

**Additional cytogenetic changes do not influence the outcome of patients with newly diagnosed acute promyelocytic leukemia treated with an ATRA plus anthracyclin based protocol. A report of the Spanish group Pethema**

**haematologica** 2001; 86:807-813

[http://www.haematologica.it/2001\\_08/0807.htm](http://www.haematologica.it/2001_08/0807.htm)

JESUS MARÍA HERNÁNDEZ, GUILLERMO MARTÍN, NORMA CARMEN GUTIÉRREZ, JOSÉ CERVERA, MARÍA TERESA FERRO, MARÍA JOSÉ CALASANZ, JOSÉ ÁNGEL MARTÍNEZ-CLIMENT, ELISA LUÑO, MAR TORMO, CONSUELO RAYÓN, JOAQUÍN DÍAZ-MEDIAVILLA, MARCOS GONZÁLEZ, JOSÉ DAVID GONZÁLEZ-SAN MIGUEL, KATY PÉREZ-EQUIZA, CONCHA RIVAS, JORDI ESTEVE, MARÍA DEL CARMEN ALVÁREZ, JESÚS ODRIOZOLA, JOSÉ MARÍA RIBERA, MIGUEL ÁNGEL SANZ  
for the PETHEMA Cooperative Group, Spain

Correspondence: Jesús M. Hernández Rivas, Servicio de Hematología, Hospital Universitario de Salamanca, Paseo San Vicente 58-182, 37007 Salamanca, Spain. Phone: international + 34.9.23291100. Fax: international + 34.9.23294624. E-mail: [jmhernandez@aejh.org](mailto:jmhernandez@aejh.org)

*Background and Objectives.* To analyze in patients with *de novo* acute promyelocytic leukemia (APL) treated with an ATRA plus anthracyclin-based protocol if the presence of additional cytogenetic aberrations to the t(15;17) influences: 1. clinical and biological presenting features; 2. disease outcome.

*Design and Methods.* One hundred and thirteen patients with newly diagnosed APL enrolled in the APL-96 protocol of the Spanish PETHEMA group were studied by conventional karyotyping, FISH and RT-PCR for the PML-RAR $\alpha$  fusion. Treatment was homogeneous in all cases and consisted of anthracyclines and ATRA.

*Results.* Additional chromosome aberrations were observed in 30% of cases. The most frequent secondary changes were +8 (14 cases), and abnormalities of chromosomes 9 or 3 (4 patients each), and of chromosomes 1 and 8 (3 cases each). No clinical, biological, morphological, immunophenotypic or molecular differences were observed between the group of APLs with t(15;17) alone and the group of patients with additional changes. Patients with additional changes had a higher rates of complete remission (CR) and 4-year disease-free survival (DFS) (97%, and 97%, respectively) than patients with t(15;17) alone (CR, 70% and DFS, 84%) but these differences were not statistically significant.

*Interpretation and Conclusions.* Patients with APL and additional cytogenetic abnormalities do not show different clinical, biological, morphological or molecular features as compared to patients with

t(15;17) alone. The prognosis of patients with APL and t(15;17) alone and those with additional changes is similar in both groups. This study indicates that there is no rationale for administering more intensive treatment in APL patients with additional cytogenetic abnormalities receiving ATRA plus anthracycline-based chemotherapy.

©2001, Ferrata Storti Foundation

Key words: acute promyelocytic leukemia; cytogenetics; prognosis; additional chromosomal changes, t(15;17)

Acute promyelocytic leukemia (APL) is a singular type of acute myelogenous leukemia (AML) displaying distinctive morphological, clinical and genetic features. APL is genetically characterized by a reciprocal translocation between the long arms of chromosomes 15 and 17, t(15;17)(q22;q21)<sup>1</sup> which results in two hybrid fusion genes, PML/RAR $\alpha$  and RAR $\alpha$ /PML.<sup>2</sup> Nevertheless, in rare APL cases (less than 1%) chromosome 17 is reciprocally translocated with other partners, such as chromosomes 5 and 11.<sup>3</sup> Conventional karyotyping on banded metaphases has shown that, in addition to the specific t(15;17), 30% to 35% of APL patients have additional cytogenetic changes, most frequently trisomy 8.<sup>4-10</sup> These studies have explored whether or not this subgroup with additional chromosomal abnormalities has distinct clinical and molecular features as well as a different outcome. The results so far provided are controversial and scarcely informative on the prognostic relevance of

these abnormalities at least in APL patients receiving state-of-the-art therapeutic approaches. In fact, with the exception of those included in the study recently reported by De Bottom *et al.*,<sup>10</sup> most of patients included in the aforementioned studies had been treated with chemotherapy (CHT) alone, and they had not benefit from what is considered the most effective modern treatment for APL, i.e. a simultaneous all-trans retinoic acid (ATRA) plus chemotherapy combination. Data from recently reported large multicenter trials<sup>11-17</sup> indicate that ATRA combined with CHT results in a significant improvement of remission, disease-free survival and cure rates, particularly when ATRA is simultaneously administered with CHT.<sup>13-17</sup> The impact of this therapeutic progress leads to a new scenario in which studies to evaluate the role of the secondary cytogenetic changes are needed. The aforementioned report by De Bottom *et al.*<sup>10</sup> has been the first study addressing this issue in APL patients treated with simultaneous ATRA and chemotherapy, including anthracyclines and cytarabine showing that additional chromosomal abnormalities had no impact on prognosis.

The aim of the present study was to compare the clinical and biological characteristics, as well as the outcome, of APL patients with or without additional chromosomal changes to the t(15;17). Our data indicate that secondary changes do not influence the prognosis of APL patients treated with an ATRA plus anthracyclin-based protocol.

## Design and Methods

### Patients

Between November 1996 and November 1999, a total of 186 newly diagnosed PML/RAR $\alpha$ -positive APL patients were enrolled in the Spanish PETHEMA/LPA96 trial, whose eligibility criteria and protocol design were reported elsewhere.<sup>16</sup> Cytogenetic analysis was unavailable or inadequate for the purposes of the study in 73 patients. All the remaining 113 patients with successful karyotype performed at diagnosis (see below for definition) were included in the present study.

### Diagnosis

In addition to the morphologic and cytochemical criteria used by the FAB classification, as well as routine immunophenotyping, the diagnosis of APL was genetically confirmed in all cases by demonstration of the PML/RAR $\alpha$  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, we occasionally employed immunohistochemical analysis of the PML protein distribution, with the monoclonal antibody PG-M3 (kindly provided by B. Falini) using the procedure reported elsewhere.<sup>18</sup>

### Cytogenetics and FISH

Bone marrow samples were cultured for 24 or 48 hours following standard procedures. The chromosomes were stained by G-banding and the karyotypes reported according to ISCN (1995) recommendations.<sup>19</sup> Karyotypes were performed in the reference hospitals. At least 15 metaphases were analyzed in each case. Studies were considered normal diploid if no clonal abnormalities were detected in a minimum of 20 mitotic cells analyzed. In cases with PML/RAR $\alpha$  rearrangement and normal karyotype by conventional cytogenetics, FISH studies were additionally carried out in metaphase and interphase nuclei. Two-colour FISH was performed using an APL t(15;17) translocation probe (Vysis, Stuttgart, Germany) to detect the PML-RAR $\alpha$  fusion. In addition, two-color FISH with painting probes for whole chromosomes 15 (Cambio, Cambridge, UK) and 17 (Oncor, Gaithersburg, MD, USA) were used.

As also reported by Slack *et al.*,<sup>7</sup> patients with a variant t(15;17) translocation as the sole cytogenetic change, or with a normal karyotype with the fusion PML-RAR $\alpha$  demonstrated by FISH, were considered as t(15;17) without additional cytogenetic changes.

### RT-PCR studies

Details on processing of bone marrow samples for RNA extraction and RT-PCR protocols for PML/RAR $\alpha$  amplification have been given in previous reports.<sup>16</sup> RT-PCR tests were carried out by 12 different Spanish laboratories, involved in an external quality control program, which included inter-laboratory exchange of samples, as reported elsewhere.<sup>20</sup> In brief, a pre-established time interval for PCR monitoring was planned to collect samples at diagnosis, after induction, after consolidation, every three months during the first two years, and every four to six months thereafter. In cases of doubtful or positive PCR during hematological complete remission (HCR) after the end of consolidation, an extra bone marrow sample was taken two to four weeks later to confirm the result.

### Treatment

The treatment schedule included an induction course with ATRA plus idarubicin, as in the original AIDA regimen,<sup>13</sup> three monthly consolidation courses and maintenance therapy with ATRA and

**Table 1. Base-line characteristics, therapy response and outcome in APL patients with and without additional cytogenetic changes.**

Characteristic	<i>t(15;17)</i> alone (n=79)		<i>t(15;17)</i> with additional changes (n=34)		p value
	Median (range)	N (%)	Median (range)	N (%)	
Age		42		38.5	NS
	(7-74)		(16-74)		
≤15		2 (3)		0	
16-60		65 (82)		27 (79)	
>60		12 (15)		7 (21)	
Gender					
Male		45 (57)		23 (68)	NS
Female		34 (43)		11 (32)	
Coagulopathy		54 (68)		24 (71)	NS
WBC ( $\times 10^9/L$ )	1.68 (0.4-16.2)		2.3 (0.7-21.0)		NS
≤3.5 $\times 10^9/L$		54 (68)		22 (65)	
3.5-10 $\times 10^9/L$		11 (14)		4 (12)	
10-50 $\times 10^9/L$		10 (13)		7 (20)	
>50 $\times 10^9/L$		4 (5)		1 (3)	
Hemoglobin (g/dL)	9.6 (4.4-15.2)		9.5 (5.5-13)		NS
≤10 g/dL		48 (61)		19 (56)	
>10 g/dL		31 (39)		15 (44)	
Platelets ( $\times 10^9/L$ )	20 (2-156)		18.5 (1-137)		NS
≤10 $\times 10^9/L$		17 (21)		9 (26)	
11-50 $\times 10^9/L$		44 (56)		21 (62)	
>50 $\times 10^9/L$		18 (23)		4 (12)	
BCR (n = 106)					NS
1		36 (50)		16 (47)	
2		5 (6)		2 (6)	
3		31 (44)		16 (47)	
FAB subtype					NS
Typical		63 (80)		28 (82)	
Variant		16 (20)		6 (17)	
Absence of Auer Rods		11 (14)		2 (7)	
CR		70 (89)		33 (97)	NS
Hematologic relapses		8 (10)		1 (3)	
Molecular relapses		11 (14)		3 (9)	
% DFS at 4 years hematologic/molecular relapses		84/79		97/91	NS

Abbreviations: NS, not significant; WBC: white blood cells; BCR: breakpoint cluster region on PML gene; DFS, disease-free survival.

low dose chemotherapy with ATRA, mercaptopurine and methotrexate. Details on the treatment schedule have been previously reported.<sup>16</sup> In brief, induction consisted of 45 mg/m<sup>2</sup> ATRA daily until complete remission (CR) and 12 mg/m<sup>2</sup> idarubicin on days 2, 4, 6 and 8. Patients in CR received three monthly chemotherapy courses: idarubicin 5 mg/m<sup>2</sup>/d  $\times$  4 (course #1), mitoxantrone 10 mg/m<sup>2</sup>/d  $\times$  5 (course #2), and idarubicin 12 mg/m<sup>2</sup>/d  $\times$  1 (course #3). Maintenance therapy consisted of 90 mg/m<sup>2</sup>/d mercaptopurine orally, 15 mg/m<sup>2</sup>/week methotrexate intramuscularly, and 45 mg/m<sup>2</sup>/d ATRA for 15 days every three months.

#### Definitions and study endpoints

Hematological complete remission and hematological relapse were defined according to the National Cancer Institute criteria.<sup>21</sup> Molecular remission was defined as the disappearance on an ethidium bromide gel of the PML/RAR $\alpha$  specific band visualized at diagnosis, using an RT-PCR assay with a sensitivity level of 10<sup>-4</sup>. Molecular relapse was defined as the reappearance of PCR-positivity in two consecutive bone marrow samples at any time after consolidation therapy. For Kaplan-Meier actuarial estimates in which the event *relapse* was the end point, such as the leukemia-free survival, it was calculated in two different ways: 1) hematological relapse was the only uncensored event considered; in such case, patients who were intensively treated because of a molecular relapse were censored for survival analysis at the time of salvage treatment; and 2) hematological and molecular relapses were equally considered as uncensored events.

#### Statistical methods

Chi-square and Fisher's exact tests were used to analyze differences in the distribution of variables among patient subsets. For univariate comparison, unadjusted time-to-event analyses were performed using the Kaplan-Meier estimate,<sup>22</sup> log-rank tests and their generalizations.<sup>23-25</sup> All survival estimates are reported plus or minus ( $\pm$ ) 1 SE. The median duration of follow-up was 31 months (range, 5 to 50 months). Patient follow-up was updated in February 2000. Computations were performed using 3D, 4F and 1L programs from the BMDP statistical library (BMDP Statistical Software Inc, Los Angeles, CA, USA).

## Results

### Cytogenetic abnormalities and disease characteristics

The main clinical and biological characteristics of

**Table 2. Secondary cytogenetic changes in patients with APL.**

Chromosome	Cytogenetic abnormality in addition to t(15;17).
8	+8 (fourteen patients) t(8;19)(q21;p13) t(X;8)(q27;q21) del(8)(q13q22)
9	del(9)(q22) (two patients) inv(9)(p11q13) t(1;9)(q32;q34) +9
3	del(3)(q23) t(3;4)(q26;p12) t(X;3)(p22;q21) add(3)(q24)
1	add(1)(p36) t(1;12)(p32;p13)
17	ider(17)(t(15;17)(q22;q21) ( t (two patients)

Other additional abnormalities were: -Y; add(2)(q36); +5; -5; t(6;20)(p21;q11); del(13)(q14); add(14)(q32); +10.

the 113 newly diagnosed PML/RAR $\alpha$ -positive APL patients who were considered eligible for the present study are shown in Table 1. There were no statistically significant differences in the distribution of clinico-biological variables between patients with t(15;17) alone and those with additional changes. No relationship was observed between the PML/RAR $\alpha$  isoforms and chromosomal abnormalities.

Additional chromosomal abnormalities were found in 34 patients (30%) while 79 (70%) had t(15;17) alone. Forty additional changes were found associated to a t(15;17) (Table 2). The most frequent additional change was trisomy 8 which was present in 14 patients (12%), either alone (12 patients) or associated to other numerical abnormalities (2 patients). Five cases had abnormalities of chromosome 9 (4%). Four of these cases had structural abnormalities of chromosome 9, including two patients with del(9)(q22), and the remaining case had a trisomy 9. One patient displayed an inv(9)(p13q13) in 40% of mitoses. Four cases had structural abnormalities of 3q. Three patients showed structural abnormalities of chromosome 8, all of them with involvement of 8q21. Other additional abnormalities are listed in Table 2. Double color FISH studies performed with a specific probe to study the PML and RAR $\alpha$  genes showed fusion of the genes in the nine cases in which the t(15;17) was not detected by conventional cytogenetics. In

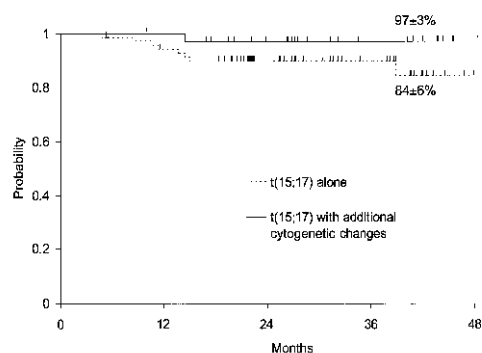
6 of these patients the karyotype was normal while in the remaining 3 cases the karyotype showed other cytogenetic changes but not the t(15;17). In all cases the absence of t(15;17) was confirmed by double color painting of chromosomes 15 and 17.

#### Therapy response and outcome

Seventy out of the 73 patients (88%) with the t(15;17) alone and 33 out of the 34 (97%) with additional changes achieved CR. This difference was not statistically significant (Table 1). Concerning the subsequent clinical outcome, a total of 14 relapses were recorded (9 clinical and 5 molecular relapses). When hematological relapse was the only uncensored event considered, the four-year Kaplan-Meier estimates of DFS for the total series was 89 $\pm$ 4%. Although DFS rate was higher in patients with secondary changes (97 $\pm$ 3%) than in patients without additional abnormalities (84 $\pm$ 6%), these differences were not statistically significant (Table 1 and Figure 1). When hematological and molecular relapses were equally considered as uncensored events, DFS was 83 $\pm$ 5% for the total series, and 91 $\pm$ 5% and 79 $\pm$ 5% for patients with and without secondary changes, respectively.

#### Discussion

The present study shows that secondary cytogenetic changes did not influence the therapy response and outcome of patients with newly diagnosed APL receiving upfront ATRA and idarubicin (AIDA) followed by an anthracycline-based consolidation.



**Figure 1. Kaplan-Meier product-limit estimate of disease free survival of patients with t(15;17) alone or with additional cytogenetic changes.**

Overall the incidence of additional abnormalities (30% of cases) besides t(15;17) is similar to that described by others in large series,<sup>7,8,10</sup> but slightly lower compared to that described in smaller series.<sup>6,9</sup> In keeping with the results of other studies, trisomy 8 was the most frequent secondary abnormality. Other structural abnormalities involved 9q, 3q and 8q. Structural abnormalities of chromosome 9, mostly deletions of 9q, are recurrently found in association with t(15;17).<sup>6,7</sup> By contrast, abnormalities in 8q, other than trisomy 8, and those of 3q are less frequent in APL.<sup>6,7,9</sup>

No clinical or biological differences were found in the patients with t(15;17) alone and patients with other cytogenetic changes. Thus the morphology of M3v was found in a similar proportion in patients with and without additional changes to the t(15;17). This result also is in agreement with previous reports<sup>7,8,10</sup> but is in contrast with the findings reported by Schoch *et al.* (1997) who found no cases with additional abnormalities and variant morphology. At the molecular level, previous reports described a relationship between the breakpoint at BCR-3 region and the presence of additional cytogenetic changes.<sup>7</sup> By contrast our results and those recently reported by De Botton *et al.* (2000) do not demonstrate a relation between the molecular breakpoint and the presence of additional chromosomal abnormalities.

It has been suggested that in some patients with *de novo* AML the primary genetic event is the creation, by translocation or inversion, of an oncogenic fusion protein.<sup>5</sup> The role of additional cytogenetic changes in the transformation process in leukemia is unknown and the impact in the outcome is still controversial. Several studies have analyzed the possible role of the additional chromosomal abnormalities to the t(15;17) in the outcome of APL.<sup>6-10</sup> However most of these studies have been performed in series of patients who were not treated with ATRA and chemotherapy.<sup>4-9</sup> Several reports did not show any difference in the prognosis of patients with additional changes to t(15;17).<sup>6,8</sup> However a single study showed that the group with secondary changes had a shorter survival,<sup>9</sup> and a large study by the CALG-B group showed a better event-free survival for APL patients displaying additional cytogenetic changes.<sup>7</sup> Recently, a study of the European APL group, in which all patients received ATRA in combination with chemotherapy, did not report differences in the outcome of APL patients with additional abnormalities to t(15;17).<sup>10</sup> Our results are in keeping with the latter findings both in terms of response to the therapy and disease free survival.

We remark however that, compared to patients in the European APL study, our patients were only given anthracyclines as chemotherapy and received no cytarabine. Thus, this is the first demonstration that presence of karyotype alterations other than t(15;17) do not confer inferior prognosis in APL patients treated with an anthracycline-based chemotherapy and ATRA.

In summary, our findings lend further support to the notion that no additional treatment is needed in patients with newly diagnosed APL who have additional karyotypic lesions besides t(15;17), particularly if they are enrolled in modern treatment programs including front-line ATRA and anthracycline-based chemotherapy.

#### *Contributions and Acknowledgments*

*JMH: conception, design. Analysis and interpretation of data and drafting the paper; GM: conception, design and drafting the paper; NCG: drafting the article and chromosome studies; JC: chromosome studies; MTF: chromosome studies; MJC: design and chromosome studies; JAMC: chromosome studies; EL: chromosome studies; MT: Design and clinical data; CR: clinical data; JDM: design and clinical data; MG: molecular studies; JDGSM: clinical data; KPE: clinical data; CR: clinical data; JE: clinical data; MCA: clinical data; JO: clinical data; JMR: design and clinical data; MAS: conception and design; analysis and interpretation of data; revising of the paper and final approval of the version to be published.*

#### *Funding*

*This work was supported in part by Grants #99/0806 and 00/1089 from the Fondo de Investigación Sanitaria (FIS), Ministerio de Sanidad of Spain, Grant FIJC PETH-99 from the International José Carreras Leukemia Foundation, Grant No. HI1998-0147 from Ministerio de Educación y Cultura of Spain*

#### *Disclosures*

*Conflict of interest: none.*

*Redundant publications: yes, <50%. (Presented in part at the Fourth Meeting of the European Hematology Association, Barcelona, June 3-7, 1999).*

#### *Manuscript processing*

*This manuscript was peer-reviewed by two external referees and by Prof. Francesco Lo Coco, who acted as an Associate Editor. The final decision to accept this paper for the publication was taken jointly by Prof. Lo Coco and the Editors. Manuscript received May 8, 2001; accepted July 31, 2001.*

### Potential implications for clinical practice

Patients with additional cytogenetic changes have the same prognosis as patients with APL and t(15;17) alone. They have to be treated with the same therapy than the APL without additional cytogenetic changes

### References

- Rowley JD, Golomb HM, Dougherty C. 15/17 translocation, a consistent chromosomal change in acute promyelocytic leukaemia. *Lancet* 1977; 1: 549-50.
- de Thé H, Chomienne C, Lanotte M, Degos L, Dejean A. The t(15;17) translocation of acute promyelocytic leukaemia fuses the retinoic acid receptor  $\alpha$  gene to a novel transcribed locus. *Nature* 1990; 347:558-61.
- Lo Coco F, Diverio D, Falini B, Biondi A, Nervi C, Pelicci PG. Genetic diagnosis and molecular monitoring in the management of acute promyelocytic leukemia. *Blood* 1999; 94:12-22.
- Berger R, Le Coniat M, Derre J, Vecchione D, Jonveaux P. Cytogenetic studies in acute promyelocytic leukemia: a survey of secondary chromosomal abnormalities. *Genes Chromosomes Cancer* 1991; 3:332-7.
- Johansson B, Mertens F, Mitelman F. Secondary chromosomal abnormalities in acute leukemias. *Leukemia* 1994; 8:953-62.
- Schoch C, Haase D, Haferlach T, et al. Incidence and implication of additional chromosome aberrations in acute promyelocytic leukaemia with translocation t(15;17)(q22;q21): a report on 50 patients. *Br J Haematol* 1996; 94:493-500.
- Slack JL, Arthur DC, Lawrence D, et al. Secondary cytogenetic changes in acute promyelocytic leukemia: prognostic importance in patients treated with chemotherapy alone and association with the intron 3 breakpoint of the PML gene: a Cancer and Leukemia Group B study. *J Clin Oncol* 1997; 15:1786-95.
- Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 Trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood* 1998; 92:2322-33.
- Hiorns LR, Swansbury GJ, Mehta J, et al. Additional chromosome abnormalities confer worse prognosis in acute promyelocytic leukaemia. *Br J Haematol* 1997; 96:314-21.
- De Botton S, Chevret S, Sanz M, et al. Additional chromosomal abnormalities in patients with acute promyelocytic leukaemia (APL) do not confer poor prognosis: results of APL 93 trial. *Br J Haematol* 2000; 111:801-6.
- Soignet S, Fleischauer A, Polyak T, Heller G, Warrell RP Jr. All-trans retinoic acid significantly increases 5-year survival in patients with acute promyelocytic leukemia: long-term follow-up of the New York study. *Cancer Chemother Pharmacol* 1997; 40 (Suppl):S25-9.
- Tallman MS, Andersen JW, Schiffer CA, et al. All-trans-retinoic acid in acute promyelocytic leukemia. *N Engl J Med* 1997; 337:1021-8.
- Mandelli F, Diverio D, Avvisati G, et al. Molecular remission in PML/RAR $\alpha$ -positive acute promyelocytic leukemia by combined all-trans retinoic acid and idarubicin (AIDA) therapy. Gruppo Italiano-Malattie Ematologiche Maligne dell'Adulto and Associazione Italiana di Ematologia ed Oncologia Pediatrica Cooperative Groups. *Blood* 1997; 90: 1014-21.
- Asou N, Adachi K, Tamura J, et al. Analysis of prognostic factors in newly diagnosed acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. Japan Adult Leukemia Study Group. *J Clin Oncol* 1998; 16:78-85.
- Burnett AK, Grimwade D, Solomon E, Wheatley K, Goldstone AH. Presenting white blood cell count and kinetics of molecular remission predict prognosis in acute promyelocytic leukemia treated with all-trans retinoic acid: result of the Randomized MRC Trial. *Blood* 1999; 93:4131-43.
- Sanz MA, Martín G, Rayón C, et al. A modified AIDA protocol with anthracycline-based consolidation results in high antileukemic efficacy and reduced toxicity in newly diagnosed PML-RAR $\alpha$ -positive acute promyelocytic leukemia. PETHEMA group. *Blood* 1999; 94:3015-21.
- Fenaux P, Chastang C, Sanz MA, et al. A randomized comparison of ATRA followed by chemotherapy and ATRA plus chemotherapy, and the role of maintenance therapy in newly diagnosed acute promyelocytic leukemia. *Blood* 1999; 94:1192-200.
- Falini B, Flenghi L, Fagioli M, et al. Immunocytochemical diagnosis of acute promyelocytic leukemia (M3) with the anti-PML monoclonal antibody PG-M3. *Blood*. 1997; 90:4046-53.
- ISCN. Guidelines for Cancer Cytogenetics. Supplement to: An International System for Human Cytogenetic Nomenclature (ed. by F. Mitelman). Karger, Basel; 1995.
- Bolufer P, Barragán E, Sanz MA, et al. Preliminary experience in external quality control of RT-PCR PML/RAR $\alpha$  detection in promyelocytic leukemia. *Leukemia* 1998; 12:2024.
- Cheson BD, Cassileth PA, Head S, et al. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. *J Clin Oncol* 1990; 8:813.
- Kaplan EL, Meier P. Nonparametric estimations from incomplete observations. *J Am Stat Assoc* 1958; 53:457-81.
- Mantel N. Evaluation of survival data and two new rank order statistics arising in its consideration. *Cancer Chemother Rep* 1966; 50:163-70.
- Harrington D, Fleming TR. A class of rank test procedures for censored survival data. *Biometrika* 1982; 69:133-43.
- Harrington D, Fleming TR. Counting Processes and Survival Analysis. New York, NY: Wiley & Sons.

*Appendix*

*The following Institutions and personnel participated in PETHEMA trial.*

Hospital Universitario La Fe, Valencia: Sanz MA, Martín G, Bolufer P, Barragán E; Hospital Clínico Universitario, Valencia: Tormo M, Marugán I; Hospital Central de Asturias, Oviedo: Rayón C; Hospital Clínico San Carlos, Madrid: Díaz-Mediavilla J, Villegas A; Hospital Universitario, Salamanca: González M, Gutiérrez NC, Hernández JM; Hospital Insular de Las Palmas, Las Palmas: González JD; Hospital General, Alicante: Rivas C; Hospital General, Pamplona: Pérez K; Hospital Clinic, Barcelona: Esteve J, Colomer D; Hospital de Cruces, Baracaldo: Alvarez MC; Hospital Ramón y Cajal, Madrid: Odriozola J, Ferro C; H. U. Germans Trias i Pujol, Badalona: Ribera JM; Hospital Dr. Peset, Valencia: Sayas MJ; Hospital Clínico Universitario, Valladolid: Fernández F; Complejo H. Xeral-Calde, Lugo: Arias J; H. Universitario Vall D'Hebron, Barcelona:

Bueno J; Complejo Hospitalario, León: Ramos F; H.U. Puerta del Mar, Cádiz: Capote FJ; H.U. Virgen de la Victoria, Málaga: Pérez I; Hospital Xeral de Galicia, Santiago de Compostela: Pérez M; Hospital 12 de Octubre, Madrid: De la Serna J, Bornstein R; Hospital San Pau, Barcelona: Brunet S; Hospital General de Murcia, Murcia: Moraleda JM; Hospital Reina Sofia, Córdoba: Rodríguez A, Román J; Hospital Xeral Cies, Vitoria: Poderós C; Hospital Son Dureta, Palma de Mallorca: Novo A; Hospital Severo Ochoa, Leganés: Sánchez P; Hospital Doctor Trueta, Gerona: Guardia R; Hospital General, Albacete: Romero JR; Hospital Rio Hortega, Valladolid: Peñarrubia MJ; Hospital do Meixoeiro, Vigo: Loureiro C; Hospital Rio Carrión, Palencia: Ortega F; Hospital General, Jerez de la Frontera: León A; H. Ntra Sra del Pino/Sabinal, Las Palmas: Mataix R, Gómez T; Basurtuko Ospitalea, Basurto: Beltrán de Heredia JM; H. San Pedro de Alcántara, Cáceres: Bergua JM.

©Ferrata Storti Foundation