whom died from it, whereas moderate DS was reported in 61 (16.3%; Table 2). In 9 additional patients (2.5%) with possible DS, an unambiguous diagnosis of DS could not be made. This was attributable to the presence of concurrent medical problems that could explain the clinical manifestations. These problems were pulmonary hemorrhage in 5 patients, pneumonia in 2, renal failure in 1, and septic shock in another. ATRA was temporarily discontinued in 78 patients (74%) with DS. Diuretics and intravenous dexamethasone were administered in 95 patients (90%) and 88 patients (83%), respectively, whereas mechanical ventilation and dialysis was needed in 8 patients (8%) and 3 patients (3%), respectively.

### Consolidation therapy

***Tolerability and treatment feasibility.*** Three hundred sixty-eight of the 372 patients who achieved CR proceeded to consolidation

**Table 1. Demographic and baseline characteristics of the study population according to PETHEMA/HOVON trial**

| | LPA99 | | LPA2005 | | |
|---|---|---|---|---|---|
| **Characteristic** | **Median (range)** | **N (%)** | **Median (range)** | **N (%)** | ***P*** |
| Overall | | 561 (100) | | 402 (100) | |
| **Age, y** | 40 (2-83) | | 42 (3-83) | | |
| Younger than 18 | | 65 (12) | | 26 (6) | .01* |
| 18-40 | | 223 (40) | | 168 (42) | |
| 41-60 | | 171 (30) | | 150 (37) | |
| 61-70 | | 68 (12) | | 36 (9) | |
| Older than 70 | | 34 (6) | | 22 (6) | |
| **Sex** | | | | | |
| Male | | 270 (48) | | 209 (52) | .22 |
| Female | | 291 (52) | | 193 (48) | |
| **ECOG** | 1 (0-3) | | 1 (0-3) | | |
| 0-1 | | 378 (73) | | 267 (80) | .02 |
| 2 | | 102 (20) | | 41 (12) | |
| 3 | | 36 (7) | | 26 (8) | |
| **Fever** | | | | | |
| No | | 334 (60) | | 238 (63) | .33 |
| Yes | | 220 (40) | | 137 (37) | |
| **WBC count, $\times 10^9$/L** | 2.2 (0.2-460) | | 3.0 (0.3-126) | | |
| 5 or less | | 373 (67) | | 248 (62) | .13† |
| 5-10 | | 47 (8) | | 36 (9) | |
| 10-50 | | 100 (18) | | 96 (24) | |
| More than 50 | | 40 (7) | | 22 (5) | |
| **Platelet count, $\times 10^9$/L** | 22 (1-207) | | 23 (1-235) | | |
| 40 or less | | 432 (77) | | 298 (74) | .24 |
| More than 40 | | 128 (23) | | 104 (26) | |
| **Relapse-risk group** | | | | | |
| Low | | 107 (19) | | 84 (21) | .14 |
| Intermediate | | 313 (56) | | 200 (50) | |
| High | | 140 (25) | | 118 (29) | |
| **Hemoglobin, g/dL** | 9.2 (3-16.9) | | 9.3 (2.4-14.5) | | |
| 10 or less | | 361 (65) | | 255 (63) | .74 |
| More than 10 | | 199 (35) | | 147 (37) | |
| **Creatinine, mg/dL** | 0.9 (0.2-2.4) | | 0.8 (0.3-3.1) | | |
| 1.4 or less | | 526 (98) | | 365 (97) | .48 |
| More than 1.4 | | 13 (2) | | 12 (3) | |
| **Uric acid, mg/dL** | 3.9 (0.29-12.7) | | 4.1 (1.0-10.5) | | |
| 7 or less | | 445 (95) | | 327 (96) | .63 |
| More than 7 | | 24 (5) | | 15 (4) | |
| **Fibrinogen, mg/dL** | 158 (0-862) | | 179 (37-777) | | |
| 170 or less | | 280 (54) | | 176 (48) | .07 |
| More than 170 | | 240 (46) | | 193 (52) | |
| **Albumin, g/dL** | 4.0 (1.7-6.7) | | 4.1 (2.0-6.0) | | |
| 3.5 or less | | 107 (24) | | 66 (20) | .15 |
| More than 3.5 | | 335 (76) | | 267 (80) | |
| **Morphologic subtype** | | | | | |
| Hypergranular | | 452 (82) | | 296 (80) | .44 |
| Microgranular | | 99 (18) | | 74 (20) | |
| **PML/RARα isoform** | | | | | |
| BCR1/BCR2 | | 300 (60) | | 146 (55) | .29 |
| BCR3 | | 204 (40) | | 117 (45) | |

BCR indicates breakpoint cluster region; ECOG, Eastern Cooperative Oncology Group; PML, promyelocytic leukemia; RAR, retinoic acid receptor; and WBC, white blood cell.

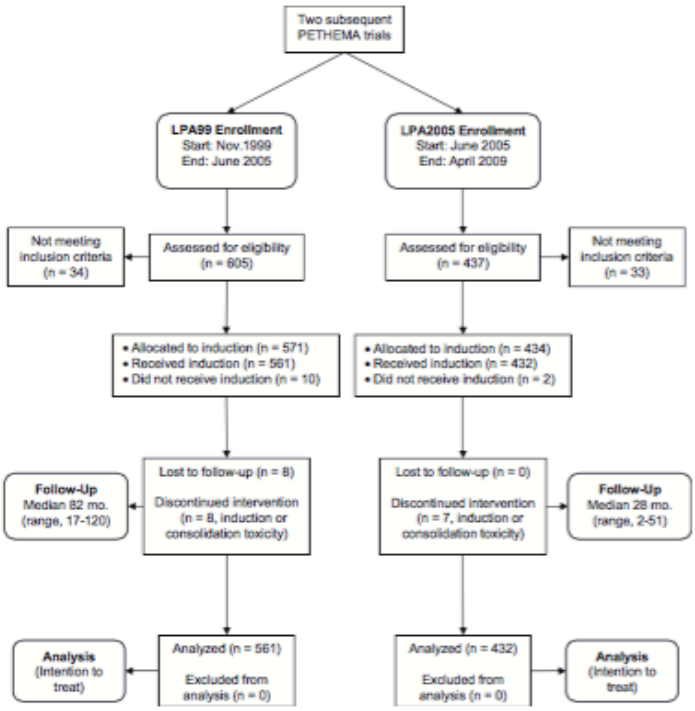
*P compares < 18 versus ≥ 18 years old.

†P compares ECOG 0 to 1 versus ECOG 2 to 3.

therapy as scheduled. Three of the 4 exceptions who developed severe cardiac toxicity during induction received a modified consolidation treatment. It consisted of substituting idarubicin and mitoxantrone for a bioequivalent dose of liposomal daunorubicin in 1 patient and arsenic trioxide in another. The remaining patient with cardiac toxicity and one additional 73-year-old patient who had a perianal abscess during induction proceeded directly to maintenance therapy. Four additional patients died during consolidation therapy (3 high risk; 1 low risk), 3 as the result of infection, and 1 to intracranial bleeding. Three further patients did not receive the third course of consolidation because of toxicity in previous courses. The remaining 361 patients (97%) completed the 3 consolidation courses as scheduled.

***Hematologic toxicity.*** Details on hematologic toxicity and length of in-hospital stay in each consolidation course are shown in Table 3. Low- and intermediate-risk patients after the second consolidation

**Figure 2. Consolidated Standards of Reporting Trials (CONSORT) diagram for the subsequent LPA99 and LPA2005 PETHEMA trials.**




course of the LPA2005 with reduced dose of mitoxantrone showed a lower rate of prolonged (> 15 days) grade 3 to 4 neutropenia and grade 4 thrombocytopenia compared with the results of the LPA99 trial ($P < .001$). This reduction of hematologic toxicities was associated with a significantly lower proportion of patients requiring a hospital stay longer than 10 days ($P < .001$). Overall, the mean length of in-hospital stay during the 3 consolidation courses in low- and intermediate-risk patients was also reduced (ie, 22 days in the LPA99 and 17 days in the LPA2005 trial; $P < .001$). In contrast, high-risk patients receiving the combination of ATRA, idarubicin, and cytarabine as part of the treatment schedule had a greater rate of prolonged grade 3 to 4 neutropenia and grade 4 thrombocytopenia during the first and third consolidation courses of the LPA2005, compared with the LPA99 trial ($P < .001$). This increase of hematologic toxicities was associated with a significantly greater proportion of patients requiring hospitalization longer than 10 days ($P < .001$). The mean length of in-hospital stay during the 3 consolidation courses in this setting was 26 days in the LPA99 and 33 days in the LPA2005 trial ($P = .10$).

***Molecular response.*** RT-PCR tests for PML/RARA at the end of consolidation therapy were available in 320 patients (89%). No patient showed evidence of molecular persistence, whereas 3 patients were positive at this point in time in the LPA99 trial

**Table 2. Induction outcome and differentiation syndrome of APL patients in the PETHEMA LPA99 and LPA2005 trials**

| | LPA99 | | LPA2005 | | |
|---|---|---|---|---|---|
| **Characteristic** | **Median (range)** | **N (%)** | **Median (range)** | **N (%)** | ***P*** |
| Overall | | 561 (100) | | 402 (100) | |
| **Morphologic CR** | | 511 (91.1) | | 372 (92.5) | .42 |
| Days to CR | 37 (21-77) | | 39 (18-81) | | .38 |
| Days to PMN > 1 × $10^9$/L | 23 (0-60) | | 24 (6-72) | | .36 |
| Days to platelets > 50 × $10^9$/L | 19 (0-50) | | 19 (4-80) | | .08 |
| **Cause of induction death** | | | | | |
| Hemorrhage | | 28 (5.0) | | 15 (3.7) | .44 |
| Infection | | 12 (2.1) | | 6 (1.5) | .62 |
| Differentiation syndrome | | 8 (1.4) | | 4 (1.0) | .55 |
| Other | | 2 (0.4) | | 5 (1.2) | .22 |
| **Differentiation syndrome** | | | | | |
| Severe | | 66 (12) | | 45 (12) | .12 |
| Moderate | | 66 (12) | | 61 (16) | |
| Absent | | 429 (76) | | 266 (72) | |

CR indicates complete response; and PMN, polymorphonuclear leukocytes.

**Table 3. Severity of hematologic toxicity and hospitalization associated with consolidation therapy in the LPA99 and LPA2005 trials**

| | Course 1 | | | Course 2 | | | Course 3 | | |
|---|---|---|---|---|---|---|---|---|---|
| Toxicity | LPA99 N (%) | LPA2005 N (%) | P | LPA99 N (%) | LPA2005 N (%) | P | LPA99 N (%) | LPA2005 N (%) | P |
| **All patients, no. evaluable courses** | 472 | 305 | | 477 | 292 | | 449 | 276 | |
| Days with grade 3-4 thrombocytopenia, median (range) | 11 (0-52) | 16 (0-52) | | 21 (0-91) | 15 (0-117) | | 18 (0-90) | 19 (0-85) | |
| Episodes > 15 d, n (%) | 205 (44) | 159 (52) | .02 | 352 (75) | 140 (48) | < .001 | 257 (53) | 156 (57) | .35 |
| Days with grade 4 neutropenia, median (range) | 17 (0-63) | 18 (0-54) | | 22 (0-68) | 18 (0-78) | | 20 (0-80) | 19 (0-78) | |
| Episodes > 15 d, n (%) | 256 (54) | 182 (60) | .12 | 403 (84) | 179 (61) | < .001 | 259 (58) | 165 (60) | .58 |
| Days of hospitalization, median (range) | 4 (0-61) | 4 (0-37) | | 10 (0-119) | 0 (0-36) | | 0 (0-63) | 0 (0-64) | |
| Episodes > 10 d, n (%) | 110 (24) | 100 (32) | .02 | 216 (46) | 75 (26) | < .001 | 70 (16) | 73 (26) | .35 |
| **Low-risk patients, no. evaluable courses** | 93 | 66 | | 97 | 68 | | 82 | 63 | |
| Days with grade 3-4 thrombocytopenia, median (range) | 0 (0-37) | 0 (0-28) | | 21 (0-36) | 0 (0-57) | | 0 (0-67) | 0 (0-31) | |
| Episodes > 15 d, n (%) | 12 (13) | 16 (24) | .08 | 60 (64) | 27 (40) | < .001 | 13 (16) | 11 (17) | .79 |
| Days with grade 4 neutropenia, median (range) | 0 (0-30) | 0 (0-31) | | 21 (0-42) | 17 (0-35) | | 0 (0-55) | 0 (0-52) | |
| Episodes > 15 d, n (%) | 21 (23) | 21 (32) | .19 | 78 (80) | 37 (54) | < .001 | 17 (21) | 12 (19) | .80 |
| **Days of hospitalization, median (range)** | 0 (0-30) | 4 (0-36) | | 8 (0-119) | 0 (0-36) | | 0 (0-36) | 0 (0-17) | |
| Episodes > 10 d, n (%) | 12 (13) | 17 (25) | .06 | 38 (39) | 15 (24) | .02 | 3 (3) | 3 (4) | .99 |
| **Intermediate-risk patients, no. evaluable courses** | 271 | 161 | | 271 | 153 | | 264 | 144 | |
| Days with grade 3-4 thrombocytopenia, median (range) | 17 (0-52) | 16 (0-52) | | 22 (0-91) | 0 (0-117) | | 24 (0-86) | 20 (0-85) | |
| Episodes > 15 d, n (%) | 149 (55) | 82 (50) | .34 | 212 (78) | 59 (38) | < .001 | 163 (62) | 84 (59) | .49 |
| Days with grade 4 neutropenia, median (range) | 19 (0-63) | 18 (0-54) | | 22 (0-68) | 17 (0-61) | | 23 (0-75) | 19 (0-78) | |
| Episodes > 15 d, n (%) | 171(63) | 96 (60) | .47 | 232 (86) | 88 (57) | < .001 | 174 (66) | 92 (64) | .68 |
| Days of hospitalization, median (range) | 5 (0-61) | 4 (0-34) | | 10 (0-49) | 0 (0-33) | | 0 (0-63) | 0 (0-64) | |
| Episodes > 10 d, n (%) | 73 (28) | 42 (26) | .51 | 124 (47) | 32 (21) | < .001 | 45 (18) | 34 (23) | .35 |
| **High-risk patients, No. evaluable courses** | 107 | 77 | | 109 | 70 | | 103 | 69 | |
| Days with grade 3-4 thrombocytopenia, median (range) | 0 (0-34) | 18 (0-33) | | 21 (0-64) | 24 (0-102) | | 21 (0-90) | 26 (0-56) | |
| Episodes > 15 d, n (%) | 43 (41) | 61 (81) | < .001 | 80 (77) | 54 (77) | .97 | 61 (61) | 61 (89) | < .001 |
| Days with grade 4 neutropenia, median (range) | 19 (0-46) | 20 (0-43) | | 24 (0-64) | 22 (0-78) | | 20 (0-80) | 22 (0-49) | |
| Episodes > 15 d, n (%) | 63 (59) | 65 (84) | < .001 | 93 (85) | 54 (77) | .16 | 68 (66) | 61 (89) | < .001 |
| Days of hospitalization, median (range) | 0 (0-34) | 13 (0-37) | | 11 (0-40) | 6 (0-28) | | 0 (0-60) | 11 (0-37) | |
| Episodes > 10 d, n (%) | 78 (23) | 41 (52) | < .001 | 53 (50) | 28 (39) | .13 | 22 (22) | 36 (51) | < .001 |

($P = .37$). Among the 41 patients who were not tested for RT-PCR at the end of consolidation therapy, one bone marrow relapse occurred at 6 months.

### Maintenance therapy

All of the 361 patients alive after completing consolidation therapy proceeded to maintenance therapy. Cytopenias, especially neutropenia, and slight liver function test abnormalities were commonly observed in this phase, often requiring dose reduction or temporary discontinuation of chemotherapy. No death in remission was reported during maintenance.

### Outcome

Median follow-up of patients in the LPA99 and LPA2005 trials were 82 months (range, 17-120 months) and 28 months (range, 2-51 months) from diagnosis, respectively. In addition to the 4 cases of death in CR, 2 patients developed colorectal adenocarcinoma at 25 and 31 months from APL diagnosis. Deaths in remission occurred in patients aged 23, 38, 45, and 52 years as the result of infection (3 patients) and cerebral hemorrhage (1 patient) belonging to high- and low-risk groups (3 and 1 patients, respectively). Twenty-one relapses occurred among the 361 patients who achieved CR (16 overt morphologic and 5 molecular relapses).

**Table 4. Postremission outcome of APL patients in the PETHEMA LPA99 and LPA2005 trials**

| Characteristic | No. of patients | | CIR | | | DFS | | | OS | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Overall | CR, n (%) | % at 3 y | % at 4 y | P | % at 3 y | % at 4 y | P | % at 3 y | % at 4 y | P |
| Overall | 963 | 883 (92) | 8 | 11 | | 88 | 85 | | 87 | 85 | |
| All patients | | | | | | | | | | | |
| LPA99 | 561 | 511 (91) | 9 | 11 | .39 | 87 | 84 | .06 | 85 | 83 | .08 |
| LPA2005 | 402 | 372 (92) | 7 | 9 | | 92 | 90 | | 89 | 88 | |
| Low-risk | | | | | | | | | | | |
| LPA99 | 107 | 103 (96) | 4 | 4 | .58 | 90 | 89 | .49 | 91 | 89 | .14 |
| LPA2005 | 84 | 83 (99) | 6 | 6 | | 93 | 93 | | 96 | 96 | |
| Intermediate-risk | | | | | | | | | | | |
| LPA99 | 313 | 294 (94) | 5 | 7 | .69 | 91 | 87 | .21 | 89 | 88 | .18 |
| LPA2005 | 200 | 191 (95) | 6 | 8 | | 94 | 92 | | 93 | 91 | |
| High-risk | | | | | | | | | | | |
| LPA99 | 140 | 113 (81) | 26 | 27 | .03 | 73 | 71 | .11 | 71 | 68 | .34 |
| LPA2005 | 118 | 98 (83) | 11 | 14 | | 82 | 82 | | 79 | 79 | |

CIR indicates cumulative incidence of relapse; CR, complete response, DFS, disease-free survival; and OS, overall survival.

Three were extramedullary relapses. These relapses occurred in the central nervous system at 17 and 20 months from diagnosis in 2 intermediate-risk patients and the skin at 6 months in 1 high-risk patient with WBC count 55 × $10^9$/L at presentation.

***Relapse rate.*** For patients in the LPA2005 study, the 3-year CIR rate was 7%, whereas for patients in the LPA99 study it was 9% ($P = .39$). For patients in the LPA2005 study, the CIR rates for low- and intermediate-risk patients were 6% and 6%, respectively, whereas in the LPA99 study, they were 4% and 5% ($P = .58$ and $P = .69$, respectively; Table 4). Among high-risk patients, the 3-year CIR rate was significantly lower in those treated with the LPA2005 protocol compared with those treated with the LPA99 protocol (11% vs 26%, $P = .03$; Figure 3). Multivariate analysis showed that the PETHEMA protocol was the sole independent risk factor for relapse in this setting ($P = .02$).

***DFS and OS.*** The 3-year DFS rates were 92% plus or minus 4% in the LPA2005 study and 87% plus or minus 3% in the LPA99 study ($P = .06$; Figure 4). Multivariate analysis showed that lower relapse-risk group ($P < .001$), female sex ($P = .04$), and the LPA2005 protocol ($P = .04$) were favorable risk factors for DFS. For patients in the LPA2005 and LPA99 study, the 3-year DFS rates for low-risk (93% ± 8% vs 90% ± 6%, $P = .49$), intermediate-risk (94% ± 4% vs 91% ± 3%, $P = .21$), and high-risk patients (82% ± 10% vs 73% ± 8%, $P = .11$) were not statistically different (Table 4). The probability of remaining alive after 3 years was 89% plus or minus 4% in the LPA2005 study and 85% plus or minus 3% in the LPA99 study ($P = .08$). In addition, for OS there were no significant differences between LPA2005 and LPA99 among low-, intermediate-, and high-risk patients (Table 4).

## Discussion

This study shows a significant improvement in the outcome of APL patients treated with a successive risk-adapted PETHEMA/HOVON trial (LPA2005) on the basis of the combination of ATRA with idarubicin for induction plus anthracycline-based chemotherapy for consolidation, followed by ATRA and low-dose methotrexate and mercaptopurine for maintenance therapy. A reduction in the dose of mitoxantrone for low- and intermediate-risk patients in the LPA2005 trial led to a significant lower toxicity during the second consolidation course without decreasing the high antileukemic efficacy achieved in the previous LPA99 trial. Furthermore, the addition of cytarabine to the combination of ATRA and idarubicin in consolidation for high-risk patients resulted in a significantly

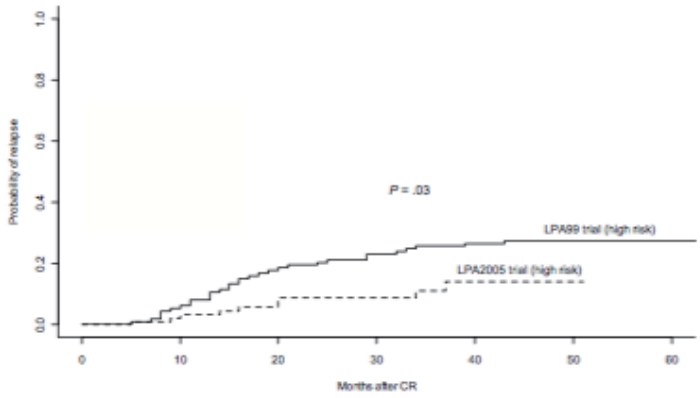



**Figure 3. Cumulative incidence of relapse in high-risk patients according to PETHEMA trial.**

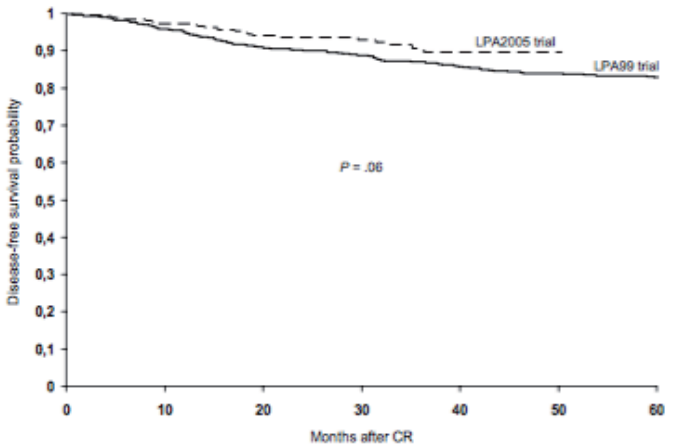



Figure 4. DFS in the overall series according to PETHEMA trial.

Number at risk

| | | | | | | |
|---|---|---|---|---|---|---|
| LPA99 trial | 488 | 459 | 447 | 424 | 407 | 355 |
| LPA2005 trial | 317 | 229 | 145 | 65 | 2 | 0 |

greater antileukemic activity coupled with an increased but tolerable toxicity. Overall, compared with the previous LPA99, the LPA2005 protocol resulted in an improvement of the outcome in APL patients.

Following standard practice of the PETHEMA Group, the LPA2005 trial was nonrandomized and designed to provide a historical comparison with the previous LPA99 trial with the minimum possibility of bias. Given the sequential nature of the studies, patients in the LPA2005 series had a shorter follow-up than those in the LPA99 study (median, 28 and 82 months, respectively). Notwithstanding, the follow-up of patients in the LPA2005 study falls within the range reported by the major trials published to date. Participating institutions in all PETHEMA trials registered all eligible and noneligible patients with newly diagnosed APL and the eligibility criteria were unchanged throughout the 2 subsequent studies. Regarding therapy, only a single change was made in each relapse risk group to facilitate the interpretation of the potential impact on outcome in comparable series of patients. In this respect, it should be noted that the proportion of non eligible and nonevaluable patients were similar in the LPA99 and LPA2005 trial. Patients in both trials were comparable for all baseline characteristics, except for the somewhat-greater proportion of pediatric patients in the LPA99 trial and those with ECOG grade 0 to 1 in the LPA2005. These findings could explain, at least in part, the slightly lower induction failure rate, although not statistically significant, observed in the LPA2005 trial. Induction results in the LPA2005 study confirmed again a practical absence of leukemia resistance, as well as a similar CR rate and time interval to CR compared with previous trials of our group using the same AIDA regimen.[6] Regarding the incidence of moderate and severe DS in the LPA2005 trial compared with the LPA99 trial, only a slight increase of the moderate form was observed (16% vs 12%), but this difference was not statistically significant. The changes made in the LPA2005, in which patients with WBC count lower than $5 \times 10^9$/L within the 2 first weeks of ATRA therapy did not received DS prophylaxis, might explain the small increase of moderate DS observed, but this is speculative.

Similar to a previous comparison between the LPA99 and the LPA96 study,[1,2] we demonstrate here again a stepwise significant further improvement in outcome in the present LPA2005 study, that appears the consequence of selected modifications in consolidation therapy. We had hypothesized that patients with APL with a comparatively low risk of relapse, such as the low- and intermediate-risk groups, might receive overtreatment. Therefore, a reduction in the dose intensity of mitoxantrone in the second consolidation course was implemented in the LPA2005 trial with the aim of decreasing short- and long-term toxicities, without sacrificing the antileukemic efficacy of the LPA99 trial. This objective was fully achieved in terms of hematologic toxicities, relapse, and survival rates. In patients treated with reduced dose of mitoxantrone, that is, all patients older than 60 years and those younger with low and intermediate relapse risk, which overall represents 86% of the study population, prolonged grade 4 neutropenia, grade 3 to 4 thrombocytopenia, and hospitalization were reported significantly less frequently in the second course of the LPA2005 trial compared with the LPA99. Besides this favorable outcome, the dose reduction of mitoxantrone did not negatively impact on the antileukemic efficacy as is apparent from the similar relapse rates, DFS, and OS rates in the LPA2005 and the previous LPA99 trials.

Concerning the role of cytarabine in APL, the study conducted by the European APL group[4] in younger patients randomly assigned to receive a state-of-the-art approach with daunorubicin alone or daunorubicin plus cytarabine, the addition of this agent resulted in a better outcome. However, the authors cautiously confined the interpretation of these results to the boundaries of the type and the cumulative dose of anthracycline given in this study. In the present LPA 2005 trial, the addition of cytarabine to the combination of ATRA and idarubicin in consolidation therapy for the high-risk patients younger than 60 years was mainly conducted to verify the outstanding but preliminary results reported by the GIMEMA group with a similar approach.[5] The results of our study also demonstrate improved antileukemic efficacy of the cytarabine

enriched schedule in high-risk APL. Also a recently updated analysis of the aforementioned GIMEMA study (Front-line treatment of acute promyelocytic leukemia with AIDA induction followed by risk-adapted consolidation: results of the AIDA-2000 trial of the Italian GIMEMA group, F. Lo-Coco, G. Avvisati, M. Vignetti, et al, manuscript submitted) has meanwhile confirmed the significant improvement of the therapeutic regimen supplemented with cytarabine.

However, the different way in which the GIMEMA and PETHEMA studies have substantiated this improvement deserves further discussion. It should be noted that the GIMEMA study showed a dramatic reduction of relapse rate in high-risk patients when, in the AIDA2000 trial, ATRA was added to the classical cytarabine-containing chemotherapy consolidation given in the.AIDA0493 trial.[17] In contrast, the PETHEMA study demonstrated a similar favorable impact on relapse rate when, in the LPA2005 trial, cytarabine was added to the consolidation therapy administered in the LPA99 trial, which was based on the combination of ATRA and anthracycline/anthraquinone monochemotherapy. Taken together, these results allow us to speculate on a possible supra-additive effect of the combination of ATRA plus cytarabine that might support the improvement observed in high-risk patients. Interestingly, in vitro studies with human NB4 promyelocytic leukemia cells have showed an increased sensitivity to cytarabine after treatment with ATRA.[18] In these experiments, the combination effect was supra-additive, with a maximum cytotoxicity and potency of cytarabine administration when it was closely followed by ATRA administration. In addition to the improved relapse rate observed in the present study, we also noticed an apparent reduction in the molecular persistence rate at the end of consolidation therapy in the LPA2005 (0 of 320 patients assessed) compared with the LPA99 trial (3 of 444). It should be noted that considering both the LPA99 and LPA2005 trials together, 3 of 764 patients assessed had molecular persistence at the end of consolidation therapy, this finding being significantly lower than in the LPA96 study (5 of 147 patients assessed, $P = .002$; data not shown). Besides the improvement in the antileukemic efficacy, as expected, the cytarabine enriched schedule increased the hematologic toxicities compared with the LPA99. However, both a high protocol compliance (97%) and a low death rate during consolidation therapy were observed in the LPA2005 trial, even when high-risk patients receiving cytarabine were analyzed separately (3 deaths of 85 patients, data not shown).

In conclusion, following a risk-adapted strategy, the LPA2005 trial provided an overall improvement of results in APL patients by means of specific changes in consolidation therapy. A decrease in dose intensity of mitoxantrone in low- and intermediate-risk patients resulted in a significant reduction of hematologic toxicities and hospital stay without compromising the antileukemic activity. Furthermore, the combination of ATRA, idarubicin, and cytarabine in consolidation therapy for high-risk APL patients demonstrated a striking antileukemic efficacy coupled with a significant but tolerable increase of toxicity. Once the use of arsenic trioxide to reinforce consolidation therapy in standard ATRA plus chemotherapy regimens has been supported by a large randomized study by the US Intergroup,[19] future studies should address the role of this agent in front-line therapy. These studies may establish whether arsenic trioxide can permit a reduction of chemotherapy intensity while maintaining cure rates or confirm the improvement on outcomes currently achieved with optimal ATRA and anthracycline-based protocols.

## Acknowledgments

We thank Leticia Llopis for data collection and management.

This study was supported in part by the Fundación para la Investigación Hospital Universitario La Fe-Ayudas Bancaja (grant 2006/0137) and Red Temática de Investigación Cooperativa en Cáncer (RD06/0020/0031).

## Authorship

Contribution: M.A.S. and P.M. conceived the study and analyzed and interpreted the data; M.A.S., P.M., and B.L. wrote the paper; P.M. performed the statistical analyses; and C.R., A.H., J.d.l.S., G.M., E.d.L., S.B., V.R., J.M.R., C.R., I.K., J.B., J.G., J.D.-M., R.R., F.M., G.O., and J.D.G. included data of patients treated in their institutions, reviewed the manuscript, and contributed to the final draft.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

For a complete list of PETHEMA and HOVON participants, see the supplemental Appendix.

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## 9.3 Artículo Anexo 3

de la Serna J, Montesinos P, Vellenga E, Rayón C, Parody R, León A, Esteve J, Bergua JM, Milone G, Debén G, Rivas C, González M, Tormo M, Díaz-Mediavilla J, González JD, Negri S, Amutio E, Brunet S, Lowenberg B, Sanz MA.

Causes and prognostic factors of remission induction failure in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and idarubicin.

Blood. 2008 Apr 1;111(7):3395-402.

CLINICAL TRIALS AND OBSERVATIONS

# Causes and prognostic factors of remission induction failure in patients with acute promyelocytic leukemia treated with *all-trans* retinoic acid and idarubicin

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**An understanding of the prognostic factors associated with the various forms of induction mortality in patients with acute promyelocytic leukemia (APL) has remained remarkably limited. This study reports the incidence, time of occurrence, and prognostic factors of the major categories of induction failure in a series of 732 patients of all ages (range, 2-83 years) with newly diagnosed APL who received *all-trans* retinoic acid (ATRA) plus idarubicin as induction therapy in 2 consecutive studies of the Programa de Estudio y Tratamiento de las Hemopatias Malignas (PETHEMA) Group. Complete remission was attained in 666 patients (91%). All the 66 induction failures were due to induction death. Hemorrhage was the most common cause of induction death (5%), followed by infection (2.3%) and differentiation syndrome (1.4%). Multivariate analysis identified specific and distinct pretreatment characteristics to correlate with an increased risk of death caused by hemorrhage (abnormal creatinine level, increased peripheral blast counts, and presence of coagulopathy), infection (age >60 years, male sex, and fever at presentation), and differentiation syndrome (Eastern Cooperative Oncology Group [ECOG] score >1 and low albumin levels), respectively. These data furnish clinically relevant information that might be useful for designing more appropriately risk-adapted treatment protocols aimed at reducing the considerable problem of induction mortality in APL. (Blood. 2008;111:3395-3402)**



## Introduction

Since the routine introduction of *all-trans* retinoic acid (ATRA) in front-line therapy of acute promyelocytic leukemia (APL), significant improvements in patient outcomes were achieved. A number of studies conducted during the past decade have contributed to the optimizing of the antileukemic efficacy of ATRA, especially when it is combined with chemotherapy.[1-3] In fact, the current standard for induction therapy is the simultaneous combination of ATRA with anthracycline-based chemotherapy, which results in extremely high antileukemic efficacy, achieving a 90% to 95% complete remission (CR) rate. Although leukemia resistance to therapy has become an uncommon cause of remission induction failure, death during induction from hemorrhage, infection, and differenjtiation syndrome (formerly retinoic acid syndrome) has remained the main problem during the early treatment phase. The frequency of induction death from medical complications has probably not changed during recent years. The relative incidence and time of occurrence of each of these categories of induction failure, as well as their pretreatment characteristics (prognostic factors), have been investigated critically and in detail in rare studies only.[4]

The present study reports the incidence, time of occurrence, and prognostic factors of the major categories of induction failure in a large series of 732 patients with newly diagnosed APL who received ATRA plus idarubicin (AIDA)[5] as induction therapy in 2 consecutive studies of the Programa de Estudio y Tratamiento de las Hemopatias Malignas (PETHEMA) Group (LPA96 and LPA99).

## Methods

### Eligibility

Patients enrolled in the consecutive PETHEMA LPA96 and LPA99 trials were required to have a diagnosis of de novo APL with demonstration of the t(15;17) or *PML/RARα* rearrangements, normal hepatic and renal function, no cardiac contraindications to anthracycline chemotherapy, and an Eastern Cooperative Oncology Group (ECOG) performance status of less than 4. Informed consent was obtained from all patients in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Board of each participating hospital. A list of the

Submitted July 10, 2007; accepted January 10, 2008. Prepublished online as *Blood* First Edition paper, January 14, 2008; DOI 10.1182/blood-2007-07-100669.

The online version of this article contains a data supplement.

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participating institutions and clinicians can be found in Document S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article).

### Induction therapy

Induction therapy consisted of oral ATRA (45 mg/m$^2$ per day) divided into 2 daily doses, which was maintained until complete hematologic remission or for a maximum of 90 days, and idarubicin (12 mg/m$^2$ per day) given as an intravenous bolus on days 2, 4, 6, and 8 (AIDA regimen). For patients 20 years of age or younger, the ATRA was adjusted to 25 mg/m$^2$ per day. Since November 1999 (LPA99 trial), the dose of idarubicin on day 8 has been omitted for patients older than 70 years of age. Treatment with ATRA was started as soon as a diagnosis of APL was made by morphologic criteria.[6,7] For patients in whom the diagnosis was not confirmed by genetic studies, ATRA treatment was withdrawn, and alternative chemotherapy was given at the physician's discretion.

### Supportive measures

***Management of coagulopathy.*** Platelet transfusions were given to maintain a platelet count of more than $30 \times 10^9$/L until resolution of the coagulopathy. Once the coagulopathy was under control, platelet transfusions were only given for patients with infectious or hemorrhagic manifestations, or when the platelet count dropped below $20 \times 10^9$/L. Patients with active coagulopathy were treated with fresh frozen plasma, cryoprecipitate, or fibrinogen to maintain a fibrinogen level higher than 1.5 mg/L and hemostatic levels of coagulation factors. Heparin prophylaxis was not recommended, except for the treatment of thrombotic events. Since November 1999 (LPA99 study), patients with platelet counts less than $50 \times 10^9$/L or evident clinical-biologic signs of coagulopathy have received tranexamic acid (100 mg/kg per day) by continuous intravenous infusion until the disappearance of signs of coagulopathy and the platelet count was higher than $50 \times 10^9$/L.

***Management of differentiation syndrome.*** At the first signs of suspected differentiation syndrome (DS), patients were given 10 mg dexamethasone every 12 hours. ATRA was discontinued only in case of progression to severe DS. In the LPA96 trial, patients with a white blood cell (WBC) count greater than $5 \times 10^9$/L received prophylaxis with dexamethasone (10 mg/12 h intravenously for 7 days), whereas in the LPA99 study, all patients received DS prophylaxis with prednisone (0.5 mg/kg per day orally) from days 1 to 15.

***Other supportive measures.*** Patients were admitted in single or double conventional rooms with no specific isolation measures. Management of febrile episodes was not standardized and varied with the practice of each institution. Treatment with granulocyte colony stimulating factors was not recommended. For patients with extreme hyperleukocytosis, hydroxyurea and leukapheresis were given at the physician's discretion. Packed red cell transfusions were recommended to maintain blood hemoglobin greater than 9 g/dL.

### Definitions and study endpoints

Remission induction response was assessed according to the recently revised criteria by Cheson et al[8] A morphologic CR designation requires less than 5% blasts and atypical promyelocytes in an aspirate sample and an absolute neutrophil count of more than $10^9$/L and platelets of more than $100 \times 10^9$/L. Hemoglobin concentration or hematocrit has no bearing on remission status, although the patient must be independent of transfusions. Treatment failure includes those patients for whom treatment has failed to achieve a CR. The causes of treatment failure were classified as follows.

***Resistant disease.*** In APL, at variance with other subtypes of acute myeloblastic leukemia, leukemia resistance can be assessed only after sufficient time has passed to allow for full terminal differentiation of the malignant promyelocytes. The bone marrow aspirate performed too early after induction therapy may reveal a hypercellular specimen with residual blasts and atypical promyelocytes and may give the misleading impression of response failure, occasionally even after some weeks after the start of treatment (up to 40-50 days).

***Death during induction therapy.*** Causes of induction deaths include the following categories.

*Infection.* When death is due to a clinical, radiologic, or microbiologically documented infection.

*Hemorrhage.* When a major bleeding occurs in a vital organ (central nervous system, lungs). Gastrointestinal tract hemorrhage requires massive melena or hematemesis accompanied by fall in blood pressure.

*Differentiation syndrome.* That is, death occurring in patients with definitely present DS and not explained by infection, hemorrhage, or any other cause. Definitely present DS is defined as the presence of at least 4 of the characteristic signs or symptoms described by Frankel et al[9]: fever, dyspnea, pleural or pericardial effusion, pulmonary infiltrates, renal failure, hypotension, and unexplained weight gain greater than 5 kg.

*Other.* That is, any other cause not classified as infection, hemorrhage, or DS that was the cause of death.

Duration of neutropenia and thrombocytopenia was defined as the time, in days, from the end of chemotherapy until the day of the first measurement of absolute neutrophil count greater than $10^9$/L and platelets greater than $50 \times 10^9$/L, respectively.

### Prognostic factors

Twenty-two patient and disease characteristics documented at initial evaluation were examined in the prognostic factor analysis to establish their relation to induction response. Basic demographic data and clinical characteristics at presentation included age, sex, ECOG score, fever, total body surface, as well as liver and spleen enlargement. Serum biochemical parameters were creatinine, uric acid, and albumin. Peripheral blood features included hemoglobin level and platelet, WBC, and blast cell counts. Bone marrow aspirate parameters evaluated were cellularity, peroxidase reactivity, French-American-British subtype, cytogenetics, and *PML/RARA* isoforms. The protocol, fibrinogen level, and the presence of coagulopathy were also included. The latter was defined as a prolonged prothrombin time or activated partial thromboplastin time, in addition to hypofibrinogenemia or increased levels of fibrin degradation products or D-dimers.

### Statistical analysis

The chi-square test with Yates correction and Fisher exact test were used for statistical analyses. *P* values were calculated using the 2-tailed test. Characteristics selected for inclusion in the multivariate analysis were those for which there was some indication of a significant association with induction failure in univariate analysis ($P < .1$) and, if available, those that prior studies had suggested a possible relation. Multivariate analysis was performed using a logistic regression model.[10] Missing data were substituted by the mean values from patients in whom data were available.[11] Computations were performed using the programs from the BMDP statistical library (BMDP Statistical Software, Los Angeles, CA).

## Results

### Accrual and patient characteristics

Between November 1996 and June 2005, 792 consecutive patients with genetic diagnosis of APL were registered from 82 institutions from Spain, The Netherlands, Argentina, Uruguay, and the Czech Republic. A total of 42 patients (5%) were considered not eligible for the treatment because of severe clinical condition contraindicating the administration of chemotherapy, 8 (4%) and 34 (6%) in the LPA96 and LPA99 trials, respectively. Thus, 750 patients met the previously defined entry criteria and were enrolled in the LPA96 and LPA99 studies. Eighteen additional patients were not evaluated because of protocol violations during induction therapy (8 of 181 in the LPA96 trial and 10 of 570 in the LPA99 trial). Causes of protocol violation were the addition of cytarabine (10 patients), premature administration of salvage therapy because of inappropriate assessment for response as resistant leukemia

**Table 1. Reasons for the exclusion from the study**

| | No. of patients |
|---|---|
| **Noneligible patients** | |
| Poor performance status/severe active infection or hemorrhage | 28 |
| Intracranial hemorrhage | 15 |
| Pulmonary hemorrhage | 4 |
| Septic shock (bacteremia) | 3 |
| Pneumonia | 1 |
| Encephalitis | 1 |
| Severe thromboembolism | 3 |
| Unfit for chemotherapy | 14 |
| Very elderly patients (75, 78, 78, 82, 88, 90 y) | 6 |
| ECOG score of 4 | 1 |
| Cardiac contraindications | 3 |
| Abnormal renal or hepatic function | 2 |
| Serious psychiatric illness | 2 |
| Hemiplegia after stroke | 1 |
| **Nonevaluable patients (protocol violations)** | 18 |
| Addition of cytarabine | 10 |
| Premature administration of salvage therapy because of inappropriate assessment for response | 6 |
| Lost to follow-up because of transfer to another hospital | 2 |

ECOG indicates Eastern Cooperative Oncology Group.

(6 patients), and lost to follow-up because of transfer to another hospital during induction therapy (2 patients). The 6 patients inappropriately assessed for response had been evaluated during pancytopenia on days 18, 20, 23, 26, 30, and 33 after chemotherapy and 4 of them had developed a definitely present DS. Five patients achieved CR after second-line therapy. Details about noneligible and nonevaluable patients are shown in Table 1.

The main clinical and biologic characteristics of the 732 patients evaluable for induction are shown in Table 2.

### Induction therapy

***Response and induction mortality.*** Six hundred sixty-six of the 732 evaluable patients achieved morphologic CR (91%; 95% CI, 89.9%-92.1%). The median time intervals to CR were 36 days (range, 21-78 days) in the LPA96 trial and 37 days (range, 21-77 days) in the LPA99 trial. The median time to reach neutrophil counts greater than $10^9$/L and platelet counts greater than $50 \times 10^9$/L was 23 days (range, 0-60 days) and 19 days (range, 0-50 days), respectively. All the 66 induction failures were attributed to death during induction.

Hemorrhage and infection accounted for most of the deaths during induction therapy (37 and 17 patients, respectively). Differentiation syndrome and acute myocardial infarction were contributing causes of death in 10 and 2 patients, respectively.

***Factors predicting induction death.*** We first set out to evaluate overall induction mortality, and in the subsequent sections we evaluated distinct types of death separately, ie, hemorrhagic death, infection-related death, and death associated with the differentiation syndrome, respectively.

The univariate analysis of prognostic factors (Table 3) identified the following characteristics predicting induction mortality: older age, with 60 years as the most significant cutoff point ($P < .001$); male sex ($P = .003$); ECOG score 2 to 3 ($P = .01$); fever at presentation ($P = .02$); increased WBC and peripheral blast counts at presentation, with $10 \times 10^9$/L and $30 \times 10^9$/L as the most statistically significant cutoff points, respectively ($P < .001$); as well as abnormal levels of serum creatinine ($P < .001$), low levels of albumin ($P = .003$), microgranular subtype ($P = .002$), and presence of coagulopathy ($P = .02$). After multivariate analysis, the following factors remained for their independent predictive significance for induction mortality: abnormal creatinine level ($P < .001$), peripheral blast count of more than $30 \times 10^9$/L ($P < .001$), age older than 60 years ($P < .001$), male sex ($P < .001$), and WBC count of more than $10 \times 10^9$/L ($P = .04$; Table 4).

### Deaths as a result of hemorrhage

Overall, 37 deaths were attributable to hemorrhages. The mortality rates resulting from hemorrhage were similar in both the LPA96 and LPA99 studies (9 of 175, 5.1% and 28 of 561, 5%, respectively), despite using tranexamic acid prophylaxis in the latter study. Hemorrhagic mortalities were almost exclusively caused by intracranial (24 patients, 65%) and pulmonary hemorrhages (12 patients, 32%). Of the 24 patients with intracranial bleeding, 2 developed the hemorrhage over an extensive cerebral thrombosis, and 2 additional patients had concomitant pulmonary hemorrhage. One case of fatal gastrointestinal tract bleeding was registered. The median time interval from start of treatment to development of intracranial and pulmonary hemorrhage was 6 days (range, 1-21 days) and 9 days (range, 1-23 days), respectively. Most lethal hemorrhages occurred during the first week (21 patients, 57%). There were 7 deaths (19%) each during the second and third week, and 2 deaths (5%) were noted during the fourth week (Figure 1). No lethal hemorrhages were recorded beyond the fourth week. Seventeen (71%) of 24 cerebral hemorrhages and 4 (33%) of 12 pulmonary hemorrhages occurred during the first week of starting induction ($P = .07$). Twenty-five of (69%) 36 patients who died from cerebral (19 of 24) or pulmonary hemorrhages (6 of 12) had a fulminant course, with death occurring within 24 hours from the onset of lethal bleeding.

### Factors predicting fatal hemorrhage

The univariate analysis (Table 3) identified the following prognostic factors for hemorrhagic mortality: older age, with 70 years as the most significant cutoff point ($P = .02$); increased WBC and peripheral blast counts at presentation, with $10 \times 10^9$/L and $30 \times 10^9$/L as the most statistically significant cutoff points, respectively ($P < .001$); as well as abnormal levels of serum creatinine ($P < .001$) and the presence of coagulopathy ($P = .01$), whereas there was a trend for microgranular subtype ($P = .09$) and short *PML/RARα* isoform (bcr3; $P = .09$). After multivariate analysis, the following factors remained significant: abnormal creatinine level ($P < .001$), peripheral blast count of more than $30 \times 10^9$/L ($P < .001$), and presence of coagulopathy ($P = .03$; Table 4).

### Deaths as a result of infection

Overall, 17 deaths were attributable to infection. Infection-associated mortality was due to pneumonia (9 patients, 53%), septicemia (6 patients, 35%), orbital cellulitis (n = 1), and clinically and radiologically suspected but not microbiologically documented hepatosplenic candidiasis (n = 1). Eleven patients had a microbiologically documented infection. Eight were bacterial infections (4 Gram-positive and 4 Gram-negative), 2 fungal infections (pulmonary aspergillosis), and 1 mixed infection (pulmonary infection by *Aspergillus* spp and *Klebsiella pneumoniae*). Deaths resulting from infection occurred at a median time of 21 days (range, 3-39 days), at a constant rate during the induction period (3-4 deaths each week; Figure 2).

### Factors predicting fatal outcome of infection

The following prognostic factors (Table 3) for infectious mortality appeared from univariate analysis: older age, with 60 years as the

**Table 2. Demographic and baseline characteristics of the study population**

| Characteristic | LPA96 Median (range) | LPA96 No. (%) | LPA99 Median (range) | LPA99 No. (%) | P | Total Median (range) | Total No. (%) |
|---|---|---|---|---|---|---|---|
| Overall | — | 172 (24) | — | 560 (76) | — | — | 732 (100) |
| **Age, y** | 39 (2-78) | — | 40 (2-83) | — | — | 40 (2-83) | — |
| 15 or younger | — | 11 (6) | — | 48 (9) | .83 | — | 59 (8) |
| 16 to 40 | — | 75 (43) | — | 240 (43) | — | — | 315 (43) |
| 41 to 60 | — | 57 (33) | — | 171 (30) | — | — | 228 (31) |
| 61 to 70 | — | 19 (11) | — | 68 (12) | — | — | 87 (12) |
| 71 or older | — | 10 (6) | — | 33 (6) | — | — | 43 (6) |
| **Sex** | | | | | | | |
| Male | — | 102 (59) | — | 270 (48) | .01 | — | 372 (51) |
| Female | — | 70 (41) | — | 290 (52) | — | — | 360 (49) |
| **ECOG score** | 1 (0-3) | — | 1 (0-3) | — | — | 1 (0-3) | — |
| 0 to 1 | — | 130 (81) | — | 377 (73) | .07 | — | 507 (75) |
| 2 | — | 20 (13) | — | 102 (20) | — | — | 122 (18) |
| 3 | — | 10 (6) | — | 36 (7) | — | — | 46 (7) |
| **Fever** | | | | | | | |
| No | — | 108 (63) | — | 333 (60) | .57 | — | 441 (61) |
| Yes | — | 64 (37) | — | 220 (40) | — | — | 284 (39) |
| **WBC count, × $10^9$/L** | 2.0 (0.3-210) | — | 2.2 (0.2-460) | — | — | 2.2 (0.2-460) | — |
| Less than 5 | — | 108 (62) | — | 336 (60) | .79 | — | 444 (61) |
| 5 to 10 | — | 21 (13) | — | 84 (15) | — | — | 105 (14) |
| 10 to 50 | — | 31 (18) | — | 99 (18) | — | — | 130 (18) |
| 50 or higher | — | 12 (7) | — | 40 (7) | — | — | 52 (7) |
| **PB blast count, × $10^9$/L** | 0.6 (0-210) | — | 0.7 (0-179) | | | 0.7 (0-210) | |
| Less than 30 | — | 151 (93) | — | 459 (90) | .35 | — | 610 (91) |
| 30 or higher | — | 12 (7) | — | 50 (10) | — | — | 62 (9) |
| **Platelet count, × $10^9$/L** | 20 (1-161) | — | 22 (1-207) | — | — | 22 (1-207) | — |
| Less than 40 | — | 132 (77) | — | 431 (77) | .37 | — | 563 (77) |
| 40 or higher | — | 40 (23) | — | 128 (23) | — | — | 168 (23) |
| **Hemoglobin level, g/dL** | 9.4 (4.3-15.2) | — | 9.2 (3-16.9) | — | — | 9.3 (3-16.9) | — |
| Less than 10 | — | 104 (60) | — | 361 (65) | .26 | — | 465 (64) |
| 10 or higher | — | 68 (40) | — | 198 (35) | — | — | 266 (36) |
| **Creatinine level, mg/dL** | 0.9 (0.2-1.7) | — | 0.9 (0.2-2.4) | — | — | 0.9 (0.2-2.4) | — |
| Less than 1.4 | — | 171 (99) | — | 525 (98) | .22 | — | 696 (98) |
| 1.4 or higher | — | 1 (1) | — | 13 (2) | — | — | 14 (2) |
| **Coagulopathy** | | | | | | | |
| No | — | 51 (30) | — | 141 (26) | .61 | — | 192 (28) |
| Yes | — | 121 (70) | — | 369 (72) | — | — | 490 (72) |
| **Uric acid level, mg/dL** | 3.8 (1-9.6) | — | 3.9 (0.29-12.7) | — | — | 3.8 (0.29-12.7) | — |
| Less than 7 | — | 152 (95) | — | 442 (94) | .65 | — | 594 (94) |
| 7 or higher | — | 8 (5) | — | 28 (6) | — | — | 36 (6) |
| **Fibrinogen level, mg/dL** | 160 (0-784) | — | 158 (0-862) | — | — | 158 (0-862) | — |
| Less than 170 | — | 91 (30) | — | 285 (28) | .89 | — | 376 (28) |
| 170 or higher | — | 76 (70) | — | 232 (72) | — | — | 308 (72) |
| **Albumin level, g/dL** | 4.1 (2.2-6.2) | — | 4 (1.7-6.7) | — | — | 4 (1.7-6.7) | — |
| Less than 3.5 | — | 22 (14) | — | 107 (24) | .01 | — | 129 (22) |
| 3.5 or higher | — | 129 (86) | — | 333 (76) | — | — | 462 (78) |
| **Morphologic subtype** | | | | | | | |
| Hypergranular | — | 143 (83) | — | 450 (82) | .7 | — | 593 (82) |
| Microgranular | — | 29 (17) | — | 100 (18) | — | — | 129 (18) |
| **PML/RARα isoform** | | | | | | | |
| BCR1/BCR2 | — | 91 (56) | — | 286 (59) | .42 | — | 377 (58) |
| BCR3 | — | 73 (44) | — | 198 (41) | — | — | 271 (42) |

ECOG indicates Eastern Cooperative Oncology Group; WBC, white blood count; PB, peripheral blood; and —, not applicable.

most significant cutoff point ($P < .001$); male sex ($P = .02$); fever at presentation ($P = .01$); and abnormal serum creatinine level ($P = .04$). Among them, multivariate analysis identified the following independent prognostic parameters: age older than 60 years ($P < .001$), male sex ($P = .003$), and fever at presentation ($P = .009$; Table 4).

### Deaths as a result of differentiation syndrome

Ten deaths attributable to DS occurred at a median of 17 days of starting induction (range, 1-26 days). The time of occurrence of deaths resulting from DS is shown in Figure 2. The mortality rates because of DS were similar in both the LPA96 and LPA99

**Table 3. Univariate analysis according to the type of induction remission failure**

| | Overall deaths during induction | | Failure rates according to the cause of death | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Hemorrhage | | Infection | | DS | |
| Characteristic | No. (%) | P | No. (%) | P | No. (%) | P | No. (%) | P |
| Overall | 66 (9.0) | — | 37 (5.0) | — | 17 (2.3) | — | 10 (1.4) | — |
| **Protocol** | | | | | | | | |
| LPA96 | 16 (9.3) | .88 | 9 (5.2) | .90 | 5 (2.9) | .77 | 2 (1.2) | 1 |
| LPA99 | 50 (8.9) | — | 28 (5.0) | — | 12 (2.1) | — | 8 (1.4) | — |
| **Age, y** | | | | | | | | |
| 15 or younger | 5 (8.5) | <.001 | 3 (5.1) | .06 | 0 (0.0) | <.001 | 2 (3.4) | .08 |
| 16 to 40 | 14 (4.4) | — | 11 (3.5) | — | 3 (1.0) | — | 0 (0.0) | — |
| 41 to 60 | 19 (8.3) | — | 11 (4.8) | — | 3 (1.3) | — | 5 (2.2) | — |
| 61 to 70 | 17 (19.5) | — | 6 (6.9) | — | 7 (8.0) | — | 2 (2.3) | — |
| 71 or older | 11 (25.6) | — | 6 (14.0) | — | 4 (9.3) | — | 1 (2.3) | — |
| **Sex** | | | | | | | | |
| Male | 45 (12.1) | .003 | 23 (6.2) | .16 | 14 (3.8) | .02 | 6 (1.6) | .79 |
| Female | 21 (5.8) | — | 14 (3.9) | — | 3 (0.8) | — | 4 (1.1) | — |
| **ECOG score** | | | | | | | | |
| 0 to 1 | 38 (7.5) | .01 | 24 (4.7) | .2 | 11 (2.2) | .66 | 3 (0.6) | .01 |
| 2 | 15 (12.3) | — | 6 (4.9) | — | 3 (2.5) | — | 4 (3.3) | — |
| 3 | 9 (19.6) | — | 5 (10.9) | — | 2 (4.3) | — | 2 (4.3) | — |
| **Fever** | | | | | | | | |
| No | 31 (7.0) | .02 | 19 (4.3) | .31 | 5 (1.1) | .01 | 6 (1.4) | .999 |
| Yes | 34 (12.0) | — | 17 (6.0) | — | 12 (4.2) | — | 4 (1.4) | — |
| **WBC count, × $10^9$/L** | | | | | | | | |
| Less than 5 | 23 (5.2) | <.001 | 12 (2.7) | <.001 | 7 (1.6) | .23 | 4 (0.9) | .21 |
| 5 to 10 | 10 (9.5) | — | 5 (4.8) | — | 3 (2.9) | — | 2 (1.9) | — |
| 10 to 50 | 21 (16.2) | — | 11 (8.5) | — | 4 (3.1) | — | 4 (3.1) | — |
| 50 or higher | 12 (23.1) | — | 10 (17.3) | — | 3 (5.8) | — | 0 (0.0) | — |
| **PB blast count, × $10^9$/L** | | | | | | | | |
| Less than 30 | 44 (7.2) | <.001 | 24 (3.9) | <.001 | 13 (2.1) | .11 | 7 (1.1) | .999 |
| 30 or higher | 17 (27.4) | — | 10 (16.1) | — | 4 (6.5) | — | 1 (1.6) | — |
| **Platelet count, × $10^9$/L** | | | | | | | | |
| Less than 40 | 55 (9.8) | .2 | 32 (5.7) | .16 | 13 (2.3) | .999 | 8 (1.4) | .999 |
| 40 or higher | 11 (6.5) | — | 5 (3.0) | — | 4 (2.4) | — | 2 (1.2) | — |
| **Hemoglobin level, g/L** | | | | | | | | |
| Less than 10 | 43 (9.2) | .79 | 26 (5.6) | .39 | 11 (2.4) | .999 | 6 (1.3) | .999 |
| 10 or higher | 23 (8.6) | — | 11 (4.1) | — | 6 (2.3) | — | 4 (1.5) | — |
| **Creatinine level, mg/dL** | | | | | | | | |
| Less than 1.4 | 52 (7.5) | <.001 | 27 (3.9) | <.001 | 15 (2.2) | .04 | 8 (1.1) | .43 |
| 1.4 or higher | 10 (71.4) | — | 7 (50.0) | — | 2 (14.3) | — | 1 (7.1) | — |
| **Coagulopathy** | | | | | | | | |
| No | 10 (5.2) | .02 | 3 (1.6) | .01 | 3 (1.6) | .57 | 3 (1.6) | .999 |
| Yes | 53 (10.8) | — | 33 (6.7) | — | 13 (2.7) | — | 6 (1.2) | — |
| **Uric acid level, mg/dL** | | | | | | | | |
| Less than 7 | 45 (7.6) | .44 | 21 (3.5) | .07 | 13 (2.2) | .77 | 9 (1.5) | .98 |
| 7 or higher | 4 (11.1) | — | 4 (11.1) | — | 0 (0) | — | 0 (0.0) | — |
| **Fibrinogen level, mg/dL** | | | | | | | | |
| Less than 170 | 32 (8.5) | .58 | 18 (4.8) | .999 | 9 (2.4) | .999 | 4 (1.1) | .52 |
| 170 or higher | 30 (9.7) | — | 15 (4.9) | — | 8 (2.6) | — | 6 (1.9) | — |
| **Albumin level, mg/dL** | | | | | | | | |
| Less than 3.5 | 19 (14.7) | .003 | 9 (7.0) | .13 | 5 (3.9) | .34 | 5 (3.9) | .02 |
| 3.5 or higher | 30 (6.5) | — | 16 (3.5) | — | 9 (1.9) | — | 3 (0.6) | — |
| **Morphologic subtype** | | | | | | | | |
| Hypergranular | 45 (7.6) | .002 | 26 (4.4) | .09 | 11 (1.9) | .11 | 8 (1.3) | .999 |
| Microgranular | 21 (16.3) | — | 11 (8.5) | — | 6 (4.7) | — | 2 (1.6) | — |
| **PML/RARα isoform** | | | | | | | | |
| BCR1/BCR2 | 27 (7.2) | .11 | 14 (3.7) | .09 | 8 (2.1) | .91 | 5 (1.3) | .75 |
| BCR3 | 29 (10.7) | — | 19 (7.0) | — | 7 (2.6) | — | 2 (0.7) | — |

ECOG indicates Eastern Cooperative Oncology Group; WBC, white blood count; PB, peripheral blood; and —, not applicable.

studies (1.1% and 1.4%, respectively), despite prednisone prophylaxis in the latter. Renal and respiratory failures, either combined or separately, were implicated in all deaths (8 combined, 1 renal, and 1 respiratory failure). Two patients underwent hemodialysis, 2 mechanical ventilation, and 3 both procedures.

**Table 4. Multivariate analysis according to type of induction death**

| | | | | Cause of death | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Induction death | | Hemorrhage | | Infection | | Differentiation syndrome | |
| Covariate | Unfavorable category | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) | P |
| Creatinine level | Abnormal levels | 23.8 (6.3-89.2) | <.001 | 24.3 (7.5-78.1) | <.001 | — | .29 | — | .22 |
| WBC count, × $10^9$/L | 10 or higher | 2.2 (1.1-4.6) | .04 | — | .12 | — | .21 | — | .49 |
| PB blast count, × $10^9$/L | 30 or higher | 3.3 (1.4-7.9) | <.001 | 4.4 (1.9-10.2) | <.001 | — | .13 | — | .85 |
| Age, y | 60 years or older | 4.4 (2.4-8.1) | <.001 | — | .14 | 11.0 (3.9-31.4) | <.001 | — | .56 |
| Sex | Male | 2.8 (1.5-5.2) | <.001 | — | .16 | 5.1 (1.4-18.3) | .003 | — | .61 |
| Coagulopathy at presentation | Yes | — | .21 | 3.3 (0.97-11.6) | .03 | — | .78 | — | .88 |
| Fever at presentation | Yes | — | .49 | — | .85 | 3.9 (1.3-11.7) | .009 | — | .47 |
| ECOG score | At least 2 | — | .45 | — | .86 | — | .98 | 4.5 (1.2-17.2) | .009 |
| Albumin level | Abnormal levels | — | .11 | — | .62 | — | .52 | 4.1 (1.1-16.1) | .045 |

OR indicates odds ratio; WBC, white blood cell; PB, peripheral blood; ECOG, Eastern Cooperative Oncology Group; and —, not applicable.

### Factors predicting fatal outcome of DS

The following prognostic factors (Table 3) correlated with death associated with DS after univariate analysis: ECOG score 2 to 3 ($P = .01$), low level of albumin ($P = .02$), whereas there was a trend for younger age, with 15 years as the most significant cutoff point ($P = .08$). Multivariate analysis identified ECOG score of at least 2 and low level of albumin ($P = .045$) as the only significant independent prognostic factors (Table 4).

### Other causes of death

In the absence of history of cardiac events and coronary risk factors, 2 male patients of 65 and 68 years of age developed a fatal acute myocardial infarction at days 15 and 33 of induction therapy, respectively. Both were diagnosed by chest pain consistent with ongoing myocardial ischemia with concomitant typical changes in electrocardiogram and creatine kinase levels.

## Discussion

Remission induction deaths continue to represent one of the major stumbling blocks in modern therapy of APL. This study shows that hemorrhage is the single most common cause of death (5%) during induction therapy, followed by infection (2.3%) and differentiation syndrome (1.4%) in patients with APL receiving ATRA and idarubicin (AIDA regimen). Typically, the majority of lethal hemorrhages occurred early during induction, whereas infection and DS caused deaths at a somewhat later time. Multivariate analysis identified pretreatment characteristics associated with an increased risk of death, which were different to predict fatal hemorrhage (abnormal creatinine level, increased peripheral blast counts, and presence of coagulopathy), death because of infection (age older than 60 years, male sex, and fever at presentation), and death because of DS (ECOG score ≥ 2 and abnormal albumin levels). Thus, apparently these different types of death seem subject to different and specific risk factors. This may provide clinically relevant information that in the future may be useful for designing more appropriately risk-adapted treatment protocols and improving treatment outcome in this type of leukemia.

A number of studies conducted during the past decade have contributed to the optimizing of the antileukemic efficacy of ATRA when used in combination with anthracycline chemotherapy for induction therapy.[1-3] In fact, this combination shows an extremely high antileukemic efficacy, leading to complete remission rates in 90% to 95% of cases, and leukemia resistance has been only reported in infrequent cases.[4,12-14]

It should be noted that 4 of the 6 nonevaluable patients who were registered by their physicians in our study as resistant leukemia and then administered salvage therapy had developed a definitely present DS, and all of them were assessed prematurely for response, that is, during pancytopenia. This is contradictory to

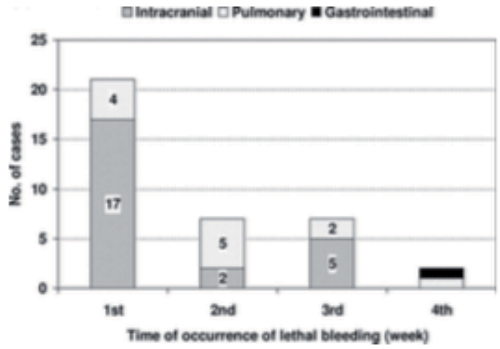


Figure 1. Chronology and site of lethal hemorrhages occurring during induction therapy with the AIDA regimen.

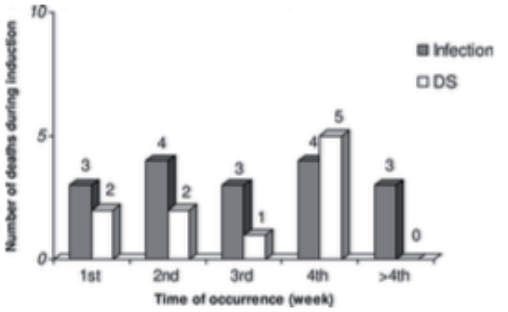


Figure 2. Chronology of deaths because of infection and differentiation syndrome (DS) occurring during induction therapy with the AIDA regimen.

the current recommendations of response assessment in APL.[8] Unfortunately, the hasty administration of salvage therapy to all these patients with delayed clearance of blasts did not allow us to verify whether they were really chemotherapy resistant or rather only exhibited a delay in terminal differentiation of blasts. In case of any doubt about the achievement of CR, it is recommended to repeat another bone marrow assessment after an additional interval of 2 to 3 weeks and meanwhile refrain from new therapeutic interventions. Since the introduction of this policy, no case of resistant leukemia was recorded among the most recent 350 patients enrolled in the PETHEMA studies.

Apart from a virtual absence of leukemia resistance and a lower mortality rate observed in APL compared with other subtypes of acute myeloblastic leukemia (AML), the causes of induction deaths showed a characteristic pattern and time of occurrence that also differ from those in AML.[15] Although infection is the predominant cause of death in AML, hemorrhage is the principal cause of death during induction therapy in APL. However, few data are available about the clinical features of this complication, such as time of onset and time interval between hemorrhage and death, and also little is still known about the prognostic factors of this particular complication. As far as we know, only a study by Kantarjian et al[16] that was performed in 60 patients with morphologically diagnosed APL treated between 1973 and 1984 has previously addressed the analysis of the prognostic factors associated with induction failure because of hemorrhage, as in the present study. Two studies of the Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) group,[4,17] one of them in the pre-ATRA era, have also analyzed the prognostic factors associated with early hemorrhagic death, but it was restricted to deaths occurring within the first 10 days of induction therapy. Finally, 2 additional studies of the ATRA era,[18,19] with 3 and 8 hemorrhagic deaths, respectively, have analyzed the prognostic factors associated with the development of severe hemorrhage but not those factors associated with an increased risk of death because of hemorrhage.

In addition to an increased WBC count, which has been recognized as an independent prognostic factor of response to induction therapy in other studies,[12-14,20] we also found that the presence of coagulopathy and abnormal levels of creatinine were significantly associated with a higher risk of mortality and, most particularly, of hemorrhagic mortality during induction remission. It should be noted that the inclusion of peripheral blast counts in multivariate analysis, which was also recognized as independent prognostic factor of early death (defined as death occurring within the first 10 days of induction treatment) by the Italian GIMEMA group,[4,17] prompted that WBC count was removed from the regression model. The reason for the association between an elevated creatinine value and death of hemorrhage is not clear, although one may speculate that it might be a reflective sign of the disseminated intravascular coagulopathy compromising the glomerular microcirculation. However, this speculation is an apparent contrast with the presence of coagulopathy as an independent prognostic factor. Whatever the explanation, it may also be noted that an abnormal level of creatinine was already found to predict poor response to induction therapy in a study reported by us in the pre-ATRA era.[21] The prognostic value of the hemorrhagic score defined by the GIMEMA group,[22] and also recognized as independent prognostic factor,[4] was not analyzed in the present study because clinical assessment of our patients did not include the use of this score.

It should be noted that the reported hemorrhagic mortality was observed despite a generalized and early aggressive supportive care given to all patients, regardless of prognostic factors. In this context, the acknowledgment of a particular set of prognostic factors can be useful to identify high-risk patients as a potential target population to explore experimental or novel approaches to minimize the risk of death because of hemorrhage. For the systematic use of tranexamic acid prophylaxis in the LPA99 trial, a historical comparison with the LPA96 trial, without tranexamic acid prophylaxis, showed no effect in decreasing hemorrhagic mortality. However, there was a trend toward a higher incidence of thrombosis.[23] Therefore, a potential benefit of the use of tranexamic acid in this setting is not supported by this study.

The type of infections associated with mortality in the present study appears similar to that seen during induction therapy in AML. However, the incidence of infectious deaths (2.3%) was lower than generally reported in other subtypes of AML. Apart from the apparently shorter time to hematologic recovery in APL compared with AML, the relatively low median age of patients and the lack of use of cytarabine, among others, might explain the low infectious mortality rate observed in our series. As far as we know, a study of prognostic factors of remission failure because of infection in APL has not been reported previously. Although age is generally recognized as a risk factor for death during induction therapy because of the greater "vulnerability" to chemotherapy toxicity of older patients, we have found 2 additional factors that contribute to a higher risk of infection-related death, ie, male sex and fever at presentation. It is presently unclear why we observed a lower mortality rate among women. We can speculate on a better tolerance to the side effects of chemotherapy linked to better organ function in female patients, particularly at older age, which would be in line with the somewhat greater life expectancy of women in general. This factor was already noted in a previous study of our group concerned with elderly patients.[24] The greater risk of death because of infection that was apparent in patients presenting with fever at time of diagnosis may be related with an intrinsic susceptibility to infections but also to a more prolonged use of antibiotics, leading to a higher risk of breakthrough bacterial and fungal infections.

It should be noted that the characteristics predisposing to lethal DS, such as ECOG of at least 2 and low levels of serum albumin, should be cautiously interpreted because of low number of events observed (10 of 732 patients at risk). These characteristics were not recognized as independent prognostic factors of remission failure, probably because of the low contribution of DS-associated mortality on the overall mortality. In this regard it is also of note that the systematic use of prednisone prophylaxis in the LPA99 trial, showed no effect on reducing mortality because of DS compared with a selective use of dexamethasone prophylaxis in patients with WBC count greater than $5 \times 10^9$/L in the LPA96 trial.

In summary, our study of a large series of patients homogeneously treated for induction with ATRA and idarubicin shows a characteristic pattern of causes of induction failure, as well as a specific set of prognostic variables that can be applied to predict separate types of induction failure. These predictive models, based on readily available baseline characteristics, may be useful for designing more appropriately risk-adapted treatment protocols aimed at reducing mortality from hemorrhage, infection, or DS.

## Acknowledgments

We thank Miguel Priego for data collection and management.

This study was supported in part by the Fundación para la Investigación Hospital Universitario La Fe-Ayudas Bancaja (grant 2006/0137), Red Temática de Investigación Cooperativa en Cáncer (RD06/0020/0031).

## Authorship

Contribution: J.d.l.S., M.A.S., and P.M. conceived the study and analyzed and interpreted the data; J.d.l.S., M.A.S., P.M., and B.L. wrote the paper; P.M. performed the statistical analyses; E.V., C. Rayón, R.P., A.L., J.E., J.M.B., G.M., G.D., C. Rivas, M.G., M.T., J.D.-M., J.D.G., S.N., E.A., and S.B. included data of patients treated in their institutions, reviewed the manuscript, and contributed to the final draft.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

For a complete list of the members of the Programa de Estudio y Tratamiento de las Hemopatias Malignas study, see Document S1.

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## 9.4 Artículo Anexo 4

Cervera J, Montesinos P, Hernández-Rivas JM, Calasanz MJ, Aventín A, Ferro MT, Luño E, Sánchez J, Vellenga E, Rayón C, Milone G, de la Serna J, Rivas C, González JD, Tormo M, Amutio E, González M, Brunet S, Lowenberg B, Sanz MA.

Additional chromosome abnormalities in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy.

Haematologica. 2010 Mar;95(3):424-31.

Original Articles

# Additional chromosome abnormalities in patients with acute promyelocytic leukemia treated with all-*trans* retinoic acid and chemotherapy

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## ABSTRACT

### Background
Acute promyelocytic leukemia is a subtype of acute myeloid leukemia characterized by the t(15;17). The incidence and prognostic significance of additional chromosomal abnormalities in acute promyelocytic leukemia is still a controversial matter.

### Design and Methods
Based on cytogenetic data available for 495 patients with acute promyelocytic leukemia enrolled in two consecutive PETHEMA trials (LPA96 and LPA99), we analyzed the incidence, characteristics, and outcome of patients with acute promyelocytic leukemia with and without additional chromosomal abnormalities who had been treated with all-*trans* retinoic acid plus anthracycline monochemotherapy for induction and consolidation.

### Results
Additional chromosomal abnormalities were observed in 140 patients (28%). Trisomy 8 was the most frequent abnormality (36%), followed by abn(7q) (5%). Patients with additional chromosomal abnormalities more frequently had coagulopathy ($P$=0.03), lower platelet counts ($P$=0.02), and higher relapse-risk scores ($P$=0.02) than their counterparts without additional abnormalities. No significant association with *FLT3*/ITD or other clinicopathological characteristics was demonstrated. Patients with and without additional chromosomal abnormalities had similar complete remission rates (90% and 91%, respectively). Univariate analysis showed that additional chromosomal abnormalities were associated with a lower relapse-free survival in the LPA99 trial ($P$=0.04), but not in the LPA96 trial. However, neither additional chromosomal abnormalities overall nor any specific abnormality was identified as an independent risk factor for relapse in multivariate analysis.

### Conclusions
The lack of independent prognostic value of additional chromosomal abnormalities in acute promyelocytic leukemia does not support the use of alternative therapeutic strategies when such abnormalities are found.

Key words: acute promyelocytic leukemia, additional chromosomal abnormalities, prognostic factors, all-*trans* retinoic acid, anthracycline.

*Citation: Cervera J, Montesinos P, Hernández-Rivas JM, Calasanz MJ, Aventín A, Ferro MT, Luño E, Sánchez J, Vellenga E, Rayón C, Milone G, de la Serna J, Rivas C, José D. González, Tormo M, Amutio E, González M, Brunet S, Lowenberg B, and Sanz MA. Additional chromosome abnormalities in patients with acute promyelocytic leukemia treated with all-*trans *retinoic acid and chemotherapy. Haematologica. 2010; 95:424-431. doi:10.3324/haematol.2009.013243*



*Funding: this study was supported in part by the Fundación para la Investigación Hospital Universitario La Fe-Ayudas Bancaja (grant 2006/0137), Red Temática de Investigación Cooperativa en Cáncer (RD06/0020/0031).*

*Acknowledgments: the authors thank Miguel Priego for data collection and management.*

*Manuscript received June 22, 2009. Revised version arrived September 1, 2009. Manuscript accepted September 2, 2009.*

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## Introduction

Cytogenetics is the most powerful single prognostic factor for outcome in acute myeloid leukemia[1-3] and the most useful guide available for stratification and planning post-remission treatment in this disease. The t(15;17), characterizing the acute promyelocytic form of acute myeloid leukemia, is considered to be a favorable cytogenetic feature. However, the prognostic significance of additional cytogenetic abnormalities (ACA) in acute promyelocytic leukemia (APL) has remained a matter of debate.

During the 1990s, some studies suggested a relationship between ACA and outcome in APL.[4,5] However, these studies were retrospective and performed in small series of patients mostly treated with chemotherapy alone. More recently, three studies undertaken in patients with APL managed with state-of-the-art treatments, that is, a simultaneous combination of all-*trans* retinoic acid (ATRA) with anthracycline-based chemotherapy, have yielded conflicting results with regard to the impact of ACA on prognosis. In two large studies ACA were not found to have an impact on prognosis,[6,7] while, in the third study, patients with ACA had a higher death rate during induction therapy compared with patients exhibiting the t(15;17) alone.[8] Although none of these studies demonstrated that ACA in APL have a significant impact on the risk of relapse, physicians may be tempted to modify the planned treatment based on the presence of these abnormalities, extrapolating strategies used for the management of other subtypes of acute myeloid leukemia.

In order to clarify the role of ACA in APL patients treated with modern treatments, we report here the characteristics, outcome and prognostic value of cytogenetics in a large cohort of successfully karyotyped patients with a long follow-up who were enrolled in two successive studies carried out by the Spanish *Programa de Estudio y Tratamiento de las Hemopatías Malignas* (PETHEMA) group (studies LPA96 and LPA99).

## Design and Methods

### Patients and eligibility

Between November 1996 and June 2005, a total of 739 patients with *de novo*, genetically confirmed APL were enrolled into two consecutive trials, LPA96 and LPA99. The eligibility criteria and protocols of these studies have been reported elsewhere.[9-11] Informed consent to participation in the studies was obtained from all patients, in accordance with the Declaration of Helsinki. The protocol was approved by the Research Ethics Board of each participating hospital.

### Diagnosis

In addition to the morphological and cytochemical criteria used by the French-American-British classification and routine immunophenotyping, the diagnosis of APL was genetically confirmed in all cases by demonstration of the *PML/RARA* hybrid gene and/or the chromosomal translocation t(15;17)(q22;q21). Immunophenotypic and cytogenetic analyses were systematically performed at presentation only. For the purpose of rapid diagnosis, an immunohistochemical analysis of PML protein distribution was performed, using the monoclonal antibody PG-M3,[12] in a subgroup of patients.

### Cytogenetics and fluorescence in situ hybridization

Bone marrow samples for cytogenetic analysis were processed after short-term culture (24 or 48 h) following standard procedures. The chromosomes were stained by G-banding and the karyotypes reported according to International System for Human Cytogenetic Nomenclature (ISCN, 1995) recommendations.[13] Whenever possible at least 20 metaphases were analyzed in each case. Cases were considered normal diploid if no clonal abnormalities were detected in a minimum of 20 mitotic cells. In most of the patients with apparently normal karyotype and *PML/RARA* rearrangement demonstrated by reverse transcriptase-polymerase chain reaction (RT-PCR), fluorescence *in situ* hybridization (FISH) studies were additionally carried out in metaphase and interphase nuclei. Two-color FISH was performed using a PML/RARA translocation probe (Abbott, Wiesbaden, Germany).

The majority of cytogenetic analyses were performed at reference laboratories. The original cytogenetics reports were requested from the centers for central review. Appropriate karyotype nomenclature (ISCN 1995) was centrally reviewed by two of the authors (JC, JMH). For the purposes of this study, patients with a normal karyotype with the *PML/RARA* fusion demonstrated by either RT-PCR or FISH, were considered as having APL without ACA.[4,14]

### Reverse transcriptase–polymerase chain reaction studies

The details on processing bone marrow samples for RNA extraction and on the RT-PCR protocols for *PML/RARA* amplification used by the participating laboratories have been described elsewhere.[15,16]

### Treatment

The induction regimen consisted of oral ATRA (45 mg/m$^2$/day), divided into two daily doses, which was maintained until complete remission, and intravenous idarubicin (12 mg/m$^2$/day) on days 2, 4, 6, and 8. For patients 20 years of age or younger, the ATRA dose was adjusted to 25 mg/m$^2$/day. From November 1999, the idarubicin on day 8 was omitted for patients older than 70 years. Patients in complete remission received three monthly consolidation courses. The first course consisted of idarubicin (5 mg/m$^2$/day for 4 days), the second of mitoxantrone (10 mg/m$^2$/day for 5 days), and the third of idarubicin (12 mg/m$^2$/day for 1 day). From November 1999 (LPA99 study), intermediate- and high-risk patients, as previously defined,[17] received ATRA (45 mg/m$^2$/day for 15 days) combined with the three chemotherapy courses;[10,11] those based on idarubicin were slightly reinforced by increasing the dose in the first course to 7 mg/m$^2$/day and by administering idarubicin for two consecutive days instead of one in the third course. Patients who tested negative for *PML/RARA* at the end of consolidation were started on maintenance therapy with oral mercaptopurine (50 mg/m$^2$/day), intramuscular methotrexate (15 mg/m$^2$/week), and oral ATRA (45 mg/m$^2$/day for 15 days every 3 months) over 2 years. Details of the supportive therapy have been described elsewhere.[9,10]

### Definitions and study end-points

Response to the remission induction therapy was assessed according to criteria recently revised by Cheson *et al.*[19] Molecular remission was defined as the disappearance on an ethidium bromide gel of the *PML/RARA*-specific band visualized at diagnosis, using an RT-PCR assay with a sensitivity level of $10^{-4}$. Molecular

persistence was defined as PCR positivity in two consecutive bone marrow samples collected at the end of consolidation therapy. Molecular relapse was defined as the reappearance of PCR-positivity in two consecutive bone marrow samples at any time after consolidation therapy. Risk of relapse was established at diagnosis according to a predictive model based on each patient's leukocyte and platelet counts at diagnosis, as reported elsewhere.[17] Low-risk patients had a white cell count less than $10\times10^9$/L and a platelet count more than $40\times10^9$/L; intermediate-risk patients had a white cell count less than $10\times10^9$/L and a platelet count less than $40\times10^9$/L; and high-risk patients had a white cell count equal to or more than $10\times10^9$/L. The presence of coagulopathy was defined as a prolonged prothrombin time and/or activated partial thromboplastin time, in addition to hypofibrinogenemia and/or increased levels of fibrin degradation products or D-dimers.

### Statistical analysis

Differences in the distribution of variables among subsets of patients were analyzed using $\chi^2$ and Fisher's exact tests. Unadjusted time-to-event analyses were performed using the Kaplan-Meier estimate,[20] and, for comparisons, log-rank tests.[21] For all estimates in which the event "relapse" was considered as an end-point, hematologic and molecular relapse, as well as molecular persistence (*PML/RARA*-positive by RT-PCR at the end of consolidation), were each considered as uncensored events. The follow-up of the patients was updated on January 15, 2009. The median follow-up of surviving patients was 85 months (range, 42 to 145 months). Multivariate analysis was performed using the Cox proportional hazards model.[22] All computations were carried out using 3D, 4F, 1L and 2L programs from the BMDP statistical library (BMDP Statistical Software Inc, Los Angeles, CA, USA).

## Results

### Incidence and characteristics of chromosomal abnormalities

Between November 1996 and June 2005, a total of 739 patients with *de novo*, genetically confirmed APL were enrolled into the consecutive LPA96 and LPA99 trials from 82 institutions in Spain, The Netherlands, Belgium, Argentina, Uruguay, and the Czech Republic (see Appendix). Cytogenetic data were not available for 244 cases because cytogenetic studies had not been performed (n=32) or had failed (n=191), or for unknown reasons. Failures in cytogenetic analysis were due to the absence of metaphases (n=124) or either poor quality or insufficient number of metaphases (n=67). All these patients were genetically diagnosed by FISH, RT-PCR or anti-PML staining. Among the remaining 495 patients (67%), 355 (72%) had the t(15;17) translocation as the sole chromosomal abnormality and 140 patients (28%) had ACA; 95 of the patients had one additional abnormality (67%) and 45 had two or more abnormalities (33%) (Table 1). Trisomy 8 (n = 51), either alone (n = 37) or associated with other aberrations (n = 14), was the most frequent abnormality (36%), followed by other less frequent numerical and structural aberrations listed in Table 1.

Dual color FISH studies, RT-PCR or both showed the *PML/RARA* fusion gene in the 71 patients with available karyotype in whom the t(15;17) was not detected by conventional cytogenetics. In 45 of these patients the karyotype was normal while in the remaining 26 cases the karyotype showed other cytogenetic changes but not the t(15;17) (Table 1).

### Cytogenetic abnormalities and disease characteristics

The main clinical and biological characteristics of patients without an available karyotype and those with either t(15;17) alone or t(15;17) with ACA are shown in Table 2. Patients with ACA had significantly lower platelet counts ($P$=0.02) and were, therefore, less frequently classified as at low-risk ($P$=0.02) compared with those without ACA. A similar association with platelet counts and relapse-risk score was also observed according to the number of ACA. Patients with two or more ACA had significantly lower platelet counts ($P$=0.02) and were classified less frequently as being at low-risk ($P$=0.02) compared with those with a single additional chromosomal abnormality. In addition, patients with ACA more frequently had coagulopathy ($P$=0.03) and, although the differences were not statistically significant, tended to be younger ($P$=0.05) and more frequently had the BCR3 *PML/RARA* isoform than patients with t(15;17) alone ($P$=0.08). The presence of trisomy 8 was significantly associated with more fever at diagnosis ($P$=0.01), coagulopathy ($P$=0.02), fibrinogen levels below 170 mg/dL ($P$=0.02), male gender ($P$=0.05), serum uric acid levels above 7 mg/dL ($P$=0.02), and greater than 70% bone marrow blasts ($P$=0.03), and

Table 1. Additional chromosomal abnormalities in patients with APL.

| Number and type of abnormality | Conventional t(15;17) (%) | Cryptic t(15;17) (%) | Total n. of patients (%) | 5-year RFS (%) | P |
|---|---|---|---|---|---|
| N. of patients | 424 (100) | 71 (100) | 495 (100) | 88 | |
| N. of chromosomal abnormalities | | | | | |
| Normal karyotype | 0 (0) | 45 (63) | 45 (9) | 93 | 0.34 |
| t(15;17) alone | 310 (73) | 0 (0) | 310 (63) | 89 | |
| One | 82 (19) | 13 (18) | 95 (19) | 86 | |
| Two | 20 (5) | 5 (7) | 25 (5) | 83 | |
| Three or more | 17 (4) | 3 (4) | 20 (4) | 78 | |
| Numerical abnormalities | 65 (15) | 11(15) | 76 (15) | | |
| Trisomy 8 | 44 (10) | 7 (10) | 51 (10) | | |
| Trisomy 8 alone | 32 (7) | 5 (7) | 37 (7) | | |
| Trisomy 8 + other | 12 (3) | 2 (3) | 14 (3) | | |
| Other numerical | 21 (5) | 4 (6) | 25 (5) | | |
| Structural abnormalities | 54 (13) | 10 (14) | 64 (13) | | |
| Abn(7q) | 6 (1) | 1 (1) | 7 (1) | | |
| Abn(9q) | 5 (1) | 0 (0) | 5 (1) | | |
| Abn(1p) | 4 (1) | 1 (1) | 5 (1) | | |
| Abn(11q) | 5 (1) | 0 (0) | 5 (1) | | |
| Abn(3q) | 3 (1) | 1 (1) | 4 (1) | | |
| i(17q) | 3 (1) | 1 (1) | 4 (1) | | |
| Abn(20q) | 4 (1) | 0 (0) | 4 (1) | | |
| Complex variant t(15;17)* | 0 (0) | 4 (6) | 4 (1) | | |
| Other structural | 25 (6) | 1 (1) | 26 (5) | | |

* complex variant t(15;17) due to a 3-way balanced translocation involving 15q22, 17q21, and another chromosome. RFS: relapse-free survival.

tended to be associated with lower platelet counts ($P$=0.07). The clinicopathological characteristics of patients with trisomy 8 alone did not differ from those of patients with trisomy 8 plus other abnormalities.

### Influence of additional cytogenetic abnormalities on outcome in acute promyelocytic leukemia

Three hundred and twenty-five of the 355 patients (91%) with the t(15;17) alone and 126 of the 140 (90%) with ACA achieved complete remission. These rates were

Table 2. Demographic and baseline characteristics of the study population.

| Characteristic | Non available cytogenetics Median (range) | N. (%) | P* | t(15;17) alone Median (range) | N. (%) | t(15;17) with other abnormalities Median (range) | N. (%) | P° |
|---|---|---|---|---|---|---|---|---|
| Overall | | 244 (100) | | | 355 (100) | | 140 (100) | |
| Age, years | 40 (2-81) | | 0.13 | 41 (2-83) | | 39 (3-73) | | 0.05 |
| 18 or younger | | 35 (14) | | | 31 (9) | | 18 (13) | |
| 19-50 | | 139 (57) | | | 207 (58) | | 83 (59) | |
| 51-60 | | 32 (13) | | | 41 (12) | | 22 (16) | |
| 61-70 | | 26 (11) | | | 47 (13) | | 14 (10) | |
| 71 or older | | 12 (5) | | | 29 (8) | | 3 (2) | |
| Gender | | | | | | | | |
| Male | | 121 (50) | 0.69 | | 177 (50) | | 76 (54) | 0.37 |
| Female | | 123 (50) | | | 178 (50) | | 64 (46) | |
| ECOG score | | | | | | | | |
| 0-1 | | 172 (76) | 0.69 | | 245 (76) | | 95 (75) | 0.84 |
| 2-3 | | 54 (24) | | | 84 (24) | | 31 (25) | |
| WBC count, ×10⁹/L | 2.4 (0.3-164) | | 0.21 | 2.0 (0.2-460) | | 2.8 (0.3-210) | | 0.17 |
| Less than or equal to 3.5 | | 143 (59) | | | 229 (64) | | 75 (54) | |
| 3.5-10 | | 34 (14) | | | 43 (12) | | 29 (21) | |
| 10-50 | | 54 (22) | | | 65 (18) | | 33 (23) | |
| Higher than 50 | | 12 (5) | | | 18 (4) | | 3 (2) | |
| Platelet count, ×10⁹/L | 20 (1-207) | | 0.32 | 23 (1-207) | | 20 (1-137) | | 0.02 |
| Less than or equal to 40 | | 191 (79) | | | 261 (73) | | 116 (83) | |
| Higher than 40 | | 52 (21) | | | 94 (27) | | 24 (17) | |
| Creatinine, mg/dL | | | | | | | | |
| Less than or equal to 1.4 | | 231 (98) | 0.96 | | 338 (98) | | 133 (98) | 0.99 |
| Higher than 1.4 | | 4 (2) | | | 7 (2) | | 3 (2) | |
| Coagulopathy | | | | | | | | |
| No | | 54 (22) | 0.66 | | 93 (26) | | 24 (17) | 0.03 |
| Yes | | 188 (78) | | | 259 (74) | | 116 (83) | |
| Fibrinogen, mg/dL | | | | | | | | |
| Less than or equal to 170 | | 132 (57) | 0.51 | | 179 (55) | | 72 (53) | 0.76 |
| Higher than 170 | | 99 (43) | | | 147 (45) | | 63 (47) | |
| Albumin, g/dL | | | | | | | | |
| Less than or equal to 3.5 | | 44 (22) | 0.83 | | 65 (23) | | 22 (19) | 0.43 |
| Higher than 3.5 | | 152 (78) | | | 221 (77) | | 93 (81) | |
| Morphologic subtype | | | | | | | | |
| Hypergranular | | 197 (82) | 0.89 | | 288 (82) | | 113 (82) | 0.92 |
| Microgranular | | 44 (18) | | | 62 (18) | | 25 (18) | |
| *PML/RARA* isoform | | | | | | | | |
| BCR1/BCR2 | | 132 (60) | 0.47 | | 186 (60) | | 64 (51) | 0.08 |
| BCR3 | | 87 (40) | | | 124 (40) | | 62 (49) | |
| Relapse-risk group | | | | | | | | |
| Low | | 45 (19) | 0.66 | | 80 (23) | | 16 (11) | 0.02 |
| Intermediate | | 132 (56) | | | 192 (54) | | 88 (63) | |
| High | | 66 (25) | | | 83 (23) | | 36 (26) | |
| *FLT3/ITD* | | | | | | | | |
| Yes | | 22 (27) | 0.34 | | 33 (23) | | 12 (19) | 0.18 |
| No | | 60 (73) | | | 112 (77) | | 51 (81) | |
| Protocol | | | | | | | | |
| LPA96 | | 54 (22) | 0.49 | | 84 (24) | | 37 (26) | 0.52 |
| LPA99 | | 190 (78) | | | 271 (76) | | 103 (74) | |

*P values of the comparison of patients without evaluable karyotype versus patients successfully karyotyped. @ P values of the comparison of patients with t(15;17) alone versus patients with t(15;17) and additional abnormalities.

not statistically different (Table 3). The complete remission rate among patients for whom cytogenetic data were unavailable or inadequate was not different (89%, *P*=0.26).

Concerning the subsequent clinical outcome of patients who achieved complete remission, a total of 53 relapses were recorded (34 clinical and 19 molecular relapses, including five with molecular disease persistence after consolidation therapy). The overall 5-year relapse-free survival, disease-free survival, and overall survival rates were 88%, 85%, and 83%, respectively. The corresponding rates among patients for whom cytogenetic data were unavailable or inadequate were 85%, 80%, and 76% (*P*=0.28, *P*=0.12, and *P*=0.08, respectively).

The results of univariate analysis of relapse-free survival are presented in Table 4. In patients with available karyotype, when both protocols LPA96 and LPA99 were considered together, several variables, such as gender, relapse-risk score, morphological subtype, and *PML/RARA* isoform, had a statistically significant prognostic value, but the presence of ACA did not (*P*=0.10). When analyzed separately, trisomy 8 was associated with a statistically lower relapse-free survival compared with the absence of trisomy 8 (78% *versus* 89%, *P*=0.03). The relapse-free survival was lower in relation to the number of chromosomal abnormalities detected by conventional karyotyping, but the differences were not statistically significant (5-year relapse-free survival of 93% in patients with a normal karyotype; 89% in patients with t(15;17) alone; 86% in those with one ACA; 83% with two ACA; and 78% with three or more ACA; *P*=0.34) (Table 1). Multivariate analysis identified relapse-risk score and male gender as the only independent adverse factors for relapse-free survival (*P*<0.0001 and *P*=0.03, respectively).

Given the better outcome of the patients treated in the LPA99 trial compared with those in LPA96, as reported in previous analyses of this series when patients with and without available karyotype were included,[10,11] we performed an analysis separately by protocol (Table 4). The

**Table 3. Complete remission, overall survival, disease-free survival and relapse-free survival rates in patients with and without additional chromosome abnormalities.**

| | t(15;17) alone | t(15;17) with other abnormalities | |
|---|---|---|---|
| Outcome | (%) | (%) | P |
| LPA96 & LPA99 patients | | | |
| Complete remission | 91 | 90 | 0.59 |
| 5-year overall survival | 84 | 81 | 0.82 |
| 5-year disease-free survival | 86 | 82 | 0.42 |
| 5-year relapse-free survival | 90 | 84 | 0.10 |
| LPA96 patients | | | |
| 5-year relapse-free survival | 81 | 89 | 0.33 |
| LPA99 patients | | | |
| 5-year relapse-free survival | 92 | 82 | 0.01 |

**Table 4. Univariate and multivariate analysis for relapse-free survival in the study population.**

| Characteristic | LPA96 & LPA99 trials (n=451) Univariate 5-years RFS (%) | P | Multivariate P | Multivariate Hazard ratio [95% CI] | LPA96 trial (n=108) Univariate 5-years RFS (%) | P | Multivariate P | Multivariate Hazard ratio [95% CI] | LPA99 trial (n=343) Univariate 5-years RFS (%) | P | Multivariate P | Multivariate Hazard ratio [95% CI] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Overall | 88 | | | | 84 | | | | 90 | | | |
| Gender | | | | | | | | | | | | |
| Male | 85 | 0.02 | 0.02 | 0.59 [0.38-0.92] | 83 | 0.79 | NS | | 85 | 0.01 | 0.01 | 0.46 [0.27-0.80] |
| Female | 92 | | | | 85 | | | | 93 | | | |
| Relapse-risk group* | | | | | | | | | | | | |
| Low risk | 95 | <0.001 | <0.001 | 3.60 [2.48-5.22] | 91 | 0.01 | 0.01 | 2.67 [1.23-5.79] | 96 | <0.001 | <0.001 | 3.49 [2.16-5.65] |
| Intermediate risk | 91 | | | | 88 | | | | 92 | | | |
| High risk | 74 | | | | 65 | | | | 77 | | | |
| Cytogenetics | | | | | | | | | | | | |
| t(15;17) | 90 | 0.10 | NS | | 81 | 0.33 | NS | | 92 | 0.01 | NS | |
| t(15;17) + other | 84 | | | | 89 | | | | 82 | | | |
| Morphological subtype | | | | | | | | | | | | |
| Hypergranular | 90 | 0.02 | NS | | 89 | <0.001 | NS | | 90 | 0.60 | NS | |
| Microgranular | 81 | | | | 56 | | | | 88 | | | |
| *PML/RARA*α isoform | | | | | | | | | | | | |
| BCR1/BCR2 | 90 | 0.02 | NS | | 83 | 0.89 | NS | | 92 | 0.004 | 0.02 | 1.85 [1.02-3.33] |
| BCR3 | 82 | | | | 82 | | | | 82 | | | |
| PETHEMA LPA trial | | | | | | | | | | | | |
| LPA96 | 84 | 0.09 | NS | | | NA | | | NA | NA | | |
| LPA99 | 90 | | | | NA | | | | | | | |

*RFS: relapse-free survival; NA: not applicable; NS: not significant. *Low risk: WBC ≤10x10⁹/L and platelets >40x10⁹/L; intermediate risk: WBC ≤10x10⁹/L and platelets ≤40x10⁹/L; high risk: WBC >10x10⁹/L.*

univariate analysis showed that the presence of ACA at diagnosis was significantly associated with lower relapse-free survival in the LPA99 trial (82% *versus* 92%, *P*=0.01) (Figure 1A), but not in the LPA96 trial (89% *versus* 81%, *P*=0.33). In the LPA99 trial, univariate analysis also showed a lower relapse-free analysis for patients with trisomy 8 (77% *versus* 91%, *P*=0.02) (Figure 1B). Multivariate analysis showed that in addition to relapse-risk score and male gender, the BCR3 isoform was an independent adverse factor for relapse-free survival in the LPA99 trial, but the presence of ACA was not.

## Discussion

This study shows that roughly one third of patients with APL have ACA besides the t(15;17). Among these secondary chromosome aberrations, trisomy 8 is by far the most frequent abnormality, accounting for about one third of the additional abnormalities. In the context of state-of-the-art treatment based on a combination of ATRA and anthracycline-based chemotherapy, the presence of ACA, particularly two or more, or of trisomy 8 was associated with lower platelet counts, a higher relapse-risk score and lower relapse-free survival. However, multivariate analysis showed that neither the presence of ACA nor trisomy 8 is an independent adverse factor for relapse.

The incidence of ACA in APL has been consistently reported to be within the range of 26% to 39%,[1,4-8,23] trisomy 8 being the most frequent abnormality (33% to 53% of secondary changes). The incidence of ACA and the proportion of trisomy 8 among these abnormalities reported in the present study, 28% and 36%, respectively, are both within the ranges reported in the literature. It should be noted that the prevalence of abn(7q), the most common abnormality after trisomy 8, is usually in the range from 5% to 8%,[4-6,24] and in our study was 5%, but in a recent study by the German Acute Myeloid Leukemia Study Group (AMLSG) the prevalence was much higher (27% of aberrations).[8] This German study was, however, based on a small series of seven patients displaying this abnormality among only 26 patients with additional changes.

The relative high frequency of some additional chromosome abnormalities, particularly trisomy 8, may suggest the appropriateness of performing a systematic FISH analysis including a centromeric probe for chromosome 8 in the diagnostic work-up of patients with APL and perhaps extending this to the detection of del(7q). As has been previously reported,[14] we found that patients with t(15;17) not detected by conventional karyotyping had the same pattern of ACA as patients with conventionally identified t(15;17), with chromosome 8 abnormalities being most common. This finding would suggest that the ACA are important cooperating lesions in the leukemogenesis of APL.

With regards to clinicopathological characteristics, the association of ACA with low platelet counts, intermediate- and high-risk disease, and the presence of coagulopathy found in the present study has not been previously reported as far as we know. At the molecular level, a previous study found a relationship between the breakpoint at the BCR3 region and the presence of ACA.[4] We did not demonstrate a statistically significant relation between the BCR3 isofom and the presence of ACA, but there was a tendency for the two to be associated (*P*=0.08). Another interesting relationship, between ACA and the mutational status of the *FLT3* gene, has been recently suggested.[24,25] A Medical Research Council study[24] revealed an inverse relationship between the frequency of *FLT3*/ITD and presence of ACA accompanying t(15;17) analyzed by conventional cytogenetics. This finding has also been reported by Akagi *et al.*[25] who analyzed ACA with high-density single-nucleotide polymorphism microarray to detect copy-number-neutral loss of heterozygosity. Interestingly, *FLT3*/ITD mutations occurred only in the group with no genomic alterations. In ours series, this mutation occurred in a lower

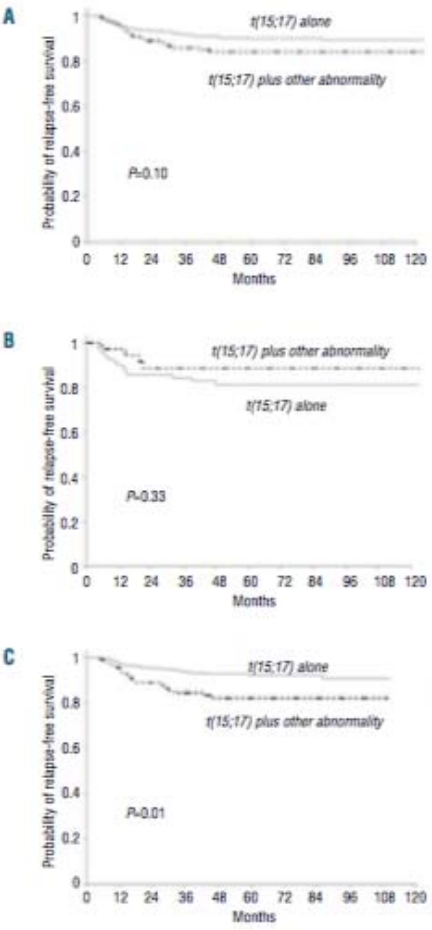


**Figure 1. Relapse-free survival of patients according to the presence of additional chromosomal abnormalities in the: (A) LPA96 and LPA99 trials, (B) LPA96 trial, and (C) LPA99 trial.**

proportion of patients with trisomy 8 (11.5%) than in those with other ACA (24.3%) or without ACA (22.8%), but the differences were not statistically significant.

Although the AMLSG study[6] reported that patients dying during induction therapy had significantly higher initial white blood cell counts and a higher likelihood of trisomy 8 or abn(7q) as ACA, no other study has found such an association between cytogenetics and induction outcome. Indeed, we found that patients with ACA had a similar induction death rate as those with only the t(15;17). The association observed in the present study between ACA and coagulopathy, which is potentially implicated in an increased risk of induction death,[18] could explain in part the results of the German study.

As far as we know, only two contradictory studies based on small series of patients treated in the pre-ATRA era have previously shown some association between ACA and relapse.[4,5] In 54 patients (44 treated without ATRA), Hiorns *et al.*[5] found that relapse-free survival was significantly correlated with karyotype: patients without ACA and with a low white blood cell count had a significant advantage in terms of relapse-free survival in comparison with patients with other combinations of these factors. In contrast, Slack *et al.*[4] in a study carried out in 80 patients treated with chemotherapy alone, found that the presence of a secondary chromosome abnormality was associated with a longer complete remission duration. Our study, based on a large series of patients treated with ATRA plus anthracycline-based chemotherapy with prolonged follow-up, does not confirm a significant relapse-free survival disadvantage in APL patients with ACA. It should be noted that the adverse prognostic impact of ACA on relapse observed in the LPA99 trial, which was not independent of relapse-risk score, male gender, and BCR3 isoform, was not observed in the LPA96 trial. Apart from differences in sample size that could explain a different impact of ACA in the LPA96 and LPA99 trials, it is well known that the efficacy of therapy can have a critical influence on the prognostic significance of other variables. In this regard, previous reports described a lower relapse-free survival in the LPA99 trial,[10,11] which may have contributed to altering the prognostic value of many variables, including ACA. We can also speculate that the adverse impact of ACA on relapse-free survival in the LPA99 trial, which was revealed by univariate analysis, could be masked in multivariate analysis because of the association of such abnormalities with intermediate- and high-risk groups and the BCR3 isoform. It is conceivable that ACA in general, or some specific abnormality in particular (e.g., trisomy 8), might have a role in generating the factors leading to a poorer risk score. Further studies to confirm and elucidate the relative importance of these variables are warranted.

In conclusion, this study confirms that one third of patients with *de novo* APL display ACA at diagnosis, trisomy 8 being the most frequent abnormality. Patients with ACA had significantly more coagulopathy, and were less frequently classified as being at a low-risk of relapse. Although ACA and trisomy 8 were significantly associated with lower relapse-free survival, they were not identified as independent risk factors for relapse, probably because of their association with relapse-risk score. Until confirmation of this hypothesis, additional therapeutic strategies are not required in APL patients with ACA, at least in the context of ATRA plus anthracycline monochemotherapy-based regimens.

## Authorship and Disclosures

JC, PM, and MS conceived the study, and analyzed and interpreted the data; JC, PM, MS, and BL wrote the paper; PM performed the statistical analyses; JC, JMH, MJC, AA, MTF, EL, and JS were responsible for the main cytogenetic laboratories; EV, CR, GM, JS, CR, JDG, MT, EA, MG and SB were clinicians responsible for the patients: they took care of the protocol, sampling and collection of clinical data for the patients treated in their institutions.

All authors reviewed the manuscript and contributed to the final draft.

The authors declare they have no conflicts of interest.

## Appendix

*The following institutions and clinicians participated in the study: Argentina (Grupo Argentino de Tratamiento de la Leucemia Aguda)—Complejo Médico Policía Federal, La Plata: L. Palmer; Fundaleu, Buenos Aires: S. Pavlovsky, G. Milone, I. Fernández; Hospital Clemente Álvarez, Rosario: S. Ciarlo, F. Bezares; Hospital de Clínicas, Buenos Aires: H. Longoni; Hospital General San Martín, La Plata: M. Gelemur, P. Fazio; Hospital Rossi, La Plata: C. Canepa, S. Saba; Hospital San Martín de Paraná, Entre Ríos: P. Negri; Instituto Privado de Hematología, Paraná: M. Giunta;Instituto de Trasplante de Médula Ósea, La Plata: J. Milone, V. Prates; Czech Republic—Faculty Hospital, Brno: M. Protivankova; Spain (Programa Español de Tratamiento de las Hemopatías Malignas)—Basurtuko Ospitalea, Bilbao: J. M. Beltrán de Heredia; Complejo Hospitalario de Segovia: J. M. Hernández; Complexo Hospitalario Xeral-Calde, Lugo; J. Arias; Complejo Hospitalario, León: F. Ramos; Fundación Jiménez Díaz, Madrid: A. Román; Hospital 12 de Octubre, Madrid: J. de la Serna; Hospital Carlos Haya, Málaga: S. Negri; Hospital Central de Asturias, Oviedo: C. Rayón; Hospital Clínic, Barcelona: J. Esteve; Hospital Clínico de Valladolid: FJ. Fernández-Calvo; Hospital Clínico San Carlos, Madrid: J. Díaz Mediavilla; Hospital Clínico San Carlos (H. Infantil), Madrid: C. Gil; Hospital Clínico Universitario, Santiago de Compostela: M. Pérez; Hospital Clínico Universitario, Valencia: M. Torno; Hospital Clínico Universitario Lozano Blesa, Zaragoza: M. Olave; Hospital de Cruces, Baracaldo: E. Amutio; Hospital del Mar, Barcelona: C. Pedro; Hospital de Navarra, Pamplona: A. Gorosquieta; Hospital Dr Negrín, Las Palmas: T. Molero; Hospital Dr Peset, Valencia: M. J. Sayas; Hospital Dr Trueta, Girona: R. Guardia; Hospital General de Albacete: J. R. Romero; Hospital General de Alicante: C. Rivas; Hospital General de Alicante (Oncología Pediátrica): C. Esquembre; Hospital General de Castellón: R. García; Hospital General de Especialidades Ciudad de Jaén: A. Alcalá; Hospital General de Jerez de la Frontera: A. León; Hospital General de Murcia: M.L. Amigo; Hospital General de Valencia: M. Linares; Hospital Germans Trias i Pujol, Badalona: J. M. Ribera; Hospital Insular de Las Palmas: J. D. González San Miguel; Hospital Juan Canalejo, A Coruña: G. Debén; Hospital Joan XXIII, Tarragona: L. Escoda; Hospital La Princesa, Madrid: R. de la Cámara; Hospital Materno-Infantil de Las Palmas: A. Molines; Hospital do Meixoeiro, Vigo: C. Loureiro; Hospital Montecelo, Pontevedra: M. J. Allegue; Hospital Mutua de Terrasa: J. M. Martí; Hospital Niño Jesús, Madrid: L. Madero; Hospital Ntra. Sra. de Sonsoles, Ávila: M. Cabezudo; Hospital Ramón y Cajal, Madrid: J. García-Laraña; Hospital Reina Sofía, Córdoba: R. Rojas; Hospital Río Carrión, Palencia: F. Ortega; Hospital Río Hortega, Valladolid: M. J. Peñarrubia; Hospital San Jorge, Huesca: F. Puente; Hospital San Rafael, Madrid: B. López-Ibor; Hospital Sant Pau, Barcelona: S. Brunet; Hospital San Pedro de Alcántara, Cáceres: J. M. Bergua; Hospital Santa María del Rosell, Cartagena: J. Ibáñez; Hospital Severo Ochoa, Leganés: P. Sánchez; Hospital Son Dureta, Palma de Mallorca: A. Novo;*

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## 9.5 Artículo Anexo 5

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Does microgranular variant morphology of acute promyelocytic leukemia independently predict a less favorable outcome compared with classical M3 APL? A joint study of the North American Intergroup and the PETHEMA Group.

Blood. 2010 Dec 16;116(25):5650-9.

MYELOID NEOPLASIA

# Does microgranular variant morphology of acute promyelocytic leukemia independently predict a less favorable outcome compared with classical M3 APL? A joint study of the North American Intergroup and the PETHEMA Group

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**Few studies have examined the outcome of large numbers of patients with the microgranular variant (M3V) of acute promyelocytic leukemia (APL) in the all-*trans* retinoic acid era. Here, the outcome of 155 patients treated with all-*trans* retinoic acid–based therapy on 3 clinical trials, North American Intergroup protocol I0129 and Programa para el Estudio de la Terapéutica en Hemopatía Maligna protocols LPA96 and LPA99, are reported. The complete remission rate for all 155 patients was 82%, compared with 89% for 748 patients with classical M3 disease. The incidence of the APL differentiation syndrome was 26%, compared with 25% for classical M3 patients, and the early death rate was 13.6% compared with 8.4% for patients with classical M3 morphology. With a median follow-up time among survivors of 7.6 years (range 3.6-14.5), the 5-year overall survival, disease-free survival, and cumulative incidence of relapse for patients with M3V were 70%, 73%, and 24%, respectively. With a median follow-up time among survivors of 7.6 years (range 0.6-14.3), the 5-year overall survival, disease-free survival, and cumulative incidence of relapse among patients with classical M3 morphology were 80% ($P = .006$ compared with M3V), 81% ($P = .07$), and 15% ($P = .005$), respectively. When outcomes were adjusted for the white blood cell count or the relapse risk score, none of these outcomes were significantly different between patients with M3V and classical M3 APL. (*Blood.* 2010;116(25):5650-5659)**

## Introduction

Approximately 15%-25% of adults and perhaps a somewhat higher incidence of children with acute promyelocytic leukemia (APL) have the microgranular variant (M3V) characterized by leukemia promyelocytes that are generally devoid of or have only sparse fine granules[1-6] and infrequent Auer rods.[7] In addition to the distinctive morphologic features, this variant form of the disease is associated with unique biological characteristics including a higher white blood cell count (WBC) at presentation[8] and frequent expression of CD2,[9-11] the stem cell marker CD34,[10,11] and *FLT3* internal tandem duplication (*ITD*) mutations.[12,13] Several series[9,14] but not all[15,16] reported an association of the S-isoform of promyelocyte (*PML*) with M3V. Classical hypergranular APL and the M3V have distinct gene expression signatures.[17] Historically, when treated with conventional chemotherapy, the M3V has been associated with a higher incidence of early death,[2] but not necessarily with an inferior outcome compared with that associated with classical APL.[18-24] However, few studies in the all-*trans* retinoic acid (ATRA) era have reported the outcome of a large number of patients with M3V.

Therefore, we sought to determine the outcome of patients with M3V when treated with ATRA-based strategies. In the present study, we undertook an analysis of 3 large series of patients treated with ATRA plus anthracycline-based regimens, North American Intergroup protocol I0129 and Programa de Estudio Tratamiento de las Hemopatias Malignas (PETHEMA) protocols LPA96 and LPA99, to have sufficient numbers of patients to definitively determine the outcome.

Submitted June 8, 2010; accepted August 30, 2010. Prepublished online as *Blood* First Edition paper, September 21, 2010; DOI 10.1182/blood-2010-02-270819.

Presented in part at the 46th annual meeting of the American Society of Hematology, December 4-7, 2004, San Diego, CA.

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## Methods

Patients with M3V registered on either North American Intergroup Protocol I0129 or PETHEMA Protocols LPA96 or LPA99 with a confirmed diagnosis of APL by either cytogenetics or molecular genetics were analyzed. The diagnosis of M3V was established when most of the leukemic cells were devoid of granules or had only sparse granules.[25,26] The abnormal promyelocytes had bilobed nucleoli with basophilic cytoplasm that varied from faint to strong. Rare cells with multiple Auer rods were found almost invariably. Myeloperoxidase and granulocyte esterase were strongly positive as in classical APL. The morphology establishing the diagnosis of M3V among patients treated on the North American Intergroup Protocol I0129 was centrally reviewed by a single author (J.M.B.). The morphology from the bone marrow of M3V patients treated on the PETHEMA protocols was not centrally reviewed. The diagnoses were confirmed either cytogenetically or molecularly in all 3 studies.

### North American Intergroup Protocol I0129

The results of the North American Intergroup Protocol I0129 have been previously reported.[23,24] Briefly, patients registered to North American Protocol I0129 were randomly assigned for induction to receive either ATRA or chemotherapy, which included daunorubicin plus cytarabine. Patients assigned to ATRA were to receive 45 mg/m$^2$/d orally in 2 divided doses given every 12 hours. Patients assigned to chemotherapy were to receive daunorubicin 45 mg/m$^2$/d by intravenous bolus on days 1-3 plus cytarabine 100 mg/m$^2$/d by continuous intravenous infusion on days 1-7 (DA). All patients achieving a complete remission (CR) with either ATRA or chemotherapy received 2 courses of consolidation. The first was identical to the first induction chemotherapy regimen, and the second included high-dose cytarabine 2 gm/m$^2$ as a 1-hour intravenous infusion every 12 hours for 4 consecutive days with daunorubicin 45 mg/m$^2$/d by intravenous infusion on days 1 and 2. For patients less than 3 years of age, the second cycle included cytarabine 67 mg/kg as a 1-hour intravenous infusion every 12 hours for 4 consecutive days with daunorubicin 1.5 mg/kg/d by intravenous infusion on days 1 and 2. Patients randomized to ATRA were to continue the drug until CR occurred or a maximum of 90 days. Patients continuing in CR after consolidation were randomized to either 1 year of daily maintenance ATRA or observation. Patients randomized to chemotherapy (DA) only for induction and not ATRA were excluded from all analyses.

### PETHEMA protocols LPA96 and LPA99

Results of the PETHEMA protocols LPA96 and LPA99 have been previously reported.[27-29] Briefly, the induction regimen consisted of oral ATRA 45 mg/m$^2$/d until CR and intravenous idarubicin (12 mg/m$^2$/d) on days 2, 4, 6, and 8 (all-*trans* retinoic acid and idarubicin [AIDA] regimen). From November 1999, the idarubicin on day 8 was omitted for patients older than 70 years. Patients in CR received 3 monthly consolidation courses. The first course consisted of idarubicin 5 mg/m$^2$/d for 4 days, the second of mitoxantrone 10 mg/m$^2$/d for 5 days, and the third of idarubicin 12 mg/m$^2$/d for 1 day. From November 1, 1999 (LPA99 study), intermediate- and high-risk patients, as previously defined,[29] received ATRA 45 mg/m$^2$/d for 15 days combined with the reinforced single-agent chemotherapy courses.[28] Risk of relapse was established at diagnosis according to a predictive model based on patient leukocyte and platelet counts at diagnosis, as reported elsewhere.[30] Low-risk patients had a WBC less than or equal to $10 \times 10^9$/L and a platelet count more than $40 \times 10^9$/L; intermediate-risk patients had a WBC less than or equal to $10 \times 10^9$/L and a platelet count less than or equal to $40 \times 10^9$/L; and high-risk patients had a WBC more than $10 \times 10^9$/L.

Patients who tested negative for *PML/RARA* fusion transcript at the end of consolidation were started on maintenance therapy with oral mercaptopurine 50 mg/m$^2$/d, intramuscular methotrexate 15 mg/m$^2$/wk, and oral ATRA 45 mg/m$^2$/d for 15 days every 3 months over 2 years. Details of the supportive therapy have been described elsewhere.[27,28]

### Definition of the APL differentiation syndrome

For patients on I0129, APL differentiation syndrome (DS) was defined as grade 2 or higher pulmonary or cardiac toxicity with unexplained fever, weight gain, respiratory distress, interstitial pulmonary infiltrates, and/or pleural or pericardial effusions.[23,24] The definition of the APL DS for patients treated on the 2 PETHEMA protocols matched that used for patients on I0129 except that renal failure, itself defined as a creatinine above the upper limit of normal, was also considered a criterion for the DS.[31]

### Statistical analysis

Descriptive statistical analysis was performed to assess patient baseline characteristics. The Cochran-Mantel-Haenszel test was used for comparison of categorical variables and a stratified Wilcoxon rank-sum test was used for comparison of continuous variables. All tests were stratified by the source of patients: I0129, LPA96, LPA99.

Overall survival (OS) and disease-free survival (DFS) were calculated using the Kaplan-Meier method. The stratified log-rank test was used for comparison of Kaplan-Meier curves, stratifying by the source of patients. OS was defined as the time between start of induction and death from any cause. DFS was defined as the time between documented date of CR and relapse or death from any cause. Cumulative incidence curves for relapse with or without death were constructed reflecting time to nonrelapse death as a competing risk. Time to relapse and time to nonrelapse death were measured from the documented date of CR to relapse or nonrelapse death. Patients who were alive without relapse were censored at the time last seen alive and relapse-free. The difference between cumulative incidence curves in the presence of a competing risk was tested using the Gray method[32] stratified by the source of patients. The impact of the APL morphology subtype (M3 vs. M3V) on the outcomes was also examined in multivariable proportional hazards model for OS and DFS and multivariable competing risks regression model[33] for relapse and nonrelapse death. In multivariable models, age, sex, WBC, platelet, and hemoglobin were included along with the APL morphology subtype. All interaction terms between the APL morphology subtype and prognostic factors were examined.

## Results

### Patient characteristics

Patient characteristics are shown in Table 1. A total of 155 patients with M3V accrued to the 3 clinical trials were analyzed. The median age was 39 years (range 3-79). The median WBC was $15.8 \times 10^9$/L (range 0.60--$550 \times 10^9$). Among 748 patients with classical M3 APL, the median age was 40 years (range 1-83) and the median WBC was $1.8 \times 10^9$/L (range 0.2-$460 \times 10^9$/L). There was a significant difference in WBC at baseline between patients with M3V and classical M3 ($P < .0001$). The proportion of the relapse risk score at diagnosis, according to PETHEMA–Gruppo Italiano Malattie Ematologiche dell'Adulto (GIMEMA) criteria, for patients with M3V was 15% low, 25% intermediate, and 61% high risk. Among patients with classical M3 APL, the proportions of patients among the 3 risk groups were 24%, 60%, and 16%, respectively. Thus, the difference in distribution of the relapse risk score between M3V and classical M3 was significant ($P < .0001$). Patients with M3V were more likely to have high-risk disease at diagnosis because of the higher WBC in these patients.

As to the difference in baseline characteristics among 3 studies with M3V, WBC was higher in LPA96 and LPA99 ($19.8 \times 10^9$/L, $15.9 \times 10^9$/L, respectively) compared with I0129 ($5.8 \times 10^9$/L) ($P = .01$), but platelet was lower ($19.5 \times 10^9$/L, $22 \times 10^9$/L, respectively) compared with I0129 ($36 \times 10^9$/L, $P = .005$).

The median follow-up of all surviving patients with M3 and M3V combined was 7.6 years (range 0.6-14.5): 11.9 and 10.9 years

**Table 1. Baseline patient characteristics**

| | M3 | | | | M3V | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | I0129 (n = 150) | LPA96 (n = 145) | LPA99 (n = 453) | All (n = 748) | I0129 (n = 24) | LPA 96 (n = 30) | LPA 99 (n = 101) | All (n = 155) | P |
| Median age, y (range) | 37 (1-81) | 42 (2-78) | 40 (2-83) | 40 (1-83) | 36 (5-76) | 40 (12-71) | 39 (3-79) | 39 (3-79) | .51 |
| Male, n (%) | 71 (47) | 87 (60) | 216 (48) | 374 (51) | 11 (46) | 16 (53) | 50 (53) | 77 (50) | .93 |
| WBC, median (range) | 1.8 (0.3-95.2) | 1.5 (0.3-148.0) | 1.8 (0.2-460) | 1.8 (0.2-460) | 5.8 (0.6-550) | 19.8 (1.4-210) | 15.9 (0.7-188.5) | 15.8 (0.6-550) | < .0001 |
| Platelets, median (range) | 36.5 (5-246) | 20 (1-161) | 22 (1-207) | 24 (1-246) | 36 (6-123) | 19.5 (4-146) | 22 (1.8-207) | 23 (1.8-207) | .52 |
| Hemoglobin, median (range) | 9.5 (2.9-16.4) | 9.5 (4.4-12.8) | 8.9 (3.0-15.3) | 9.2 (2.9-16.4) | 9.6 (2.1-13.2) | 9.1 (4.3-15.2) | 10.0 (4.4-16.9) | 9.8 (2.1-16.9) | .0005 |
| Relapse risk, n (%) | | | | | | | | | < .0001 |
| Low | 56 (37) | 31 (21) | 94 (21) | 181 (24) | 8 (33) | 3 (10) | 12 (12) | 23 (15) | |
| Intermediate | 72 (48) | 91 (63) | 284 (63) | 447 (60) | 7 (29) | 6 (20) | 25 (25) | 38 (25) | |
| High | 22 (15) | 23 (16) | 75 (17) | 120 (16) | 9 (38) | 21 (70) | 64 (64) | 94 (61) | |
| RAS, n (%) | 43 (29) | 41 (28) | 102 (23) | 186 (25) | 1 (4.2) | 40 (31) | 40 (31) | 41 (26) | .66 |
| CR rate, % | 80* | 92 | 92 | 89* | 79* | 77 | 84 | 82* | .004 (.64)† |
| Follow-up time among survivors, median (range) | 11.8 (0.6-14.3) | 10.6 (9.4-12) | 6.4 (3.5-9) | 7.6 (0.6-14.3) | 10.9 (4.3-14.5) | 10.6 (9.7-12.1) | 6.9 (3.6-9.3) | 7.6 (3.6-14.5) | .34 |

*P* value: comparison between M3 and M3V stratified by study (I0128, LPA96, LPA99).
*Accounting for ATRA patients who achieved CR after crossing over to induction chemotherapy.
†After controlling for WBC.

for M3 and M3V, respectively, on I0129 (range 0.6-14.5) and 10.6 and 10.7 years, respectively, (range 9.4-12.1) on LPA96 and 6.4 and 6.9 years, respectively (range 3.5-9.3) on LPA99. The median follow-up time among survivors was similar between M3V and M3 (7.6 years) (Table 1).

### Induction therapy

***Complete remission.*** The CR rate among all patients with M3V treated with ATRA-based regimens was 82%, 79% for patients treated with ATRA alone on I0129, 77% on LPA96, and 84% among patients treated with AIDA on LPA99. Among patients with classical M3 treated with ATRA-based regimens, the CR rate was 89%, 80% among those treated with ATRA alone on I0129 and 92% among those treated with AIDA on LPA96 and LPA99 ($P = .004$ compared with M3V). However, when the CR rate was controlled for WBC, the difference is no longer significant. Among patients with M3V, the CR rates for those who presented with a WBC less than $5 \times 10^9$/L, 5-10 $\times 10^9$/L, and greater than or equal $10 \times 10^9$/L were 89%, 92%, and 77%, respectively, compared with 92%, 80%, and 82%, respectively, for patients with classical M3 APL ($P = .64$). The CR rate did not differ among the 3 protocols in patients with the M3V.

***APL differentiation syndrome.*** Among all M3V patients treated with ATRA-based regimens for induction, the incidence of the APL DS was 26%, 4% among patients treated on I0129 and 31% among patients treated on LPA96 and LPA99 combined. Among patients with classical M3 APL, the APL DS developed in 25% of the 748 patients, 29% of patients on I0129 and 24% of patients treated on the 2 PETHEMA protocols ($P = .66$) (Table 1).

***Early death rate.*** The death rate within 30 days of the induction therapy for all M3V patients was 13.6%: 8.3% on I0129 and 20% on LPA96 and 12.9% on LPA99 (Table 2). Among patients with classical M3, the induction death rate within 30 days was 8.4%: 10.7% on I0129 and 6.9% on LPA96 and 7.5% on LPA99 ($P = .02$). This difference was no longer significant when the early death rate accounted for WBC ($P = .87$). Cause of 30-day induction death is listed in Table 3. There appears to be no apparent correlation between hemorrhage and the morphology subtype. Among M3V patients, hemorrhage was the main cause of early death in all 3 protocols. The same is true for patients with classical M3 morphology.

**Table 2. Early death rate (within 30 days of induction)**

| | M3, % | M3V, % | P |
|---|---|---|---|
| I0129 | 10.7 (2.5) | 8.3 (5.6) | — |
| LPA96 | 6.9 (2.1) | 20 (7.3) | — |
| LPA99 | 7.5 (1.2) | 12.9 (3.3) | — |
| All | 8.4 (1.0) | 13.6 (2.8) | .02* |

Standard error given in parentheses.
*Stratified by study (I0129, LPA96, LPA99).

**Table 3. Cause of death (within 30 days of induction)**

| | M3, % | M3V, % |
|---|---|---|
| I0129 | | |
| Hemorrhage | 10 (62.5) | 1 (50) |
| DS | 2 (12.5) | 0 (0) |
| Other | 4* (25) | 1† (50) |
| LPA96 | | |
| Infection | 2 (20) | 3 (50) |
| Hemorrhage | 6 (60) | 3 (50) |
| DS | 2 (20) | 0 (0) |
| LPA99 | — | — |
| Infection | 8 (23.5) | 3 (23) |
| Hemorrhage | 20 (58.8) | 7 (53.8) |
| DS | 6 (17.6) | 2 (15.4) |
| Other | 0 (0) | 1‡ (7.7) |

DS indicates APL differentiation syndrome.
*One respiratory arrest, 1 myocardial infarction, 1 multiorgan thrombosis, 1 liver failure.
†One myocardial infarction.
‡One myocardial infarction.

### Outcome

***Overall survival.*** The OS at 5 years was 70% for M3V patients (63% for I0129, 57% for LPA96, and 75% for LPA99, $P = .007$) and 80% for M3 patients (71% for I0129, 80% for LPA96, and 84% for LPA99, $P = .006$) (Table 4 and Figure 1). However, when the OS was calculated accounting for the WBC, the difference is no longer present. Among patients with M3V, the 5-year OS rates for those who presented with a WBC less than $5 \times 10^9$/L, 5-10 $\times 10^9$/L, and greater than or equal to $10 \times 10^9$/L were 80%, 84%, and 62%, respectively, compared with patients with classical M3 APL: 84%, 70%, and 68%, respectively ($P = .70, .15, .47$ for WBC less than $5 \times 10^9$/L, 5-10 $\times 10^9$/L, and greater than or equal to $10 \times 10^9$/L, respectively, and $P = .87$ for overall WBC adjusted) (Table 5). The same result was seen in a multivariable Cox model. When age, male sex, WBC, platelet, hemoglobin, and APL morphology subtype were included in the stratified Cox regression model, the APL morphology subtype was not significant in OS (HR = 1.04 for M3V compared with M3, $P = .84$) (Table 6). In this model, the unfavorable prognostic factors for OS were age greater than or equal to 60 (HR = 3.13, $P < .0001$), male sex (HR = 1.55, $P = .003$), and high WBC (HR = 2.38, $P < .0001$) (Figure 2).

***Disease-free survival.*** The DFS at 5 years was 73% for M3V patients and 81% for M3 patients ($P = .07$) (Table 3A and Figure 3). As in OS, when the DFS was adjusted for the WBC, the difference in DFS is no longer seen. Among patients with M3V, the 5-year DFS rates for those who presented with a WBC less than $5 \times 10^9$/L, 5-10 $\times 10^9$/L, and greater than or equal to $10 \times 10^9$/L were 84%, 79%, and 67%, respectively, compared with 85%, 80%, and 63%, respectively, for patients with classical M3 APL ($P = .87, .96, .45$ for WBC less than $5 \times 10^9$/L, 5-10 $\times 10^9$/L, and greater than or equal to $10 \times 10^9$/L, respectively, and $P = .50$ for overall, WBC adjusted) (Table 5). A similar result was seen in a multivariable model. When age, male sex, WBC, platelet hemoglobin, and APL morphology subtype were included in the stratified Cox regression model, the APL morphology subtype was not significant in DFS (HR = 0.91, $P = .67$) (Table 4). In this model, the unfavorable prognostic factors for DFS were age greater than or equal to 60 (HR = 2.31, $P < .001$), male sex (HR = 1.57, $P = .005$), and high WBC (HR = 2.70, $P < .0001$) (Figure 4). The model was repeated with relapse risk score instead of WBC and platelet counts. The APL morphology subtype was virtually unchanged (HR = 0.95, $P = .82$), and high relapse risk was an unfavorable prognostic factor (HR = 3.27, $P < .0001$)

***Cumulative incidence of relapse.*** The 5-year cumulative incidence rate of relapse (CIR) was 24% for all M3V patients and 15% for all classical M3 patients (relapse risk unadjusted $P = .005$) (Table 7 and Figure 5). However, the 5-year CIR for low-, intermediate-, and high-relapse risk groups were 15%, 14%, and 32%, respectively, among M3V patients and 9.5%, 12%, and 35%, respectively, for classical M3 patients ($P = .64, .71, .62$ for low,

**Table 4. Overall survival and disease-free survival**

| | | M3 | | | M3V | | | |
|---|---|---|---|---|---|---|---|---|
| | | n | 5-year, % (SE) | 10-year, % (SE) | n | 5-year, % (SE) | 10-year, % (SE) | P |
| OS | I0129 | 150 | 71 (3.7) | 67 (3.9) | 24 | 63 (9.9) | 63 (9.9) | .7 |
| | LPA96 | 145 | 80 (3.3) | 78 (3.4) | 30 | 57 (9.1) | 57 (9.1) | .007 |
| | LPA99 | 453 | 84 (1.8) | — | 101 | 75 (4.3) | — | .06 |
| | All | 748 | 80 (1.5) | — | 155 | 70 (3.7) | — | .006 (.87*) |
| DFS | I0129 | 120 | 65 (4.4) | 61 (4.5) | 19 | 63 (11.1) | 63 (11.1) | .79 |
| | LPA96 | 133 | 80 (3.4) | 79 (3.5) | 23 | 57 (10.3) | 57 (10.3) | .01 |
| | LPA99 | 416 | 86 (1.7) | — | 85 | 80 (4.3) | — | .24 |
| | All | 669 | 81 (1.5) | — | 127 | 73 (3.9) | — | .07 (0.5*) |

*Comparison between M3 and M3V, stratified by WBC.

**Table 5. Overall survival and disease-free survival by WBC count**

| | | M3 | | | M3V | | | |
|---|---|---|---|---|---|---|---|---|
| | WBC | n | 5-year, % (SE) | 10-year, % (SE) | n | 5-year, % (SE) | 10-year, % (SE) | P |
| OS | | | | | | | | .87* |
| | 0-< 5 | 566 | 84 (1.6) | 81 (1.7) | 35 | 80 (6.8) | 80 (6.8) | .7 |
| | ≤ 5-< 10 | 61 | 70 (5.8) | 70 (5.8) | 26 | 84 (7.2) | 84 (7.2) | .15 |
| | ≥ 10 | 121 | 68 (4.2) | 66 (4.4) | 94 | 62 (5.0) | 62 (5.0) | .47 |
| DFS | | | | | | | | .5* |
| | 0-< 5 | 521 | 85 (1.6) | 82 (1.8) | 31 | 84 (6.6) | 84 (6.6) | .87 |
| | ≤ 5-< 10 | 49 | 80 (5.8) | 80 (5.8) | 24 | 79 (8.3) | 79 (8.3) | .96 |
| | ≥ 10 | 99 | 63 (4.9) | 62 (5.0) | 72 | 67 (5.6) | 67 (5.6) | .45 |

*Comparing overall M3 versus M3V, adjusted for by WBC category.

intermediate, and high risk group, respectively, and $P = .84$ for overall, relapse risk adjusted) (Table 7 and Figure 6). When age, sex, relapse risk, hemoglobin, and morphology were included in a multivariable competing risks regression analysis, the APL morphology subtype was again not significant in CIR (HR = 0.94, $P = .81$). The unfavorable prognostic factors for relapse were male sex (HR = 1.78, $P = .003$) and high relapse risk score (HR = 4.06, $P < .0001$).

***FLT3 ITD mutations.*** Of 903 patients, only 332 (37%) patients had *FLT3 ITD* status available. Among patients with *FLT3 ITD* status available, WBC was higher in patients with *FLT3 ITD* mutation (median 15.4, range 0.6-550) compared with patients without mutation (median 1.9, range 0.2--133, $P < .0001$), and the incidence rate of *FLT3 ITD* mutation was 17% in M3 and 52% in M3V ($P < .001$).

Among 155 patients with M3V, 62 (40%) had *FLT3 ITD* status available. The 5-year OS was 72% for patients with a *FLT3 ITD* mutation and 63% for patients without a mutation ($P = .56$). The 5-year DFS was 78% for patients with *FLT3 ITD* mutation and 68% for patients without mutation ($P = .46$). The 5-year CIR was 22% for patients with *FLT3 ITD* mutation and 28% for patients without mutation ($P = .64$). Among 748 patients with M3, 270 (36%) had *FLT3 ITD* status available. The 5-year OS was 78% for patients with a *FLT3 ITD* mutation and 85% for patients without a mutation ($P = .44$). The 5-year DFS was 74% for patients with *FLT3 ITD* mutation and 84% for patients without mutation (P = .33). The 5-year CIR was 26% for patients with *FLT3 ITD* mutation and 12% for patients without mutation ($P = .03$).

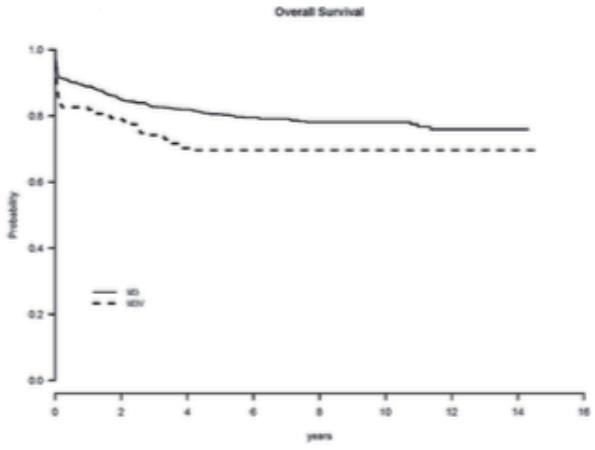


**Figure 1. Overall survival by morphology.**

**Table 6. Stratified Cox regression model**

| | OS | | DFS | |
|---|---|---|---|---|
| | Hazard ratio (95 confidence interval) | *P* | Hazard ratio (95 confidence interval) | *P* |
| Age ≥ 60 y vs age < 60 y | 3.13 (2.32–4.21) | < .0001 | 2.13 (1.48–3.07) | < .001 |
| Male vs female | 1.55 (1.17–2.05) | .003 | 1.57 (1.15–2.15) | .005 |
| Intermediate vs low WBC | 1.50 (0.95–2.39) | .08 | 1.16 (0.67–2.03) | .59 |
| High vs low WBC | 2.38 (1.71–3.32) | < .0001 | 2.70 (1.88–3.88) | < .0001 |
| Platelet (≤ 40 vs < 40) | 0.78 (0.56–1.08) | .14 | 0.77 (0.53–1.11) | .16 |
| Hemoglobin | 0.99 (0.93–1.05) | .70 | 1.00 (0.94–1.07) | .93 |
| M3V vs M3 | 1.04 (0.72–1.50) | .84 | 0.91 (0.60–1.38) | .67 |

WBC: Low if WBC <5 × $10^9$/L; intermediate if 5 to <10 × $10^9$/L; high if ≥ 10 × $10^9$/L.

To investigate whether the patient cohort with *FLT3 ITD* information represents a random subset, OS was compared between the patients without *FLT3 ITD* mutation information available (n = 571) and those with *FLT3 ITD* available (n = 332). The 5-year OS for the patients without *FLT3 ITD* information was 77% and 81% for patients with *FLT3 ITD* information available ($P = .46$). Similarly, the 5-year DFS was 79% for the patients without the information and 81% for those with ($P = .80$); the 5-year CIR was 16% for the patients with the information and 16% for those without ($P = .87$). When the analysis was repeated by APL morphology subtype, a similar result was found (data not shown).

***PML isoform.*** Of 174 I0129 pts, 108 (62%) patients had the isoform information. Of 729 PETHEMA patients, 651 (89%) had the isoform information. The detailed distribution of *PML* isoform is presented in Table 8. Based on these 759 patients with isoform data available, WBC was higher in patients with S-isoform (median 3.5 × $10^9$/L, range 0.3-210 × $10^9$/L) compared with patients with other (L/V/VL) isoforms (median 1.8 × $10^9$/L, range 0.2-550 × $10^9$/L, $P = .002$). The incidence of S-isoform is higher in M3V compared with M3 (58% in M3V vs 35% in M3, $P < .001$). However, there was no difference between S-isoform and other isoforms in CR rate (89% in S-isoform vs 90% in other isoforms, $P = .59$), OS (HR = 1.13, $P = .45$ in univariable; HR = 0.91, $P = .55$ in multivariable Cox model), and DFS (HR = 1.34, $P = .08$ in univariable, HR = 1.15, $P = .42$ in multivariable Cox model).

## Discussion

This study of a large number of patients with long-term follow-up showed that the outcome of patients with the M3V was not different from that of patients with classical morphology when treated with ATRA plus anthracycline-based regimens when adjusted for WBC or relapse risk score. A potential limitation of the data reported herein is the fact that precise quantitative criteria to establish a definitive diagnosis of M3V is lacking, and not all patients had the diagnosis of M3V established centrally. Indeed,

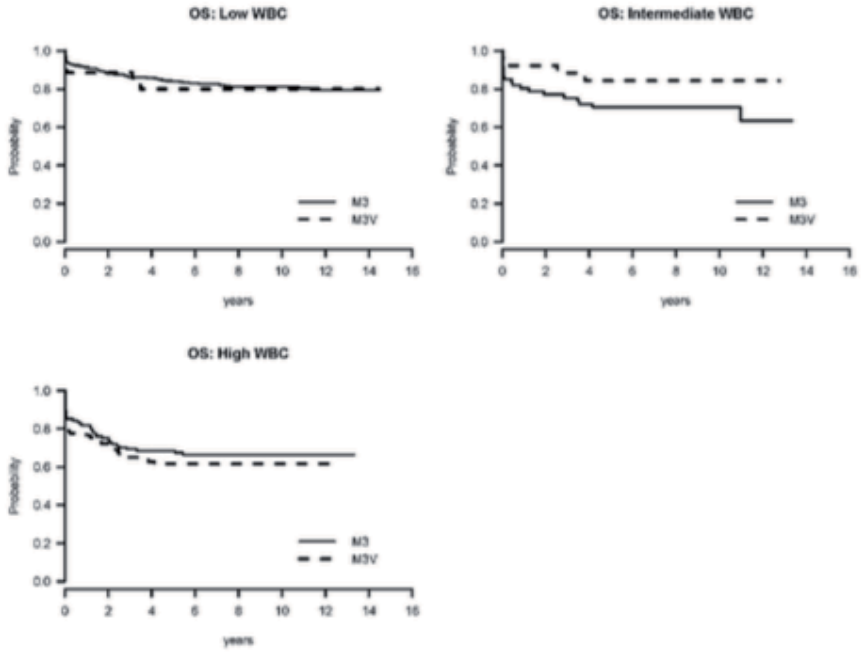


**Figure 2. Overall survival by WBC.**

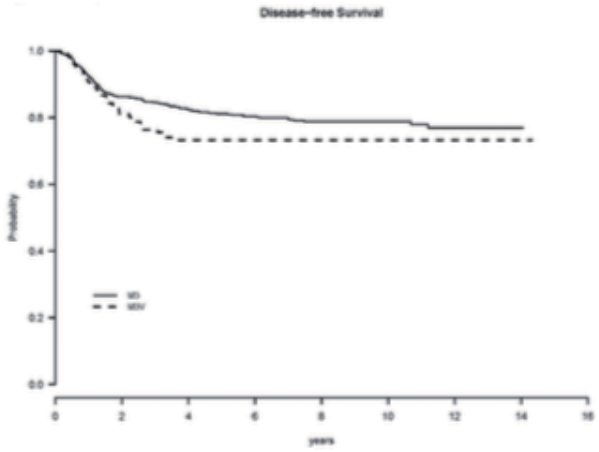


Figure 3. Disease-free survival by morphology.

there are several recognized microgranular variants with subtleties in establishing the diagnosis.[1] Some patients may have had the M3V but may not have been recognized and not included in the analysis. Furthermore, patients were not treated identically, though this issue was handled by performing stratified analysis. Patients treated on the PETHEMA protocols received idarubicin whereas those on the North American protocol were given daunorubicin. In addition, the number of cycles and intensity of consolidation differed. Nevertheless, the patients reported here represent the largest series of M3V patients treated with ATRA plus anthracycline-based therapy.

Historically, before the introduction of ATRA, the early mortality among patients with M3V APL was reported to be higher than that among patients with classical morphology, but the CR rate, except for one series,[21] and OS were not clearly inferior.[2,18-20] Early mortality among patients with M3V may be attributable to extensive hemostatic abnormalities and fatal bleeding, particularly intracerebral hemorrhage.[8] Patients with M3V morphology often have hyperleukocytosis.[4,6] The outcome of such patients appears to be influenced more by the WBC than the specific morphology of M3V. Other factors may also influence outcome among patients with M3V. It is possible that expression of CD2, associated in some

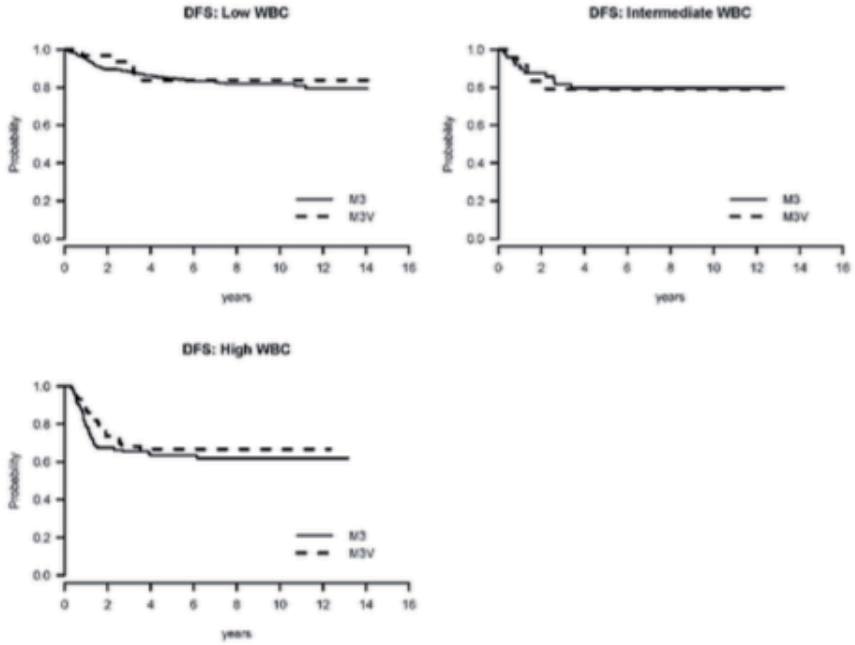


Figure 4. Disease-free survival by WBC.

**Table 7. Cumulative incidence of relapse***

| | M3, n (%) | | | M3V, n (%) | | | |
|---|---|---|---|---|---|---|---|
| | n | 5-year | 10-year | n | 5-year | 10-year | P |
| I0129 | 120 | 33 (4.3) | 35 (4.4) | 19 | 37 (11.4) | 37 (11.4) | .9 |
| LPA96 | 133 | 15 (3.1) | 15 (3.1) | 23 | 39 (10.5) | 39 (10.5) | .005 |
| LPA99 | 416 | 9 (1.4) | — | 85 | 18 (4.2) | — | .03 |
| ALL | 669 | 15 (1.4) | — | 127 | 24 (3.8) | — | .005† |
| Relapse risk | | | | | | | .84‡ |
| Low | 169 | 9.5 (2.3) | 9.5 (2.3) | 20 | 15 (8.2) | 15 (8.2) | .64† |
| Intermediate | 402 | 12 (1.6) | 13 (1.8) | 35 | 14 (6) | 14 (6) | .71† |
| High | 98 | 35 (4.9) | — | 72 | 32 (5.5) | — | .62† |

Standard error given in parentheses.
*Taking nonrelapse death as a competing risk.
†Stratified by study (I0129, LPA96, LPA99).
‡Stratified by relapse risk.

reports with M3V[3,6,8,10,11,15,34,35] may be related to the hyperleukocytosis observed. This may be attributable to interaction with its ligand CD58 or lymphocyte function-associated antigen 3, a cell surface glycoprotein, which induces proliferation of T cells to which it mediates adhesion.[35] In our study, CD2 expression was not determined. It is also possible that the hyperleukocytosis associated with the M3V is attributable in part to expression of the *FLT3* gene mutation.[12,13] Some,[12] but not all, reports[13,36,37] have demonstrated a relationship between the presence of the *FLT3* gene mutation and induction death in patients with APL. Furthermore, a recent study suggested that increased ITD mutant/wild-type ratio or longer ITD size was associated with a shorter 5-year relapse-free survival.[38] We did not examine the correlation of patients with high allelic ratio of the *FLT3* gene mutation with M3V, which may be important in unraveling a potential association of FLT3 mutations with M3V and/or hyperleukocytosis in APL. However, we did find that the mutation rate appears to be higher in M3V compared with M3, although the sample size that resulted from missing data is a limiting factor. Telomerase activity and telomere length appear to correlate with disease progression and relapse among arsenic trioxide–treated patients.[39] In our analysis, there was a correlation between the S-isoform subtype of PML and M3V morphology There may well be other as yet unidentified factors, likely molecular and currently elusive, for which WBC serves as a surrogate, which determine the prognosis of patients with the M3V.

The significantly higher induction death rate, attributable to hemorrhage in 50% or more of patients, observed in patients with M3V compared with those with classical M3 was influenced by the association of morphologic subtype with WBC. This finding was reported in a previous PETHEMA study, in which morphologic subtype had a prognostic impact on induction death rate in univariable, but not in multivariable, analysis.[40] Regarding other outcomes, such as DFS, CIR, and OS, a similar finding of the impact of morphology on prognosis was observed in univariable analysis, but again it was a result of its association with WBC and relapse risk score, as previously reported.[27,28]

The difference in the incidence of the APL DS between the I0129 and LPA trials requires comment. The incidence of the APL DS on the I0129 trial was 29% (43 of 150) among all patients with APL receiving ATRA during induction.[23,24] Of these 43 patients, 41 had classical M3, and only 2 had M3V. One of these 2 patients with M3V was erroneously identified as having APL DS and thus subsequently excluded from further analysis.[41] Few patients with the APL DS in this early trial were identified as having the M3V despite central morphologic review by one person. It does not appear that the difference can be explained by a higher incidence of pediatric patients, suggested as having a higher incidence of M3V morphology, in the LPA trials because the incidence of the APL DS was 6.2% on the PETHEMA trials, and on the I0129 trial it was 13%. In addition, the expanded definition of the APL DS in the PETHEMA trials does not provide an explanation because there

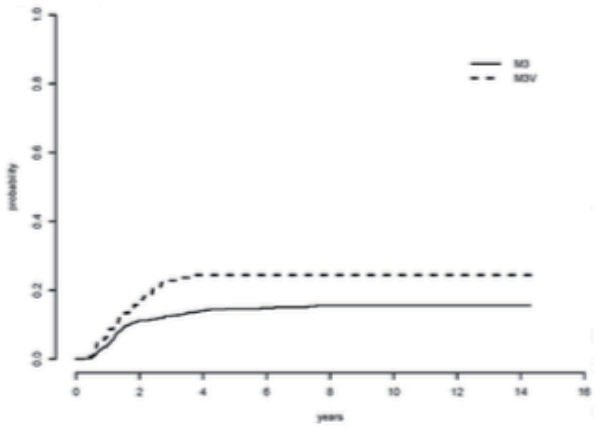


Figure 5. Cumulative incidence of relapse.

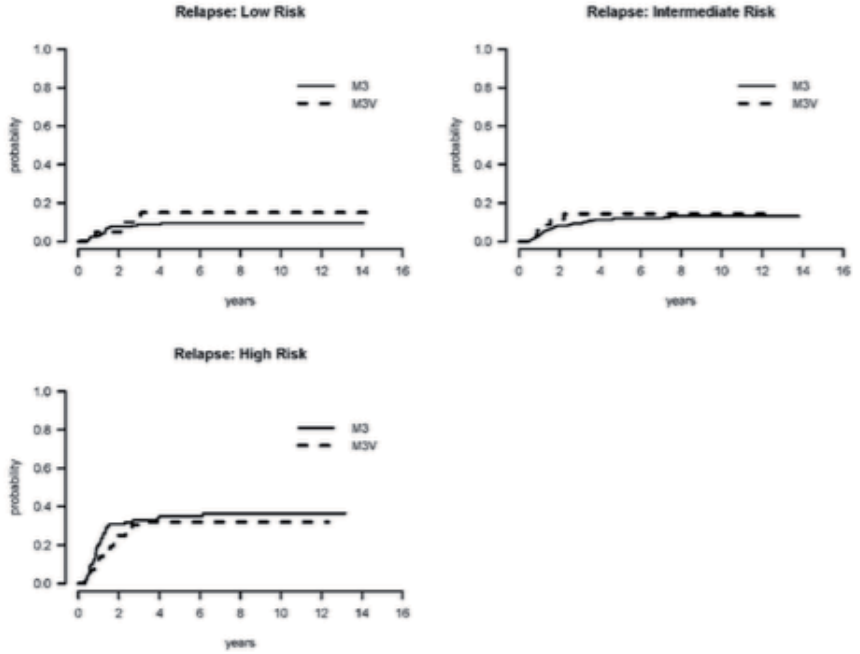


Figure 6. Incidence of relapse by relapse risk score.

Table 8. Distribution of isoform type

| | M3, n (%) | | | | M3V, n (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Isoform | I0129 (n = 91) | LPA96 (n = 137) | LPA99 (n = 395) | All (n = 623) | I0129 (n = 17) | LPA96 (n = 30) | LPA99 (n = 89) | All (n = 136) | P |
| L | 51 (56) | 72 (53) | 201 (51) | 324 (52) | 9 (53) | 10 (33) | 36 (40) | 55 (40) | — |
| S | 32 (35) | 53 (39) | 148 (37) | 233 (37) | 8 (47) | 20 (67) | 51 (57) | 79 (58) | < .001* |
| V | 8 (9) | 9 (7) | 10 (3) | 27 (4) | 0 | 0 | 1 (1) | 1 (1) | — |
| VL | 0 | 3 (2) | 36 (9) | 39 (6) | 0 | 0 | 1 (1) | 1 (1) | — |

*S-isoform type vs other, stratified by study (I0129, LPA96, LPA99).

were no patients diagnosed with DS based solely on the presence of renal failure. A contributing factor may be the fact that there was central pathology review for patients entered in the North American Trial and not for the PETHEMA patients.

ATRA remains the mainstay for all subtypes of APL, including M3V. Despite the higher risk of complications reported among patients with M3V, the addition of ATRA markedly improves outcome as demonstrated in the large series reported here. At the present time, patients with the M3V do not require treatment modification based on the morphology alone. Given the apparent high incidence of the *FLT3* gene mutation in M3V and the less favorable prognosis among patients with a high WBC, it is of interest to speculate as to a possible role for FLT3 inhibitors in treatment of patients with the M3V. However, the availability of several very effective agents likely makes this possibility premature. Interestingly, coexpression of the *PML-RAR*-alpha with the FLT3 ITD in myeloid progenitors in a mouse model leads to a disease with morphologic features resembling M3V.[42] Nevertheless, the M3V itself does not independently predict for a less favorable outcome compared with classical M3 APL.

## Acknowledgments

This work was coordinated by the Eastern Cooperative Oncology Group (Robert L. Comis, MD, Chair) and supported in part by Public Health Service Grants ECOG CA23318, CA66636, CA21115, CA17145, CALGB CA31936 and CA33601, SWOG Grant CA32102, CA38926, CA20319, CA12213, CA14028, CA56771, and from the National Cancer Institute, National Institutes of Health and the Department of Health and Human Services. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute.

## Authorship

Contribution: M.S.T. and M.A.S. were the principal investigators of the clinical trial; M.S.T. and M.A.S. participated in the

conception and design of the trial; J.d.l.S., G.D., J.G., J.H.F., M.G., J.D.G., G.M., J.M.R., C. Rayon, L.S., C. Rivas, and E.V. provided study materials and/or patients for the trial; M.S.T., H.T.K., R.G., E.P., and P.M. participated in the analysis and interpretation of data; M.S.T., H.T.K., and M.A.S. wrote the manuscript; and P.M., F.R.A., C.A.S., J.d.l.S., J.M.B., G.D., C.D.B., J.G., J.H.F., M.G., R.A.L., J.D.G., G.M., J.M.R., C. Rayon, L.S., C. Rivas, E.V., P.H.W., R.G., E.P., J.S., and C.L.W. participated in manuscript review and approval.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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## 9.6 Artículo Anexo 6

Montesinos P, Rayón C, Vellenga E, Brunet S, González J, González M, Holowiecka A, Esteve J, Bergua J, González JD, Rivas C, Tormo M, Rubio V, Bueno J, Manso F, Milone G, de la Serna J, Pérez I, Pérez-Encinas M, Krsnik I, Ribera JM, Escoda L, Lowenberg B, Sanz MA; PETHEMA; HOVON Groups.

Clinical significance of CD56 expression in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline-based regimens.

Blood. 2011 Feb 10;117(6):1799-805.

CLINICAL TRIALS AND OBSERVATIONS

# Clinical significance of CD56 expression in patients with acute promyelocytic leukemia treated with all-*trans* retinoic acid and anthracycline-based regimens

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**The expression of CD56 antigen in acute promyelocytic leukemia (APL) blasts has been associated with short remission duration and extramedullary relapse. We investigated the clinical significance of CD56 expression in a large series of patients with APL treated with all-*trans* retinoic acid and anthracycline-based regimens. Between 1996 and 2009, 651 APL patients with available data on CD56 expression were included in 3 subsequent trials (PETHEMA LPA96 and LPA99 and PETHEMA/HOVON LPA2005). Seventy-two patients (11%) were CD56$^+$ (expression of CD56 in ≥ 20% leukemic promyelocytes). CD56$^+$ APL was significantly associated with high white blood cell counts; low albumin levels; BCR3 isoform; and the coexpression of CD2, CD34, CD7, HLA-DR, CD15, and CD117 antigens. For CD56$^+$ APL, the 5-year relapse rate was 22%, compared with a 10% relapse rate for CD56$^-$ APL ($P = .006$). In the multivariate analysis, CD56 expression retained the statistical significance together with the relapse-risk score. CD56$^+$ APL also showed a greater risk of extramedullary relapse ($P < .001$). In summary, CD56 expression is associated with the coexpression of immaturity-associated and T-cell antigens and is an independent adverse prognostic factor for relapse in patients with APL treated with all-*trans*-retinoic acid plus idarubicin–derived regimens. This marker may be considered for implementing risk-adapted therapeutic strategies in APL. The LPA2005 trial is registered at http://www.clinicaltrials.gov as NCT00408278. (*Blood*. 2011;117(6):1799-1805)**

## Introduction

Several investigators have suggested a relationship between the expression of CD56 (neural adhesion factor) antigen in the surface of leukemic blasts and both short remission duration[1,2] and development of extramedullary relapse[3] in patients with acute promyelocytic leukemia (APL). However, this relationship has not been yet established. In fact, only one of these studies was performed in a relatively large population of APL patients receiving a state-of-the-art treatment with all-*trans* retinoic acid (ATRA) and anthracycline-based chemotherapy.[2] With regard to the incidence of CD56-positive (CD56$^+$) APL and the association with other clinical and biologic variables, very little information has been published.[1-5]

In this study, we set out to assess the frequency of CD56 expression, its relationship with a broad variety of clinical and hematologic features, as well as its prognostic value in a large series of patients with newly diagnosed APL who were enrolled in 3 consecutive trials of the Programa Español para el Tratamiento de Enfermedades Hematológicas (PETHEMA) and Hemato-Oncologie voor Volwassenen Nederland (HOVON) groups.

## Methods

### Eligibility

Patients enrolled in the consecutive multicenter PETHEMA LPA96 and LPA99 trials and PETHEMA/HOVON LPA2005 were required to have a diagnosis of de novo APL with demonstration of the t(15;17) or *PML/RARA* rearrangements. More details about general exclusion and inclusion criteria have been reported elsewhere.[5] Of the 1208 patients included in the 3 trials (LPA96, n = 172; LPA99, n = 560; LPA2005, n = 476), 651 patients (54%) had available the percentage of APL leukemic promyelocytes expressing CD56 surface antigen and were evaluable for the present study. Informed consent was obtained from all patients. In accordance with the Declaration of Helsinki, the protocol was approved by the Research Ethics Board of each participating hospital.

### Therapy of APL

Induction therapy consisted of oral ATRA and idarubicin given as an intravenous bolus on days 2, 4, 6, and 8 (ATRA plus idarubicin, ie, AIDA regimen). In the LPA99 and LPA2005 trials, patients older than 70 years of

Submitted April 1, 2010; accepted November 27, 2010. Prepublished online as *Blood* First Edition paper, December 8, 2010; DOI 10.1182/blood-2010-04-277434.

The online version of this article contains a data supplement.

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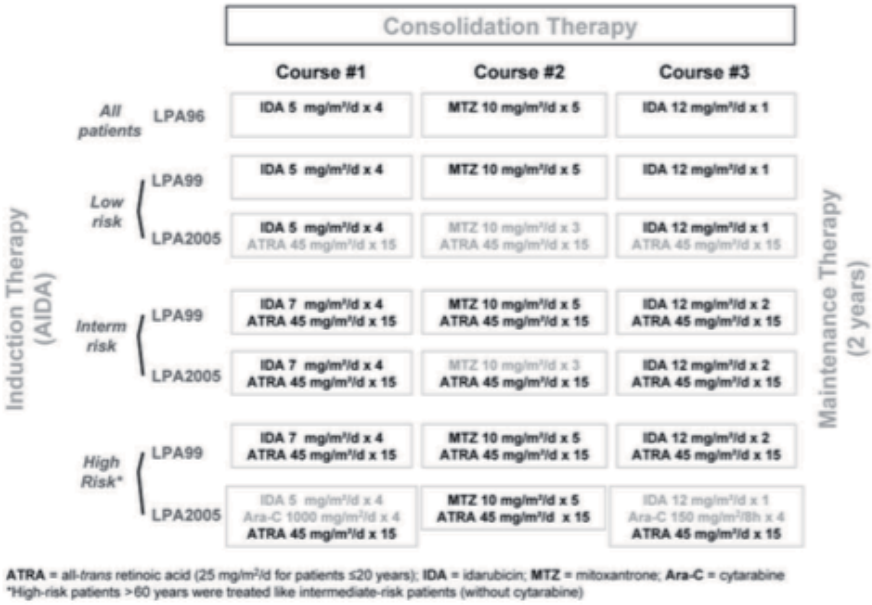


**ATRA** = all-*trans* retinoic acid (25 mg/m²/d for patients ≤20 years); **IDA** = idarubicin; **MTZ** = mitoxantrone; **Ara-C** = cytarabine
*High-risk patients >60 years were treated like intermediate-risk patients (without cytarabine)

**Figure 1. Therapeutic schedule of the PETHEMA LPA96, LPA99, and LPA2005 trials.**

age received only the 3 first doses of idarubicin.[6,7] All patients in complete remission (CR) received 3 monthly consolidation courses with anthracycline-based chemotherapy. The consolidation schedule in the 3 consecutive protocols has been previously described[6,7] and is shown in the Figure 1. After completion of consolidation, patients who tested negative for *PML/RARA* were started on maintenance therapy, as described elsewhere,[6] with intermittent ATRA and low-dose chemotherapy with 6-mercaptopurine and methotrexate for 2 years.

### Multiparameter flow cytometry

Inmunophenotyping was performed on bone marrow samples collected at APL diagnosis. Leukemic cell analysis was performed at local or reference laboratories by standard inmunofluorescence methods by the use of monoclonal antibodies directed against CD2, CD7, CD9, CD11b, CD13, CD15, CD19, CD33, CD34, CD56, CD117, and HLA-DR surface antigens. The inclusion of anti-CD56 in the panel antibodies was performed at the center's discretion. Multiparametric flow analysis was performed by the use of 3 or 4 colors on a flow cytometer. Leukemic promyelocytes were gated on the basis of their unique side-scatter/CD45-positive and/or $CD33^{hi}$/homogeneous immunophenotypic profile, in both hyper and hypogranulated cases; CD45hi expression with SSClo was used to further exclude T cells and other lymphocytes from the gate. In addition, CD3 and/or CD5 antigens were routinely included in the diagnostic set to rule out contamination of the gated leukemic promyelocytes by mature T cells. Following the EGIL criteria,[8] a sample was defined as positive if ≥ 20% of leukemic promyelocytes expressed a specific antigen in the cell surface. The only exception was the CD34 marker, for which a cutoff level of ≥ 10% expressing cells was required in line with previous reports on APL.[4,9] Expression of CD56 was systematically assessed with sensitive (eg, phycoerythrin) fluorochrome-conjugated antibody reagents.

### Definitions and study end points

Remission induction response was assessed according to the revised criteria by Cheson et al[10] For morphologic assessment of leukemia resistance, it was required that sufficient time had passed to allow for full terminal differentiation of the malignant promyelocytes (up to 40-50 days). Molecular remission was defined as the disappearance on an ethidium bromide gel of the PML/RARA-specific band visualized at diagnosis by use of reverse transcription polymerase chain reaction (PCR) assays with a sensitivity level of one cell in $10^{-4}$. Molecular persistence was defined as PCR positivity in 2 consecutive bone marrow samples collected at the end of consolidation therapy. Molecular relapse was defined as reported elsewhere.[11] Genetic diagnosis of APL with the use of reverse transcription PCR, anti-PML staining, or cytogenetic tests was required for the diagnosis of overt hematologic relapse. Central nervous system (CNS) relapse was confirmed by lumbar puncture and cytologic examination of cerebrospinal fluid, which was performed only in patients with clinically suspected CNS relapse. Extramedullary relapse in other localization (eg, skin) was confirmed by histopathology.

Data were collected and registered prospectively. Forty patient and disease characteristics were examined to establish their relationship to CD56 expression. We analyzed the characteristics listed in Tables 1 and 2. In addition we analyzed also the following variables: total body surface; liver and spleen enlargement; coagulopathy; hemorrhagic syndrome at presentation; serum levels of lactate dehydrogenase, creatinine, uric acid, alkaline phosphatases, and total bilirubin; peripheral blood blast count and blast cell percentage; bone marrow aspirate cellularity, peroxidase reactivity, and blast cell percentage; and CD13, CD19, and CD33 surface antigen markers.

Diagnosis and gradation of the differentiation syndrome was made according to the previously defined criteria.[12] Coagulopathy was defined as a prolonged prothrombin time and/or activated partial thromboplastin time in addition to hypofibrinogenemia and/or increased levels of fibrin degradation products or D-dimers. Patients were classified as having t(15;17) with or without additional chromosomal abnormalities accordingly to previously defined criteria.[13] The patient performance status at diagnosis was measured using the Eastern Cooperative Oncology Group (ECOG) scale. Risk of relapse was established at diagnosis according to a predictive model on the basis of patient leukocyte and platelet counts at diagnosis, as reported elsewhere.[14]

### Statistical analysis

The $\chi^2$ test, with Yates correction if necessary, was used to analyze differences in the distribution of categorical variables between patient

**Table 1. Demographic and baseline patient characteristics according to CD56 expression**

| Characteristic | CD56-positive | | CD56-negative | | P |
|---|---|---|---|---|---|
| | Median (range) | No. (%) | Median (range) | No. (%) | |
| Overall | | 72 (100) | | 579 (100) | |
| **PETHEMA trial** | | | | | |
| LPA96 | | 4 (6) | | 75 (13) | .17 |
| LPA99 | | 41 (57) | | 289 (50) | |
| LPA2005 | | 27 (37) | | 215 (37) | |
| **Age, y** | 41 (5-74) | | 40 (2-81) | | |
| ≤ 18 | | 5 (7) | | 62 (11) | .44 |
| 19-40 | | 31 (43) | | 235 (41) | |
| 41-60 | | 21 (29) | | 189 (33) | |
| > 60 | | 15 (21) | | 92 (17) | |
| **Sex** | | | | | |
| Male | | 33 (46) | | 312 (54) | .20 |
| Female | | 39 (54) | | 267 (46) | |
| **ECOG** | 1 (0-3) | | 1 (0-3) | | |
| 0-1 | | 48 (71) | | 431 (79) | .06 |
| 2-3 | | 20 (29) | | 106 (21) | |
| **Fever** | | | | | |
| No | | 48 (69) | | 356 (62) | .17 |
| Yes | | 22 (31) | | 220 (38) | |
| **WBC count, × $10^9$/L** | 3.6 (0.4-162) | | 2.6 (0.2-460) | | .03* |
| ≤ 5 | | 39 (54) | | 360 (62) | .19 |
| 5-10 | | 10 (14) | | 50 (9) | |
| 10-50 | | 15 (21) | | 132 (23) | |
| > 50 | | 8 (11) | | 37 (6) | |
| **Platelet count, × $10^9$/L** | 20 (2-155) | | 23 (1-222) | | |
| ≤ 40 | | 56 (78) | | 444 (77) | .83 |
| > 40 | | 16 (22) | | 135 (23) | |
| **Relapse-risk group** | | | | | |
| Low | | 12 (17) | | 109 (19) | .85 |
| Intermediate | | 37 (51) | | 301 (52) | |
| High | | 23 (32) | | 169 (29) | |
| **Hemoglobin, g/dL** | 9.5 (4.6-14.3) | | 9.4 (2.4-15.9) | | |
| ≤ 10 | | 47 (65) | | 349 (60) | .41 |
| > 10 | | 25 (35) | | 230 (40) | |
| **Fibrinogen, mg/dL** | 174 (40-622) | | 159 (0-777) | | |
| ≤ 170 | | 32 (49) | | 301 (54) | .45 |
| > 170 | | 33 (51) | | 255 (46) | |
| **Albumin, g/dL** | 3.9 (2.3-4.9) | | 4.0 (1.7-6.0) | | .002* |
| ≤ 3.5 | | 19 (31) | | 97 (19) | .04 |
| > 3.5 | | 43 (69) | | 406 (81) | |

ECOG indicates Eastern Cooperative Oncology Group; PETHEMA, Programa Español para el Tratamiento de Enfermedades Hematológicas; and WBC, white blood cell.
*P compares continuous variables (mean WBC 17.5 vs 13.5, P = .03; and mean albumin 3.8 vs 4.0, P = .002).

subsets. The Student *t* test was used to analyze continuous variables following a normal distribution and the Mann-Whitney *U* test for data that failed the normality test. Unadjusted time-to-event analyses were performed by use of the Kaplan-Meier estimate[15] and, for comparisons, log-rank tests.[16] The probability of relapse also was estimated by the cumulative incidence method (for marginal probability).[17,18] Overall survival (OS) was calculated from the date of starting induction therapy, whereas cumulative incidence of relapse (CIR) and disease-free survival (DFS) were calculated from the date of CR. In the analysis of DFS, relapse, development of secondary myelodysplastic syndrome or acute leukemia (t-MDS/t-AL), and death in CR were considered uncensored events, whichever occurred first. For cumulative incidence analysis, death in CR and development of t-MDS/t-AL were considered as a competing cause of failure. For all estimates in which the event "relapse" was considered as an end point, overt morphologic and molecular relapse, as well as molecular persistence at the end of consolidation, were each considered as uncensored events. Patient follow-up was updated on March 15, 2010. Characteristics selected for inclusion in the multivariate analysis were those for which there was some indication of a significant association in univariate analysis ($P < .1$) and, if available, those for which previous studies had suggested a possible relationship. Multivariate analyses were performed by use of Cox model for DFS and OS[19] and Fine and Gray model for CIR.[20] Missing data were substituted by the mean values from patients in whom data were available.[21] All *P* values reported are 2-sided. Computations were performed by use of the 3D, 4F, 1L, LR, and 2L programs from the BMDP statistical library (BMDP Statistical Software), and R 2.9.2 software package for CIR and Fine and Gray model.

## Results

### Patient characteristics according to CD56 expression

Between December 1996 and December 2009, 651 consecutive patients in whom the results of the analysis of CD56 surface antigen expression at diagnosis were available are the subject of the study (79 patients in the LPA96, 330 in the LPA99, and 242 in

**Table 2. Biological features of APL according to CD56 expression**

| Characteristic | CD56-positive, no. (%) | CD56-negative, no. (%) | P |
|---|---|---|---|
| Overall | 72 (100) | 579 (100) | |
| **Morphologic subtype (n = 645)** | | | |
| Hypergranular | 51 (72) | 461 (80) | .09 |
| Microgranular | 20 (28) | 113 (20) | |
| **Cytogenetics (n = 476)** | | | |
| t(15;17) | 37 (74) | 307 (72) | .77 |
| t(15;17) plus other* | 13 (26) | 109 (28) | |
| **FLT3-ITD mutations (n = 243)** | | | |
| Positive | 10 (31) | 54 (26) | .49 |
| Negative | 22 (69) | 157 (74) | |
| **PML/RARα isoform (n = 576)** | | | |
| BCR1/BCR2 | 25 (38) | 308 (60) | < .001 |
| BCR3 | 40 (62) | 203 (40) | |
| **CD2 (n = 538)** | | | |
| Positive | 25 (46) | 117 (24) | < .001 |
| Negative | 29 (54) | 367 (76) | |
| **CD7 (n = 505)** | | | |
| Positive | 9 (18) | 22 (5) | < .001 |
| Negative | 40 (82) | 434 (95) | |
| **CD9 (n = 189)** | | | |
| Positive | 11 (65) | 64 (37) | .06 |
| Negative | 6 (35) | 108 (63) | |
| **CD11b (n = 464)** | | | |
| Positive | 8 (17) | 34 (8) | .07 |
| Negative | 38 (83) | 384 (92) | |
| **CD15 (n = 555)** | | | |
| Positive | 26 (43) | 128 (26) | .004 |
| Negative | 34 (57) | 367 (74) | |
| **CD34 (n = 607)** | | | |
| Positive | 30 (48) | 117 (21) | < .001 |
| Negative | 32 (52) | 428 (79) | |
| **CD117 (n = 538)** | | | |
| Positive | 50 (88) | 357 (74) | .02 |
| Negative | 7 (12) | 124 (26) | |
| **HLA-DR (n = 569)** | | | |
| Positive | 10 (18) | 28 (5) | .001 |
| Negative | 46 (82) | 485 (95) | |

*Plus other additional chromosomal abnormalities.

the LPA2005 trial). Patients were from 85 institutions from Spain, The Netherlands, Poland, Argentina, and the Czech Republic (see supplemental Appendix, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Median follow-up of the series was 70 months (range, 3-158 months) from diagnosis.

Seventy-two of 651 patients (11%) showed expression of CD56 ranging from 20% to 100% (median, 70%). The main clinical and biologic characteristics of $CD56^+$ APL patients are shown in Tables 1 and 2. Concerning the demographic and clinical characteristics, the white blood cell (WBC) count at baseline was greater among patients with $CD56^+$ APL ($P = .03$), whereas serum albumin levels were lower ($P = .002$). There was a trend toward a greater proportion of patients with ECOG performance status grade 2-3 in the $CD56^+$ group ($P = .06$; Table 1).

Regarding other biologic features of APL, patients with $CD56^+$ APL presented more frequently with BCR3 isoform ($P < .001$), $CD2^+$ ($P < .001$), $CD34^+$ ($P < .001$), $CD7^+$ ($P < .001$), $HLA\text{-}DR^+$ ($P = .001$), $CD15^+$ ($P = .004$), and $CD117^+$ ($P = .02$). There was also a trend toward a greater frequency of microgranular morphology ($P = .09$), $CD11b^+$ ($P = .07$), and $CD9^+$ ($P = .06$; Table 2).

### Induction results

Overall, 595 of the 651 evaluable patients achieved morphologic CR (91.4%). As shown in Table 3, 61 of 72 patients (85%) achieved CR in the $CD56^+$ subgroup, compared with 534 of 579 patients (92%) in the $CD56^-$ group ($P = .04$). No significant differences were observed in the distribution of the different causes of death between the $CD56^+$ and $CD56^-$ cohorts. The incidence and severity of differentiation syndrome were similar among patients with $CD56^+$ and those with $CD56^-$ APL (Table 3).

After multivariate analysis, the regression model for induction death selected the following adverse factors: abnormal creatinine level ($P < .0001$), WBC count greater than $10 \times 10^9$/L ($P < .0001$), age older than 60 years ($P < .0001$), male sex ($P = .0004$), ECOG more than 1 ($P = .009$), and CD56 positivity ($P = .02$).

### Postremission outcomes

Sixty relapses occurred among the 595 patients who had achieved CR (4 molecular persistence, 15 molecular relapses, and 41 clinical relapses), 12 among 61 $CD56^+$ patients and 48 among 534 $CD56^-$ patients. Seven relapses involved extramedullary sites (6 in CNS and 1 in skin), of which 4 occurred among $CD56^+$ patients. In addition, 13 patients died in CR (1 in the $CD56^+$ group), and 10 patients developed t-MDS/t-AL (2 in the $CD56^+$ group).

***Relapse rate.*** The 5-year CIR rate in the $CD56^+$ cohort was 22%, whereas for $CD56^-$ APL it was 10% ($P = .006$; Table 3 and Figure 2A). For patients in the LPA96 trial, the 5-year CIR rates for $CD56^+$ and $CD56^-$ patients were 50% and 10%, whereas in the LPA99 trial they were 18% and 11%, and in the LPA2005 they were 25% and 7%, respectively ($P = .003$, $P = .29$, and $P = .01$, respectively). According to relapse risk groups, the 5-year CIR rates for $CD56^+$ and $CD56^-$ patients were 21% and 5% ($P = .07$) in the low-risk group, 17% and 6% ($P = .02$) in the intermediate group, and 35% and 22% ($P = .24$) in high-risk patients (Figures 2B-D). In the multivariate analysis, CD56 expression retained the independent predictive value along with the WBC counts (Table 4). The 5-year cumulative incidence of extramedullary relapse was significantly greater in $CD56^+$ patients compared with those $CD56^-$ (7.0% vs 0.7%, $P < .001$; Table 3 and Figure 3).

**Table 3. Treatment results according to CD56 expression**

| Characteristic | CD56-positive No. of patients | CD56-positive % | CD56-negative No. of patients | CD56-negative % | P |
|---|---|---|---|---|---|
| Overall | 72 | 100 | 579* | 100 | |
| **Induction outcome** | | | | | |
| Complete remission | 61 | 85 | 534 | 92 | .04 |
| Causes of induction death | | | | | |
| Hemorrhage | 5 | 6.9 | 26 | 4.5 | .53 |
| Infection | 3 | 4.2 | 11 | 1.9 | .41 |
| Differentiation syndrome | 1 | 1.4 | 5 | 0.9 | .99 |
| Thrombosis/infarction | 2 | 2.8 | 2 | 0.4 | .09 |
| **Differentiation syndrome†** | | | | | |
| Severe | 11 | 16 | 69 | 12 | .69 |
| Moderate | 10 | 14 | 80 | 14 | |
| Absent | 49 | 70 | 417 | 74 | |
| **Postremission outcomes at 5 y** | | | | | |
| CIR | | 22 | | 10 | .006 |
| CIR (extramedullary) | | 7.0 | | 0.7 | < .001 |
| Disease-free survival | | 73 | | 85 | .03 |
| Overall survival | | 78 | | 84 | .09 |

CIR indicates cumulative incidence of relapse.
*One patient among the $CD56^-$ cohort was considered as resistant.
†A total of 636 patients were evaluable for differentiation syndrome.

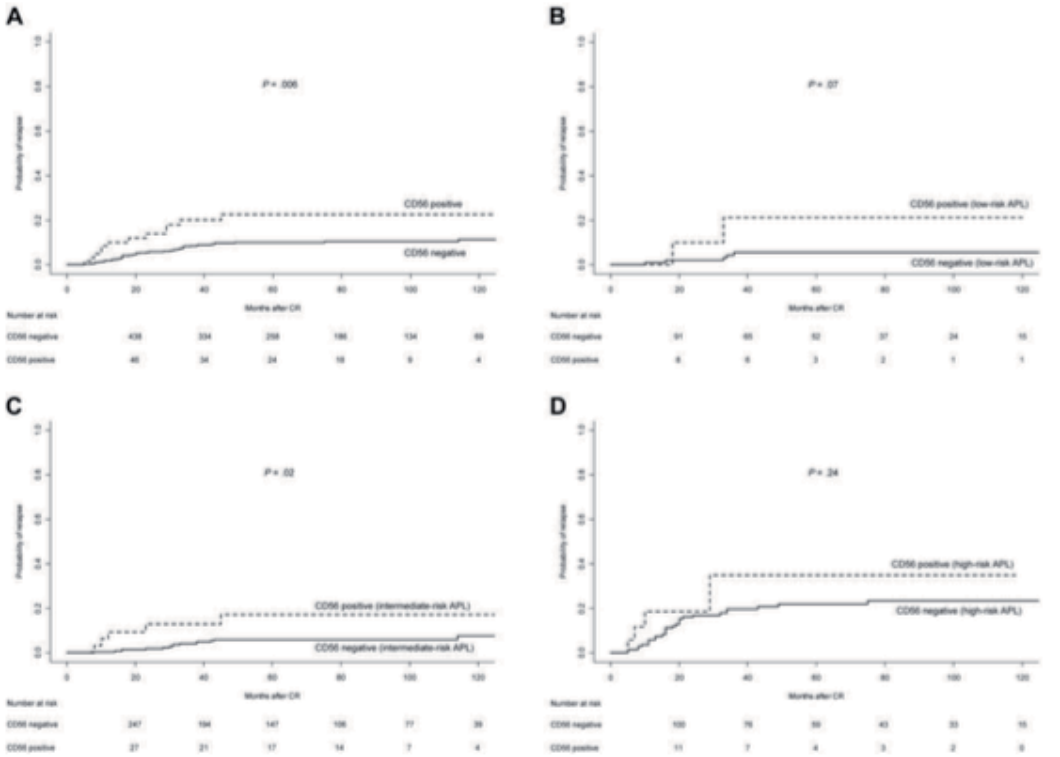


**Figure 2. Cumulative incidence of relapse in APL patients according to CD56 expression.** (A) Overall series; (B) low-risk patients; (C) intermediate-risk patients; and (D) high-risk patients.

***DFS and OS.*** The 5-year DFS rates were 73% in the CD56$^+$ cohort and 85% in the CD56$^-$ cohort ($P = .03$). The probability of remaining alive after 5 years was 78% in the CD56$^+$ group and 84% in the CD56$^-$ group ($P = .09$; Table 3).

## Discussion

This study shows a prevalence of CD56$^+$ APL in 11% of newly diagnosed patients with APL. The expression of CD56 antigen was correlated with the BCR3 isoform and the coexpression of other surface antigens, such as CD2, CD34, HLA-DR, and CD7. The results presented here confirm that the expression of CD56 antigen is an independent risk factor for predicting relapse in patients with APL treated with ATRA and anthracycline-based regimens, along with the APL relapse-risk score, which is a composite of WBC and platelet counts.[14] In addition, CD56$^+$ APL had a significantly higher risk of extramedullary relapse.

The present study analyzes the clinical significance of CD56 expression in a significantly larger series of APL patients compared with previous studies, in which only the Gruppo Italiano Malattie

**Table 4. Statistically significant variables in univariate and multivariate analysis for relapse**

| Covariate | Unfavorable category | Univariate analysis *P* | Multivariate analysis HR (95% CI) | Multivariate analysis *P* |
|---|---|---|---|---|
| WBC count | > 10 × 10⁹/L | < .001 | 3.9 (1.2-12.9) | .03 |
| Relapse risk category | High > intermediate > low | < .001 | | |
| *PML/RARA* isoform | BCR3 | .002 | | |
| Morphologic subtype | Microgranular | .001 | | |
| CD56 | Positive | .01 | 2.3 (1.2-4.6) | .01 |
| CD2 | Positive | .04 | | |
| CD34 | Positive | .002 | | |

CI indicates confidence interval; HR, hazard ratio; and WBC, white blood cell.

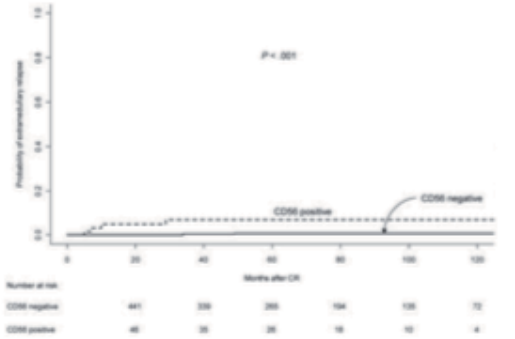


**Figure 3. Cumulative incidence of extramedullary relapse in APL patients according to CD56 expression.**

Ematologiche dell'Adulto (GIMEMA) study included a sizable number of patients (100 patients). As in previous studies, we used the cut-off level of 20% of leukemic promyelocytes expressing CD56 antigen to define CD56$^+$ APL.[1-3] The frequency of 11% of CD56$^+$ APL here reported is not dissimilar to the12% to 15% values found in other studies.[2-4] It should be noted that in the current multicenter study, as in previous studies that analyzed the prognostic impact of CD56 in APL,[1,2] immunophenotypic analyses were not performed centrally, preventing a systematic standardization of flow cytometry. A further limitation of our study is the possible selection bias because not all centers performed cytometric analysis that included anti-CD56 in the diagnostic panel. This limitation leads to a considerable reduction in the sample size to almost half compared with the total series.

Concerning the biologic features of CD56$^+$ APL, our study shows a significant correlation with the BCR3 isoform[1,5] and CD34 coexpression,[3] as has been previously observed. Furthermore, we found an association between CD56$^+$ APL and expression of additional immaturity-associated markers, such as CD117 and HLA-DR antigens, as well as natural killer (NK) and T-cell antigens, such as CD2 and CD7, which have not been previously reported. The higher frequency of coexpression of stem-cell and NK-cell antigens in CD56$^+$ APL may suggest that in some of these cases the APL might have arisen in progenitors that have not undergone lineage restriction.[4,22] Interestingly, we found a trend indicating an association with the M3 variant morphology, which has been previously related to CD2 and CD34[4,5,9] but never CD56 expression. The relationship between CD56 expression and WBC counts confirmed in our series had been suggested in previous studies, although without statistical significance, probably because of the small sample size in those studies.[1,2] However, we were unable to confirm the relationship between fibrinogen levels and CD56 expression suggested by others.[1]

The higher induction mortality rate in CD56$^+$ patients observed in our series treated with the AIDA regimen confirms a similar observation previously reported,[1] although many of these patients did not receive a state-of-the-art treatment. Probably, the higher induction mortality rate in CD56$^+$ APL was attributable to its association with other recognized adverse factors for induction response[23]; however, multivariate analysis showed that this immunophenotypic feature had an independent prognostic value.

As suggested in previous studies,[1-3] we demonstrated that CD56 expression has an impact on the relapse rate and also confirmed its independent prognostic value as in the GIMEMA study.[2] Interestingly, the CD56 expression was able to distinguish a subset of patients with a greater risk of relapse in the intermediate-risk category, whereas its usefulness in low-risk patients needs to be confirmed in larger series. Moreover, the prognostic value of CD56 in high-risk APL seems insignificant. Of note, the expression of CD56 was associated with a higher relapse rate in all 3 PETHEMA trials, although the differences were significant in the LPA96 and LPA2005 trials, but not in the LPA99.

The reason why an elevated CD56 expression leads to a greater risk of relapse remains uncertain but at least 2 hypotheses can be proposed. First, as we have alluded to previously, CD56$^+$ APL may emerge from a more immature undifferentiated and pluripotent leukemic stem cell that is less sensitive to the combination of ATRA and anthracyclines. CD56 expression has been associated with the expression of the multidrug resistant marker P-glycoprotein in patients with acute myeloid leukemia with t(8;21).[24] Unfortunately, in the current study the expression of the multidrug resistance markers has not been assessed. Second, the implication of CD56 expression in the development of extramedullary relapses found in our study has already been hypothesized in a previous study by Ito et al.[3] In this study, 3 of the 4 CD56$^+$ APL patients developed extramedullary relapse, compared with none of the 24 CD56$^-$ patients. Of note in this respect, the expression of CD56 has been associated with extramedullary involvement in nonpromyelocytic acute myeloid leukemia.[25-29]

In addition to confirm the prognostic value of CD56 expression, this study also provides new insights into the clinical features of CD56$^+$ APL. CD56 expression is associated with some clinical and biologic features, such as increased WBC counts, BCR3 isoform, and coexpression of immaturity and NK-cell antigen markers. It should be noted that, despite this association with WBC counts, the regression model for relapse risk selected both as independent adverse factors, WBC count greater than $10 \times 10^9$/L and CD56 positivity. If the independent prognostic value of CD56 expression in APL cells is confirmed, it should be considered for designing future risk-adapted strategies.

## Acknowledgments

We thank Alberto Orfao for the critical review of the section addressing the immunophenotypic analyses and Leticia Llopis for data collection and management.

This study was supported in part by the Fundación para la Investigación Hospital Universitario La Fe-Ayudas Bancaja (grant 2006/0137) and Red Temática de Investigación Cooperativa en Cáncer (RD06/0020/0031).

A complete list of participating institutions and clinicians appears in the online supplemental Appendix.

## Authorship

Contribution: P.M. and M.A.S. conceived the study and analyzed and interpreted the data; P.M., B.L., and M.A.S. wrote the paper; P.M. performed the statistical analyses; and C.R., E.V., S.B., J.G., M.G., A.H., J.E., J.B., J.D.G., C.R., M.T., V.R., J.B., F.M., G.M., J.d.l.S., I.P., M.P.-E., I.K., J.M.R., and L.E. included data of patients treated in their institutions, reviewed the manuscript, and contributed to the final draft.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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## 9.7 Artículo Anexo 7

Montesinos P, Díaz-Mediavilla J, Debén G, Prates V, Tormo M, Rubio V, Pérez I, Fernández I, Viguria M, Rayón C, González J, de la Serna J, Esteve J, Bergua JM, Rivas C, González M, González JD, Negri S, Brunet S, Lowenberg B, Sanz MA.

Central nervous system involvement at first relapse in patients with acute promyelocytic leukemia treated with all-trans retinoic acid and anthracycline monochemotherapy without intrathecal prophylaxis.

Haematologica. 2009 Sep;94(9):1242-9.

Original Article

# Central nervous system involvement at first relapse in patients with acute promyelocytic leukemia treated with all-*trans* retinoic acid and anthracycline monochemotherapy without intrathecal prophylaxis

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## ABSTRACT

### Background

The prevalence of and risk factors for central nervous system recurrence in patients with acute promyelocytic leukemia are not well established and remain a controversial matter.

### Design and Methods

Between 1996 and 2005, 739 patients with newly diagnosed acute promyelocytic leukemia enrolled in two consecutive trials (PETHEMA LPA96 and LPA99) received induction therapy with all-*trans* retinoic acid and idarubicin. Consolidation therapy comprised three courses of anthracycline monochemotherapy (LPA96), with all-*trans* retinoic acid and reinforced doses of idarubicin in patients with an intermediate or high risk of relapse (LPA99). Central nervous system prophylaxis was not given.

### Results

Central nervous system relapse was documented in 11 patients. The 5-year cumulative incidence of central nervous system relapse was 1.7% (LPA96 3.2% and LPA99 1.2%; $p$=0.09). The cumulative incidence was 0%, 0.8%, and 5.5% in low-, intermediate-, and high-risk patients, respectively. Relapse risk score ($p$=0.0001) and the occurrence of central nervous system hemorrhage during induction (5-year cumulative incidence 18.7%, $p$=0.006) were independent risk factors for central nervous system relapse.

### Conclusions

This study shows a low incidence of central nervous system relapse in patients with acute promyelocytic leukemia following therapy with all-*trans* retinoic acid and anthracycline without specific central nervous system prophylaxis. Central nervous system relapse was significantly associated with high white blood cell counts and prior central nervous system hemorrhage, which emerged as independent prognostic factors.

Key words: acute promyelocytic leukemia, central nervous system relapse, all-*trans* retinoic acid, idarubicin, prognostic factors.

*Citation: Montesinos P, Díaz-Mediavilla J, Debén G, Prates V, Tormo M, Rubio V, Pérez I, Fernández I, Viguria M, Rayón C, González J, de la Serna J, Esteve J, Bergua JM, Rivas C, González M, González JD, Negri S, Brunet S, Lowenberg B, and Sanz MA. Central nervous system involvement at first relapse in patients with acute promyelocytic leukemia treated with all-*trans *retinoic acid and anthracycline monochemotherapy without intrathecal prophylaxis. Haematologica 2009;94:1242-1249. doi:10.3324/haematol.2009.007872*



*Acknowledgments: the authors thank Miguel Priego for data collection and management.*

*Funding: this study was supported in part by the Fundación para la Investigación Hospital Universitario La Fe-Ayudas Bancaja (grant 2006/0137), Red Temática de Investigación Cooperativa en Cáncer (RD06/0020/0031).*

*Manuscript received on February 24, 2009. Revised version arrived on March 26, 2009. Manuscript accepted on April 9, 2009.*

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## Introduction

The central nervous system (CNS) is the most common site of extramedullary relapse in acute promyelocytic leukemia (APL), and at least 10% of relapses involve the CNS.[1] However, there is an apparent lack of documented knowledge about many aspects of this challenging clinical complication in the context of state-of-the-art treatments with all-*trans* retinoic acid (ATRA) and chemotherapy. The reported incidence of CNS relapses in APL ranges from 0.6% to 2%.[2,3] Although CNS involvement is associated with age[3] and BCR[3] isoform,[3,4] and possibly with the use of ATRA and the development of differentiation syndrome,[5] high white blood cell (WBC) count at presentation appears to be the most consistent predictive factor.[3,6] For patients without leukocytosis, who have an extremely low risk of CNS relapse, there is a general consensus that CNS prophylaxis is not indicated.[7] In contrast, in patients with APL and high-risk features presenting with a high WBC count, some have argued in favor of CNS prophylaxis with intrathecal chemotherapy.[6,8] Available data about disease outcome after CNS relapse are limited. The GIMEMA group reported that the survival rates of patients after CNS relapse and after isolated bone marrow relapse were similar.[2] However, in a joint study by the European APL and PETHEMA groups, survival rates after CNS relapse were significantly lower than after bone marrow relapse.[3] The optimal management of APL patients with CNS involvement at first relapse, whether isolated or associated with bone marrow involvement, has not been assessed critically.

We analyzed the incidence of and prognostic risk factors for CNS involvement at first relapse in a large series of newly diagnosed patients with APL who were enrolled in two consecutive Programa Español de Tratamiento en Hematología (PETHEMA) trials (LPA96 and LPA99) and treated with ATRA and anthracycline monochemotherapy without CNS prophylaxis. We also evaluated the outcome of these patients.

## Design and Methods

### Eligibility for inclusion in the study

The eligibility criteria for enrollment of patients with genetically diagnosed APL have been described elsewhere.[9] The study protocol was approved by the Research Ethics Board of each participating hospital according to the Declaration of Helsinki.

### Therapy of acute promyelocytic leukemia

Patients were included in two successive protocols (LPA96 and LPA99) that have been previously described.[9] Briefly, treatment consisted of induction therapy with ATRA and idarubicin (AIDA regimen), three consolidation courses with idarubicin (two courses) and mitoxantrone (one course) with or without ATRA according to a risk-adapted strategy,[9] followed by maintenance therapy with ATRA and low-dose chemotherapy with methotrexate and 6-mercaptopurine.

### Prophylaxis and treatment of central nervous system relapse

Patients did not receive CNS prophylaxis. When CNS relapse occurred, treatment comprised weekly intrathecal triple therapy (ITT) with methotrexate, hydrocortisone, and cytarabine until complete clearance of blasts in the cerebrospinal fluid, followed by less frequent ITT treatments as consolidation. Some patients received further craniospinal irradiation at the physician's discretion. Systemic treatment with ATRA plus chemotherapy or arsenic trioxide was also given as induction or consolidation or both, even for patients with isolated CNS involvement. Autologous or allogeneic stem cell transplant was given, in some cases, as intensification therapy.

### Study definitions and end-points

The remission-induction response was assessed according to the criteria revised recently by Cheson *et al.*[10] Molecular remission was defined as the disappearance on an ethidium bromide gel of the *PML–RARA*-specific band visualized at diagnosis, using a previously described nested reverse-transcriptase polymerase chain reaction (RT-PCR) assay for *PML–RARA* amplification[11] with a sensitivity level of $10^{-4}$. Persistent molecular disease and molecular relapse were defined as PCR positivity in two consecutive bone marrow samples collected at the end of consolidation therapy and at any time after consolidation therapy, respectively. CNS relapse was confirmed by lumbar puncture and cytological examination of cerebrospinal fluid, which was performed only in patients with clinically suspected CNS relapse. Morphological and molecular status was assessed by examining bone marrow aspirates from all patients at the time of CNS relapse.

### Prognostic factors

Twenty-seven characteristics of the patients and disease were analyzed to establish their relationship to CNS relapse. In addition to the characteristics listed in Table 1, the following variables were also considered: serum lactate dehydrogenase (LDH), peripheral blast count, fibrinogen level, relapse-risk score, cytogenetics, *FLT3*-internal tandem duplication (*FLT3*-ITD) mutation, and a number of surface antigen markers (CD2, CD7, CD11b, CD34, CD13, HLA-DR, and CD56). Occurrence of clinically and radiologically evident CNS hemorrhage at diagnosis or during induction, and development of differentiation syndrome were also included. Differentiation syndrome was diagnosed according to the presence of two or more of the symptoms and signs described by Frankel *et al.*[12]

### Statistical methods

The $\chi^2$ test, with Yates' correction if necessary, was used to analyze differences in the distribution of variables between subsets of patients. The probability of CNS involvement at first relapse was estimated by the cumulative incidence method, and univariate comparison between the cumulative incidence curves was performed using the Gray test.[14] Isolated bone marrow relapse (molecular or morphological), death in complete remission, and development of secondary acute leukemia or myelodysplastic syndrome was each considered as a competing risk event. Multivariate analysis was performed using a logistic

regression model.[34] Overall survival after relapse was estimated by the Kaplan–Meier method, and comparisons were made by the log-rank test.[35] The patients' follow-up was updated in June 2008, and the median follow-up was 83 months (range, 32-136 months).

## Results

### Accrual and patients' characteristics

Between November 1996 and June 2005, 793 consecutive patients with a genetic diagnosis of APL were registered from 82 institutions in Spain, The Netherlands, Belgium, Argentina, Uruguay, and the Czech Republic (*see Appendix*). Details about the non-eligible, non-evaluable, and evaluable patients have been reported elsewhere.[16,17]

The main demographic and baseline characteristics of the 667 patients who achieved complete remission are shown in Table 1. Patients enrolled in the LPA96 and LPA99 trials were similar for all baseline characteristics except for sex (more females were included in the LPA99 trial, $p$=0.02).

### Incidence and characteristics of central nervous system relapse

CNS involvement at first relapse was documented in 11 patients: in eight of these it was isolated (although three had positive *PML–RARA* in the bone marrow by RT-PCR)

Table 1. **Main demographic and baseline characteristics of 667 acute promyelocytic leukemia patients who achieved complete remission with the AIDA regimen (study population).**

| | LPA96 | | LPA99 | | | Total | |
|---|---|---|---|---|---|---|---|
| Characteristic | Median (range) | n (%) | Median (range) | n (%) | p value | Median (range) | n (%) |
| Overall | | 156 (100) | | 511 (100) | | | 667 (100) |
| Age, years | 40 (2-73) | | 39 (2-83) | | | 40 (2-83) | |
| <18 | | 16 (10) | | 62 (12) | 0.65 | | 78 (12) |
| 18-50 | | 92 (59) | | 308 (60) | | | 400 (60) |
| 51-60 | | 25 (16) | | 61 (12) | | | 86 (13) |
| 61-70 | | 17 (11) | | 53 (10) | | | 70 (10) |
| >70 | | 6 (4) | | 27 (5) | | | 33 (5) |
| Sex | | | | | | | |
| Male | | 89 (57) | | 238 (47) | 0.02 | | 327 (49) |
| Female | | 67 (43) | | 273 (53) | | | 340 (51) |
| ECOG score | 1 (0-3) | | 1 (0-3) | | | 1 (0-3) | |
| 0-1 | | 117 (81) | | 353 (75) | 0.28 | | 470 (77) |
| 2 | | 19 (13) | | 88 (19) | | | 107 (17) |
| 3 | | 8 (6) | | 29 (6) | | | 37 (6) |
| Fever | | | | | | | |
| No | | 101 (65) | | 310 (61) | 0.45 | | 411 (62) |
| Yes | | 55 (35) | | 195 (39) | | | 250 (38) |
| WBC count, ×10⁹/L | 1.9 (0.3-162) | | 2.1 (0.2-460) | | | 2.1 (0.2-460) | |
| <3.5 | | 100 (64) | | 321 (63) | 0.51 | | 421 (63) |
| 3.5-10 | | 19 (12) | | 76 (15) | | | 95 (14) |
| 10-50 | | 30 (19) | | 80 (16) | | | 110 (17) |
| >50 | | 7 (4) | | 33 (6) | | | 40 (6) |
| Platelet count, ×10⁹/L | 21 (1-161) | | 22 (1-207) | | | 22 (1-207) | |
| <10 | | 31 (20) | | 90 (18) | 0.74 | | 121 (18) |
| 10-40 | | 87 (56) | | 301 (59) | | | 388 (58) |
| > 40 | | 38 (24) | | 119 (23) | | | 157 (24) |
| Hemoglobin, g/dL | 9.6 (4.3-15.2) | | 9.1 (3-16.9) | | | 9.2 (3-16.9) | |
| ≤10 | | 93 (60) | | 329 (64) | 0.27 | | 422 (63) |
| > 10 | | 63 (40) | | 181 (36) | | | 244 (37) |
| Creatinine, mg/dL | 0.9 (0.2-1.4) | | 0.9 (0.2-2.4) | | | 0.9 (0.2-2.4) | |
| ≤1.4 | | 156 (100) | | 489 (99) | 0.58 | | 645 (99) |
| > 1.4 | | 0 (0) | | 4 (1) | | | 4 (1) |
| Albumin, mg/dL | 4.2 (2.2-6.2) | | 4 (1.7-6.7) | | | 4 (1.7-6.7) | |
| ≤3.5 | | 21 (15) | | 89 (22) | 0.07 | | 110 (20) |
| >3.5 | | 120 (85) | | 313 (78) | | | 433 (80) |
| Coagulopathy | | | | | | | |
| No | | 34 (22) | | 127 (25) | 0.82 | | 161 (24) |
| Yes | | 122 (78) | | 369 (75) | | | 501 (76) |
| FAB subtype | | | | | | | |
| Hypergranular | | 133 (85) | | 416 (83) | 0.74 | | 549 (84) |
| Microgranular | | 23 (15) | | 85 (17) | | | 108 (16) |
| PML/RARα isoform | | | | | | | |
| BCR1/BCR2 | | 82 (55) | | 268 (60) | 0.30 | | 350 (59) |
| BCR3 | | 66 (45) | | 177 (40) | | | 253 (41) |

*WBC: white blood cell count; FAB: French-American-British.*

whereas in three it was simultaneous with overt involvement of the bone marrow. The median time to CNS relapse was 16 months (range, 6-49 months) compared with 16 months (range, 5-74 months) for isolated bone marrow relapse. The characteristics of the patients who experienced CNS relapse are summarized in Table 2. Seven patients were female, and four were male. At the time of the primary APL diagnosis, the median age was 33 years (range, 6-70 years) and WBC count was $34.5\times10^9$/L (range, 1.9–$162\times10^9$/L). Eight of the 11 CNS relapses occurred in the 149 patients classified at diagnosis as being at high-risk (cumulative incidence at 5 years, 5.45%), and the other three were in the 381 patients classified as being at intermediate-risk (cumulative incidence at 5 years, 0.8%). No CNS relapses were observed in low-risk patients. Three patients experienced clinical CNS hemorrhage during induction therapy. One additional patient (9%) had differentiation syndrome compared with 22 of 73 relapsed patients (30%) without CNS involvement. The overall 5-year cumulative incidence of CNS relapse was 1.7%, with a trend toward a higher incidence in the LPA96 trial (3.2%) than in the LPA99 trial (1.2%) ($p$=0.09). The cumulative incidence of isolated bone marrow relapse was 14.7% in the LPA96 trial and 10.6% in the LPA99 trial ($p$=0.08). The relative frequency of CNS involvement among relapsed patients overall was 13%: five of 28 relapses (18%) in the LPA96 trial and six of 56 relapses (11%) in the LPA99 trial. No extramedullary sites of relapse other than CNS were observed in either PETHEMA trial.

### Treatment of central nervous system relapse

Treatment of CNS relapse included ITT for all patients except one, who was treated with intrathecal liposomal cytarabine. Six patients received further CNS irradiation (4 holocranial and 2 craniospinal). Except for two patients, both with CNS relapse without morphological bone marrow involvement, all remaining patients received systemic treatment (Table 2).

### Outcome of patients with central nervous system relapse

Of the eight patients with CNS involvement without morphological relapse in the bone marrow (3 PCR positive) one died during CNS treatment. The remaining seven patients achieved a complete clearance of blasts from the cerebrospinal fluid and relief of symptoms. The subsequent outcome of these patients was as follows: (i) two patients experienced a second relapse and died despite receiving consolidation therapy with liposomal ATRA or intensive chemotherapy; (ii) three patients are alive in complete remission after salvage therapy for a second CNS or bone marrow relapse; and (iii) two patients did not relapse after receiving ATRA plus intensive chemotherapy or arsenic trioxide followed by autologous stem cell transplantation.

The three patients with simultaneous CNS and bone marrow involvement died during systemic therapy with ATRA plus intensive chemotherapy (2 patients) or during aplasia following autologous stem cell transplantation.

**Table 2.** Pretreatment characteristics of patients who had a central nervous system relapse and time of occurrence, treatment, and outcome the relapse.

| Sex/age (years) | LPA trial | WBC ($x10^9$/L) | Risk score | BCR | DS | CNS bleeding | Time to CNS relapse (months) | BM relapse | CNS therapy | Systemic therapy | Outcome | Survival from CNS relapse (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| F/6 | 96 | 13.6 | High | 3 | No | Yes | 49 | No | ITT (x8)+ RTh (18 Gy) | LPA99 protocol | Alive in CR2 | 59 |
| M/32 | 99 | 6.2 | Interm. | 3 | No | No | 10 | No | ITT (x12) | No | Alive in CR3 | 52 |
| F/22 | 99 | 66.5 | High | 3 | No | No | 29 | PCR+ | ITT (x9) | MTZ+AraC (x2)+AutoSCT | Alive in CR3 | 50 |
| M/29 | 99 | 34.5 | High | 3 | No | No | 13 | PCR+ | ITT (x6) | MTZ+AraC (x1)+AlloSCT | Alive in CR3 | 47 |
| F/49 | 99 | 41 | High | 1/2 | Yes | No | 27 | PCR+ | IT L-AraC (x5)+ RTc (16 Gy) | ATO (x2) + AutoSCT | Alive in CR2 | 8 |
| M/33 | 96 | 67.9 | High | 1 | No | No | 16 | No | ITT (x15) + RTc (16 Gy) | L-ATRA (x28) + AutoSCT | Death in 2nd relapse | 14 |
| F/50 | 99 | 1.9 | Interm | 3 | No | No | 41 | No | ITT (x6) + RTh (18 Gy) | AIDA + HIDAC | Death in 2nd relapse | 13 |
| F/43 | 96 | 162 | High | 3 | No | No | 7 | Yes | ITT (x3) + RTh (20 Gy) | MTZ+AraC (x1)+AutoSCT | Death in CR2 | 5 |
| F/70 | 99 | 68.8 | High | 1 | No | Yes | 14 | No | ITT (x6) + RTh (36 Gy) | No | Death in 1st relapse | 3 |
| F/57 | 96 | 7.7 | Interm. | 1 | No | No | 32 | Yes | ITT (x4) | MTZ + AraC | Death in 1st relapse | 2 |
| M/16 | 96 | 26.7 | High | 3 | No | Yes | 6 | Yes | ITT (x1) | AIDA + HIDAC | Death in 1st relapse | 0.5 |

*M: male; F: female; WBC: white blood cell count; CNS: central nervous system; APL: acute promyelocytic leukemia; DS: differentiation syndrome; IT: intrathecal; ITT: intrathecal triple chemotherapy; RTc: craniospinal radiotherapy; RTh: holocranial radiotherapy; BM: bone marrow; CR: complete remission; ATO: arsenic trioxide; ATRA: all-trans retinoic acid; SCT: stem cell transplant; MTZ: mitoxantrone; AIDA: ATRA + idarubicin; AraC: cytarabine; HIDAC: high-dose cytarabine; L-AraC: liposomal cytarabine; L-ATRA: liposomal ATRA.*

The median survival was 13 months in patients who had a CNS relapse and 20 months in those who had isolated bone marrow relapse ($p$=0.61).

### Prognostic factors for central nervous system relapse

The univariate analysis identified the following characteristics as being associated with CNS relapse: high WBC count in the peripheral blood at presentation ($p$<0.0001), high blast count in the peripheral blood ($p$=0.0009), both with a count of $10\times10^9$/L as the most significant cut-off; relapse-risk score ($p$=0.001); clinical CNS hemorrhage during induction chemotherapy ($p$<0.0001); elevated serum LDH concentration ($p$=0.005); *FLT3*-ITD mutations ($p$=0.015); and CD56 positivity ($p$=0.047) (Table 3). Multivariate analysis was also carried out: insufficient data were available for *FLT3*-ITD mutation status and CD56 expression. Multivariate analysis identified the following independent prognostic factors for CNS relapse: relapse-risk score ($p$=0.0001) (Figure 1A) and clinical CNS hemorrhage during induction chemotherapy ($p$=0.006) (Figure 1B). Because univariate analysis showed a trend for a lower cumulative incidence of CNS relapse among patients treated in the LPA99 trial ($p$=0.09), the LPA96 and LPA99 trials were compared according to the relapse-risk group. For high-risk patients, the 5-year cumulative incidences of CNS relapse were 10.8% in the LPA96 trial and

**Table 3. Main comparisons of cumulative incidence of central nervous system relapse according to the characteristics of the patients and disease.**

| Characteristic | Total number of patients | CNS relapse | 5-year CI of CNS relapse | p value univariate analyses | Multivariate analysis Hazard ratio (95% confidence interval) | p value multivariate analysis |
|---|---|---|---|---|---|---|
| | n | n | % | | | |
| Overall | 667 | 11 | 1.68 | | | |
| LPA trial | | | | | | |
| LPA 96 | 156 | 5 | 3.21 | 0.09 | | 0.08 |
| LPA 99 | 511 | 6 | 1.19 | | | |
| Age, years | | | | | | |
| ≤45 | 406 | 7 | 1.76 | 0.79 | | NI |
| >45 | 261 | 4 | 1.55 | | | |
| Sex | | | | | | |
| Male | 327 | 4 | 1.22 | 0.48 | | NI |
| Female | 340 | 7 | 2.12 | | | |
| WBC count, $\times10^9$/L | | | | | | |
| ≤ 10 | 517 | 3 | 0.59 | < 0.0001 | | 0.19 |
| >10 | 149 | 8 | 5.45 | | | |
| Platelet count, $\times10^9$/L | | | | | | |
| ≤40 | 497 | 9 | 1.85 | 0.59 | | NI |
| >40 | 169 | 2 | 1.18 | | | |
| Blasts in PB, $\times10^9$/L | | | | | | |
| ≤10 | 495 | 4 | 0.85 | 0.0009 | | 0.83 |
| >10 | 119 | 6 | 5.08 | | | |
| Relapse-risk score | | | | | | |
| Low | 136 | 0 | 0.00 | 0.001[a] | 7.0 (1.42–20.70) | 0.0001 |
| Intermediate | 381 | 3 | 0.80 | | | |
| High | 149 | 8 | 5.45 | | | |
| CNS hemorrhage | | | | | | |
| No | 649 | 8 | 1.24 | < 0.0001 | 9.9 (3.22–57.40) | 0.006 |
| Yes | 18 | 3 | 18.75 | | | |
| Coagulopathy | | | | | | |
| No | 161 | 0 | 0.00 | 0.07 | | 0.81 |
| Yes | 501 | 11 | 2.22 | | | |
| LDH, xULN | | | | | | |
| ≤1 | 331 | 1 | 0.32 | 0.005 | | 0.06 |
| >1 | 302 | 10 | 3.35 | | | |
| FAB subtype | | | | | | |
| Hypergranular | 549 | 8 | 1.49 | 0.32 | | NI |
| Microgranular | 108 | 3 | 2.78 | | | |
| PML/RARα isoform | | | | | | |
| BCR1 | 300 | 4 | 1.33 | 0.37[b] | | NI |
| BCR2 | 17 | 0 | 0.00 | | | |
| BCR3 | 244 | 6 | 2.54 | | | |
| *FLT3*-ITD | | | | | | |
| Negative | 206 | 1 | 0.49 | 0.015 | | NI[c] |
| Positive | 57 | 3 | 5.39 | | | |
| CD56 | | | | | | |
| Negative | 374 | 4 | 1.13 | 0.047 | | NI[c] |
| Positive | 41 | 2 | 4.88 | | | |

*[a]Low risk vs. intermediate risk (p=0.31); low risk vs high risk (p=0.008), intermediate risk vs high risk (p=0.001); [b]BCR1 vs. BCR3 (p=0.37); [c]Not included in multivariate analysis because of missing data in a significant number of patients. CNS: central nervous system; ULN: upper limit of normal value; PB: peripheral blood; WBC: white blood cell; FAB: French-American-British; CI: cumulative incidence; NI: not included in multivariate analysis.*

3.6% in the LPA99 trial ($p$=0.02; Figure 1C). For intermediate-risk patients, the 5-year cumulative incidences were 1.2% and 0.7% in the LPA96 and LPA99 trials, respectively ($p$=0.73).

## Discussion

This study shows a relatively low incidence of CNS involvement at first relapse in APL patients receiving frontline therapy with ATRA and anthracycline monochemotherapy without specific CNS prophylaxis. A comparison of this incidence in two sequential PETHEMA trials showed that risk-adapted consolidation with ATRA and reinforced anthracycline for intermediate- and high-risk patients (LPA99 trial) significantly reduced the incidence of CNS relapse in the latter setting. The relapse-risk score, which is a composite of WBC and platelet counts,[18] and the occurrence of clinical CNS hemorrhage during induction were the main risk factors for CNS relapse. Although a sizable proportion of patients experiencing CNS involvement at first relapse will develop subsequent bone marrow relapse, some of these patients will still respond to treatment and may survive over the long term.

Despite the sequential nature of the two trials, there was an apparent unbiased improvement in the 5-year cumulative incidence of CNS involvement from 3.2% in the LPA96 trial to 1.2% in the LPA99 trial. The difference was not significant for this crude comparison, which included all patients, but became significant when high-risk patients were analyzed separately. One explanation of the lower CNS relapse rate in the LPA99 study could be that the more dose-intensive chemotherapy in this trial reduced the systemic disease burden, decreasing the risk of seeding of APL cells in the CNS and, thereby, the incidence of CNS relapse. Another explanation is that the higher-dose intensity of anthracyclines of the LPA99 trial may offer a more effective treatment of the CNS sanctuary. Notably, in contrast to other anthracyclines, the active metabolite of idarubicin, idarubicinol, can penetrate the blood–brain barrier.[19-21]

The variable incidence of CNS involvement at first relapse observed in different series should be interpreted with caution. Variables with potential impact on the incidence of this complication may have been unbalanced and may bias the direct comparison between different series. Different proportions of patients with leukocytosis at presentation, a well-recognized risk factor for CNS relapse,[3] is the most obvious potential cause of bias, but other more subtle factors should also be considered. In this respect, the sample size and the length of follow-up are particularly important and usually considered, but some other factors related to competing events, particularly bone marrow relapse, should also be considered. Theoretically, a high incidence of bone marrow relapse can lead to an apparent low incidence of CNS involvement at first relapse. This may explain, at least in part, the different incidence rates of CNS relapse reported for series with similar sample size and follow-up.[2,3] The apparently lower incidence of CNS relapse reported by the European APL Group[5] (4 cases among 582 patients) than in the GIMEMA Group[2] (16 cases among 740 patients) was observed in the context of a higher incidence of hematologic relapses in the former (150/582 *vs.* 131/740). Interestingly, the European APL Group reported a 2-year cumulative incidence of CNS relapse of 0% in 340 patients treated with ATRA combined with chemotherapy, including high-dose cytarabine and intrathecal prophylaxis in patients with WBC counts

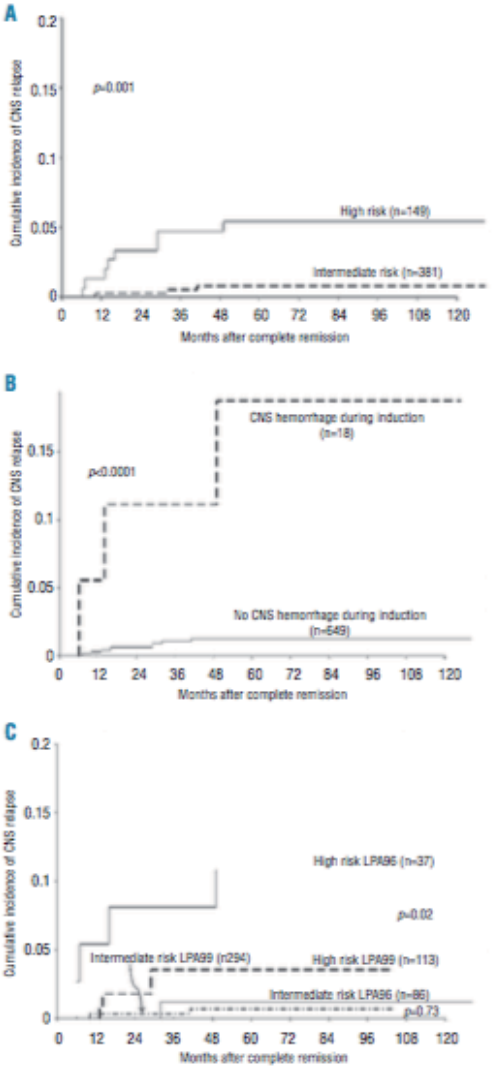


**Figure 1. Cumulative incidence of central nervous system relapse according to (A) relapse-risk group: intermediate (white blood cell count ≤10×10⁹/L and a platelet count ≤40×10⁹/L) versus high (white blood cell count >10×10⁹/L). No relapse occurred among low-risk patients (WBC count ≤10×10⁹/L and a platelet count >40×10⁹/L); (B) occurrence of central nervous system hemorrhage during induction; and (C) LPA trial and relapse-risk group: LPA96 versus LPA99.**

greater than $10\times10^9$/L.[8] However, full evaluation of these results requires both independent confirmation and a longer follow-up because a considerable proportion of CNS relapses may occur more than 2 years after the time of attainment of the first complete remission (e.g., 5 of 11 cases in our present study).[3]

As reported previously, our study confirms that APL patients with high WBC count at diagnosis (>$10\times10^9$/L) have an increased risk of CNS relapse.[3] However, the relapse-risk score[18] remained as an independent risk factor in multivariate analysis after removing WBC count from the regression equation. An additional and novel finding is the association between the occurrence of clinical CNS hemorrhage before or during remission induction and subsequent CNS relapse. This has not been reported before and could have potential therapeutic implications. Regarding other potential risk factors, some authors have suggested previously that *FLT3*-ITD mutations, which correlate with leukocytosis,[22] and an increased expression of adhesion molecules, such as CD56, can promote leukemic infiltration in the CNS.[23-25] In this study, we were unable to assess CD56 expression and *FLT3*-ITD mutation as predictive factors for CNS relapse in multivariate analysis because of an insufficient number of patients with adequate data. Other factors previously related to extramedullary relapse, such as age (< 45 years),[3] BCR3 isoform,[3,4] and development of differentiation syndrome[5] were not significant determinants of CNS relapse in our study.

The low incidence of CNS relapse does not argue in favor of systematic CNS prophylaxis even in patients with high-risk APL. The burden for these patients and the potential risks of additional medical complications (e.g., hemorrhage or neurological toxicity)[26] following lumbar puncture would be unlikely to outweigh the possible benefits. Because there is no clear benefit of systematic CNS prophylaxis, its indication in APL is still a matter of debate. Based on our results, however, prophylaxis could be considered for those patients with clinical or radiological signs of CNS hemorrhage during induction.

In conclusion, we found a low incidence of CNS involvement at first relapse in newly diagnosed patients with APL following therapy with ATRA and anthracycline monochemotherapy without specific CNS prophylaxis. CNS relapse was significantly associated with WBC counts greater than $10\times10^9$/L and CNS hemorrhage during induction treatment, which emerged as independent prognostic factors.

## Appendix

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## Authorship and Disclosures

PM and MAS, conceived the study, and analyzed and interpreted the data; PM, BL and MAS wrote the paper; PM performed the statistical analyses; JD-M, GD, VP, MT, VR, IP, IF, MV, CRi, JG, JdlS, JE, JMB, CRa, MG, JDG, SN and SB included data on patients treated in their institutions, reviewed the manuscript and contributed to the final draft.

The authors reported no potential conflicts of interest.

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