Leukemia (2003) 17, 76–82

www.nature.com/leu

Acute myeloid leukemia with MLL rearrangements: clinicobiological features, prognostic impact and value of flow cytometry in the detection of residual leukemic cells

L Muñoz[1], JF Nomdedéu[1], N Villamor[2], R Guardia[3], D Colomer[2], JM Ribera[4], JP Torres[5], JJ Berlanga[6], C Fernández[3], A Llorente[7], MP Queipo de Llano[8], JM Sánchez[9], S Brunet[1] and J Sierra[1] for the Spanish CETLAM Group

[1]*Department of Hematology, Hospital de la Santa Creu, Sant Pau, Barcelona, Spain;* [2]*Department of Hematology, Hospital Clinic i Provincial, Barcleona, Spain;* [3]*Department of Hematology, Hospital Josep Trueta, Girona, Spain;* [4]*Department of Hematology, Hospital Germans Trias I Pujol, Badalona, Spain;* [5]*Department of Hematology, Hospital Juan Canalejo, La Coruna, Spain;* [6]*Department of Hematology, Institut Catala d'Oncologia, l'Hospitalet, Barcelona, Spain;* [7]*Department of Hematology, Hospital Joan XXIII, Tarragona, Spain;* [8]*Hospital Clinico, Malaga, Spain; and* [9]*Hospital Arnau Vilanova, Lleida, Spain*

The MLL gene, located at 11q23 band, is frequently disrupted by different chromosomal rearrangements that occur in a variety of hematological malignancies. MLL rearrangements are associated with distinct clinical features and a poor prognosis. The aim of this study was to analyze the incidence and the prognostic significance of MLL rearrangements in a consecutive series of adult AML patients and to determine the immunophenotypic features of these cases. The identification of abnormal immunophenotypes could be used for the detection of minimal residual disease (MRD). Ninety-three adult patients with *de novo* acute myeloid leukemia (AML) were analyzed by Southern blot in order to detect MLL rearrangements (MLL+). RT-PCR and genomic long-range PCR were performed to further characterize MLL partial tandem duplication (PTD) in those patients in whom conventional karyotype did not show 11q23 chromosomal translocations. All the patients were homogeneously immunophenotyped at diagnosis. MLL rearrangements were detected in 13 (14%) patients. Four patients (5%) showed 11q23 translocations by karyotypic conventional analysis. Nine patients (10%) revealed PTD of MLL and one patient showed a MLL cleavage pattern. The MLL+ patients usually expressed myeloid and monocytic antigens CD33 (12/13 cases), CD13 (9/13), CD117 (9/13), CD64 (11/13) and in some cases CD14 (4/11). HLA-DR was also positive in (12/13). Eight out of 13 cases expressed the stem cell marker CD34. Only one patient revealed lymphoid marker reactivity (CD7) and CD56 was expressed in 5/13 cases. All the MLL+ patients showed at least one aberrant phenotype at diagnosis, which allowed us to set out a simple panel for the MRD studies. Twenty-seven samples from eight patients in morphologic complete remission (CR) were analyzed using the aberrant immunologic combinations detected at diagnosis. Phenotypically abnormal cells were detected in all the patients who subsequently relapsed, whereas only one patient with MRD+ remained in CR. Owing to the high level of residual leukemic cells, the MLL+ patients showed a short CR duration and a poor survival. In conclusion, immunophenotyping may be a suitable approach to investigating MRD status in AML patients with PTD of the MLL gene.
Leukemia (2003) **17,** 76–82. doi:10.1038/sj.leu.2402708
Keywords: acute myeloid leukemia; 11q23 translocations; MLL rearrangements; MLL self-fusion; immunophenotype; minimal residual disease

Introduction

Structural lesions involving chromosome 11, band q23, are among the most common cytogenetic abnormalities associated with hematopoietic malignancies. MLL rearrangements include non-constitutional or acquired deletions, duplications, inversions and reciprocal translocations at 11q23. Translocations involving 11q23 with more than 30 different chromosomal sites resulting in MLL fusion genes have been described in ALL, as well as in 5–10% of AML.[1–7] A new genetic mechanism for leukemogenesis is the direct partial tandem duplication of the MLL gene (PTD).[5,6] The PTD was first described in AML patients with trisomy 11 and a normal karyotype with a frequency of nearly 10%.[8] Recently, MLL duplications have been described in AML patients with chromosome abnormalities other than those involving the 11q23 band.[9] Regardless of their association with other high-risk factors at presentation, 11q23 rearrangements are strongly predictive of a poor clinical outcome.[10,11] However, the prognostic significance of structural chromosome aberration involving the 11q23 band is not uniform. Previous studies have shown that the outcome of patients with AML t(9;11) is more favorable than that of patients with other 11q23 abnormalities.[12]

Phenotypic aberrations detected by multiparameter flow cytometry (FC) may be associated with specific genetic abnormalities. It has been suggested that FC could be used for the screening of specific genetic lesions such as t(12;21), t(15;17) and t(9;22).[13–15] A number of studies have characterized the immunophenotype of AML with 11q23 chromosomal translocations.[16–18] However, the immunophenotype characteristics of the PTD of MLL gene have only been described in one report.[19] Another application of FC is the study of minimal residual disease (MRD) in these patients. It has been demonstrated that the detection of leukemia-associated phenotypes in patients with acute leukemia in complete remission (CR) is useful for predicting relapse.[20,21]

The present study analyzes the incidence of MLL rearrangements in a series of adults with *de novo* AML, describing the biological features and the clinical outcome of these cases. The immunophenotypic characteristics of MLL+ patients were investigated in order to identify phenotypic aberrations associated with this genetic lesion.

Patients and methods

Patients and treatment protocol

Ninety-three adult patients with *de novo* AML, who were enrolled on a LMA-99 protocol study of the Spanish CETLAM cooperative group between November 1998 and April 2001, were analyzed by Southern blot to detect MLL rearrangements. Pretreatment marrow and/or blood samples were submitted to the Hospital de Sant Pau laboratory for immunophenotyping and molecular studies. Karyotype analyses were

Correspondence: JF Nomdedéu, Laboratori d'Hematologia, Hospital de la Santa Creu i Sant Pau, Avda Sant Antoni M Claret, 167, 08025 Barcelona, Spain; Fax: 34 93-2919192

Received 23 October 2001; accepted 19 June 2002

performed in the laboratory of each hospital. No patient had a history of chemotherapy or radiotherapy, or previous hematologic disorders. Patients were uniformly treated according to the LMA-99 protocol. The remission induction therapy included one or two courses of idarubicin (12 mg/m^2 × 3), cytarabine (500 mg/m^2/12 h × 4) and etoposide (100 mg/m^2 × 3). The patients who achieved morphologic CR received one course of intensification therapy with cytarabine (500 mg/m^2/12 h × 6) and mitoxantrone (12 mg/m^2 × 3). Finally, consolidation therapy was tailored depending on the karyotype. Those patients with a favorable karyotype were treated with one course of high-dose of cytarabine. The patients with other cytogenetic abnormalities and an HLA-identical sibling received an allogeneic stem cell transplantation (allo-SCT), and the remaining patients received an autologous-SCT (auto-SCT).

Morphologic studies

The diagnosis of AML and the assignment of FAB subtypes were based on standard morphological and cytochemical criteria.[22] Morphologic CR was defined in accordance with the criteria proposed by Cheson *et al*.[23]

Cytogenetic analysis

Cytogenetic G-banding analysis was performed in 89/93 (96%) of patients with standard methods. The definition of a cytogenetic clone and the descriptions of karyotype followed the International System for Human Cytogenetic Nomenclature.[24]

Flow cytometry analysis

Sample preparation: In all cases immunophenotyping studies were performed at diagnosis on erythrocyte-lysed BM samples upon staining with monoclonal antibodies (MoAbs) directly conjugated with fluorochromes. The number of cells was quantified by microscopy and adjusted to 2×10^6 in each tube. Antigen expression was analyzed using triple stainings with the following fluorochrome-conjugated (fluorescein isothicyanate (FITC), phycoerytrin (PE), peridinin chlorophyll protein (PerCp) or phycoerytrin-cyanine 5 (PE/Cy 5)) combinations of MoAbs: CD15/CD34/HLA-DR, CD10/CD20/CD19, CD2/CD33/CD19, CD22/CD13/CD3, CD7/CD117/CD45, CD66/CD56/CD64, CD36/GA/CD45, CD34/CD41/CD45, CD34/CD11b/CD45, CD4/CD123/HLA-DR, CD14/CD135/CD45, CD5/CD16/CD45, MPO/CD79a/CD3, Tdt/MPO.

The MoAbs used in the study were: CD22 (4KB128 FITC), glycophorin A (JC 159 PE), CD41 (5B 12 PE), IgM (rabbit anti-human, PE), CD79a (HM57 PE) and TDT (HT-6 FITC), CD117 (104 02 PE), CD64 (10.1 PerCp), CD66 (Kat 4c PE) from DAKO, Glostrup, Denmark; CD15 (MMA-FITC), CD34 (8G12-FITC, PE), HLA-Dr (L243 PetCp), CD10 (W8E7 FITC), CD 20 (L27 PE), CD2 (S5.2 FITC), CD33 (67.6 PE), CD7 (4H9 FITC), CD45 (2D1 PerCp), CD13 (L138 PE), CD14 (M0P9 FITC), CD3 (SK7 PerCp), CD4 (Leu 3 FITC), CD5 (Leu 1 FITC), CD8 (Leu 2 PE), CD56 (NCAM 16.2 PE), CD16 (3G8 PE) purchased from Becton Dickinson, San Jose, CA, USA (BDIS); CD19 (SJ25-C1 PE/Cy 5) and MPO (H-43-5 FITC) from Caltag Laboratories, Burlingame, CA, USA; CD123 (9F5 PE), CD10 (HI10a, Cy-Chrome) from Pharmingen, San Diego, CA, USA; CD36 (FAG-52 FITC) from Immunotech, Marseilles, France.

Direct immunofluorescence was performed by incubating 2×10^6 cells with the specific MoAb for 15 min in the dark at room temperature. An isotype-matched negative control (BDIS) was used in all cases to assess background fluorescence intensity. Cells were lysed (FACS Lysis solution, BDIS) for 3 to 5 min and centrifuged 250 *g* for 5 min. The cells were washed twice with phosphate buffered saline (PBS) before being resuspended in PBS and examined. The immunologic criteria for the lineage assignment followed the EGIL recommendations.[25]

Data acquisition and analysis: Measurements were performed on a FACSscalibur flow cytometer (BDIS). For data acquisition the CELLQuest (BD) software program (BDIS) was used. At least 10 000 events/tube were measured. The PAINT-A-GATE PRO software program (BDIS) was employed for further data analysis. Thresholds for positivity were based on isotype negative controls. Analytical gates were set on desired viable cells based on forward light scatter and side light scatter. The positivity threshold was 20% for all markers except for cytoplasmic or intranuclear antigens for which a 10% threshold was used.

MRD analysis: MRD study was performed in BM samples from patients in CR after the induction, intensification and SCT. The strategy for MRD detection included two criteria based on previously published data:[26] (1) detection exceeding 1×10^{-3} cells coexpressing an aberrant phenotype; and (2) abnormal myeloid/lymphoid CD34$^+$ ratio.[21] A live gate was performed on a SSC/CD34-FITC dot plot and only the information on the CD34$^+$ cells was stored for further analysis. Lymphoid precursors showed CD34$^+$CD33$^-$CD19$^+$ and low FSC/SSC and myeloid precursors showed CD34$^+$CD33$^+$CD19$^-$ and high FCS/SSC. Myeloid/lymphoid CD34$^+$ ratio was considered normal when the ratio was <10. Evaluation of cells displaying leukemia-associated phenotypes and the myeloid/lymphoid CD34$^+$ ratio was performed using a two-step acquisition procedure according to previously defined methods.[26]

Southern blot analysis

Genomic DNA from 93 patients was analyzed for MLL rearrangements as previously described.[9] Genomic DNAs were digested with restriction enzymes *Bam*HI, *Hin*dIII and BglII.

RT-PCR analysis and genomic long range PCR

Reverse transcription PCR and long range genomic PCR were performed in 10 patients MLL+ by Southern blot in which karyotypic data did not show 11q23 chromosomal translocations. The techniques were performed as previously described.[9]

Statistical methods

Continuous variables were compared by the Student's *t*-test and discrete variables were compared by χ^2 or Fisher's exact

test. Curves of survival and duration of complete remission were plotted by the Kaplan–Meier method; differences between curves were analyzed by the long-rank test.

Results

Morphologic, cytogenetic and molecular features

MLL rearrangements by Southern blot were detected in 13 (14%) patients analyzed. The FAB classification, the cytogenetic data and the different MLL gene rearrangements are shown in Table 1. Three cases were AML-M0 (3%), 19 M1 (20%), 20 M2 (21%), 17 M4 (19%), 6 M4 Eo (7%), 25 M5 (27%), one M6 (1%) and two cases (2%) were unclassified (93 patients included in this series). Patients with promyelocytic leukemia had been excluded from the study. Our data showed a higher percentage of M5 in MLL+ cases (61%) than in MLL– cases (20%). As regards the PTD cases, most of these patients were also observed in the M5 category (55%); nevertheless, 45% were classified as M1 and M2.

As for the cytogenetic data, we detected four patients (5%) in the MLL+ group with reciprocal 11q23 translocations, two cases with 6q27 and two cases with 9p22. We did not detect MLL rearrangements in patients with typical chromosomal alterations such as inv(16), t(8;21), or t(6;9). Nine patients (10%) showed PTD of MLL by RT-PCR and long PCR analysis. The karyotype was normal in six out of nine MLL self-fusion cases and fewer than two chromosomal alterations were detected in the remaining three cases.

Immunophenotype characteristics

The immunophenotype of MLL+ cases is shown in Table 2. A consistent myeloid immunophenotype was found in all the patients. The myeloid antigens CD15, CD117, CD13 and CD33 were expressed in most cases. Some monocytic antigens, such as CD64 were frequently expressed and others (CD14 and CD4) less frequently. HLA-DR was expressed in all but one patient and the early stem-cell antigen CD34 was positive in 8/13 cases. As regards lymphoid markers, the CD7 expression was observed in one patient and no cases with positivity of CD2, CD3, CD5, CD19, CD20 and CD22 were detected. The NK lineage antigen CD56 was positive in 5/13 patients.

The antigen expression in the 13 MLL+ patients was compared with 79 of MLL– cases. Although $CD56^+$,$CD64^+$,$CD4^+$,$CD14^+$ and MPO– were more frequently expressed in the MLL+ cases, we found no statistically significant differences in the phenotype expression between the two groups. Nevertheless, monocytic antigens were more frequently expressed in MLL+ cases classified as M1-M2 when compared with M1-M2 MLL– cases. Of the aforementioned cases, three M2 cases showed CD64 and CD4 expression (two and one patient, respectively) and one M1 case showed a monocytic differentiation pathway with coexpression of CD34 and CD15.

Interestingly, a high frequency of aberrant phenotype at diagnosis were observed in the MLL+ cases. All the MLL+ patients displayed at least one aberrant phenotype. This finding was in sharp contrast with observations in the MLL– group. The aberrant phenotype was detected only in 63% of the MLL– cases ($P = 0.01$). As shown in Table 3, the most frequent abnormal immunophenotype was an asynchronous antigen expression which was detected in all patients; two cases also displayed antigen overexpression (CD34 and CD33 antigens) and in one patient the leukemic clone showed an abnormal FSC/SSC distribution according to its phenotype (strong expression of CD33 with low FSC/SSC).

Table 1 FAB classification, cytogenetic data and 11q23 rearrangement pattern in MLL+ patients

Patient	*FAB*	*Karyotype*	*MLL rearrangement*
1	M5	46,XY	Self-fusion
2	M5	46,XY	Self-fusion
3	M2	46,XY/46,XY,del(6)(q25)	Self-fusion
4	M2	46,XY	Self-fusion
5	M5	46,XY	Self-fusion
6	M5	46,XX,t(9;11)(p22;q23)/46,XX	t(9;11)
7	M2	46,XY	Self-fusion
8	M5	46,XX/46, XX t(6;11)(q27;q23)	t(6;11)
9	M5	46,XX,t(9;11)(p22;q23)/46,XX	t(9;11)
10	M1	46,XX/46 XX; 18 p+	Self-fusion
11	M5	46,XX	Self-fusion
12	M5	46,XX, der(1)	Self-fusion
13	M4	46, XY, t(6;11)(q27;q23)	t(6;11)

Clinical outcome

Clinical data and outcome are summarized in Table 4. There were no significant differences in age and white blood cell counts between the MLL+ and MLL– cases. In the FAB classification, there was a significantly higher percentage of M5 subtypes in the MLL+ group.

All but one patient with MLL rearrangements achieved a CR in contrast to MLL– patients who experienced induction failure in 19% instances. This difference, however, did not attain statistical significance. One MLL+ and five MLL– patients died during induction therapy. All the patients who achieved a CR received intensive post-CR therapy. One MLL+ patient died in CR during intensification therapy. In the MLL+ group, eight patients received SCT: four allo-SCT and four auto-SCT. Despite the good response to induction therapy, MLL+ patients had a lower event-free survival (EFS) and a higher probability of relapse (REL) than MLL– patients. Overall survival at 2 years was 22 (17% in MLL+ *vs* 52 ± 7% in MLL– ($P = 0.4$), EFS at 2 years was 21 ± 13% in MLL+ *vs* 56 ± 8% in MLL– cases ($P = 0.01$) (Figure 1) and the REL was 69 ± 17% in MLL+ patients *vs* 43 ± 8% in MLL– cases ($P = 0.07$). Of the patients with a normal karyotype, five were MLL self-fusion positive and 25 negative: EFS at 2 years was 20 ± 17% in self-fusion patients *vs* 66 ± 12% in non-self-fusion patients ($P = 0.03$) (Figure 2).

Minimal residual disease studies

Twenty-seven BM samples from eight MLL+ patients in morphologic CR were analyzed for the assessment of MRD (Figure 3). Two patients showed the t(6;11), six the MLL self-fusion and one a MLL cleavage pattern. All the patients were studied after the induction and intensification therapies and five cases after SCT. The most frequently encountered immunologic

Table 2 Immunophenotype characteristics of patients with MLL rearrangements

Patient	*CD34*	*CD15*	*HLA-DR*	*CD33*	*CD13*	*CD117*	*CD7*	*CD2*	*CD45*	*CD64*	*CD56*	*CD14*	*CD4*	*CD135*	*CD36*	*CD11b*	*MPO cyt*
1	+	+	+	+	+	+	–	–	+	+	+	+	ND	ND	+	ND	+
2	+	–	+	+	+	+	–	–	+	+	–	ND	+	ND	+	+	+
3	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+
4	+	–	–	+	+	+	–	–	+	+	–	+	–	–	–	–	+
5	+	+	+	+	+	+	–	–	+	+	–	+	+	–	+	+	–
6	–	+	+	–	–	+	–	–	+	+	+	–	+	+	–	–	–
7	+	–	+	+	+	+	+	–	+	+	–	–	–	–	–	–	–
8	+	+	+	+	+	–	–	–	+	+	–	+	+	–	+	+	+
9	–	+	+	+	–	–	–	–	+	+	+	ND	ND	ND	+	ND	–
10	+	+	+	+	+	+	–	–	+	–	–	–	–	–	–	–	+
11	–	+	+	+	–	–	–	–	+	+	+	–	–	–	–	–	–
12	–	+	+	+	–	–	–	–	+	+	+	–	+	–	–	–	–
13	–	+	+	+	+	+	–	–	+	+	–	–	+	–	+	+	+

ND, not done; cyt, cytoplasmic.

Table 3 Aberrant antigen expression at diagnosis in AML patients with MLL rearrangements

Aberrant phenotype	*% of patients*	
	MLL+	*MLL–*
Asynchronous antigen expression		
CD15+ CD117+	46	44
CD34+ CD56+	8	2
CD34+ CD64+	46	21
CD34+ CD11B+	46	4
CD34+ CD33+ CD13+ HLA-DR-	8	0
CD34+ CD33+ CD117+ CD7+	8	2
CD34+ CD14+	15	0
CD33+ HLA-DR+ CD13–	31	4
CD34– CD117+ CD64+ CD14+	8	0
CD34– CD117+ CD64+ CD4+	15	3
Aberrant light-scatter pattern		
CD33++ SSC low	8	0
Antigen overexpression		
CD34	8	8
CD33	8	4

Table 4 Correlation of MLL gene rearrangements with clinical features and outcome

	MLL rearranged (n = 13)	*MLL germ line (n = 80)*	*P*
Median (range) age, years	37 (16–58)	42 (17–60)	NS
FAB M5 subtype (%)	8 (61)	13 (16)	0.04
Median (range) WBC ($\times 10^9$ /l)	61 (1.3–232)	33 (0.8–360)	NS
CR (%) (after one course of induction therapy)	11 (85.7)	43 (63.8)	NS
(after two courses of induction therapy)	1 (7)	14 (19.0)	NS
Induction therapy failure	0	14 (19.0)	NS
OS	21 ± 2%	52 ± 7%	NS
EFS	21 ± 13%	56 ± 8%	0.001
REL	69 ± 17%	43 ± 8%	0.07

WBC, white bloof count; CR, complete remission; OS, overall survival; EFS, event-free survival; REL, relapse probability.

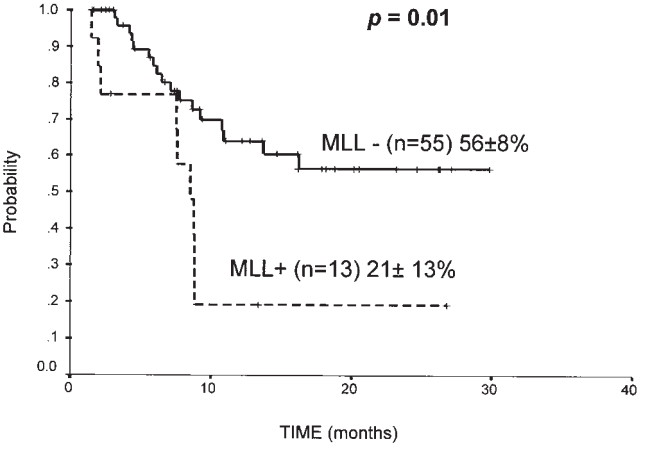

Figure 1 Event-free survival of AML patients.

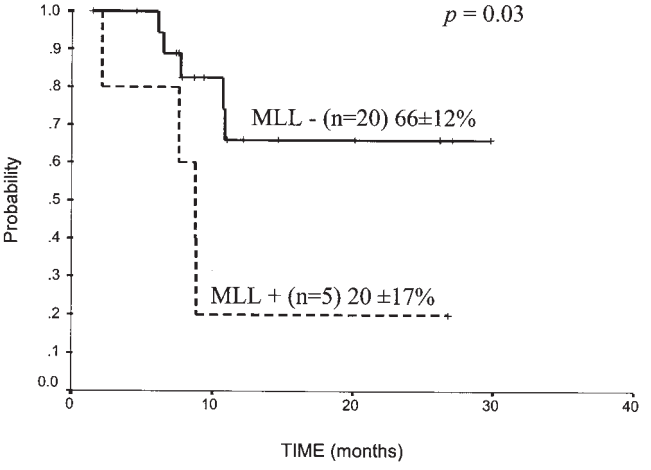

Figure 2 Event-free survival of patients with a normal karyotype.

aberration which enabled us to detect residual leukemic cells was the coexpression of CD15 and CD117 followed by the coexpression of immature antigens such as CD34 or CD117 with mature monocytic and granulocytic markers (CD64 or CD11b). A median number of 3×10^{-3} residual leukemic cells

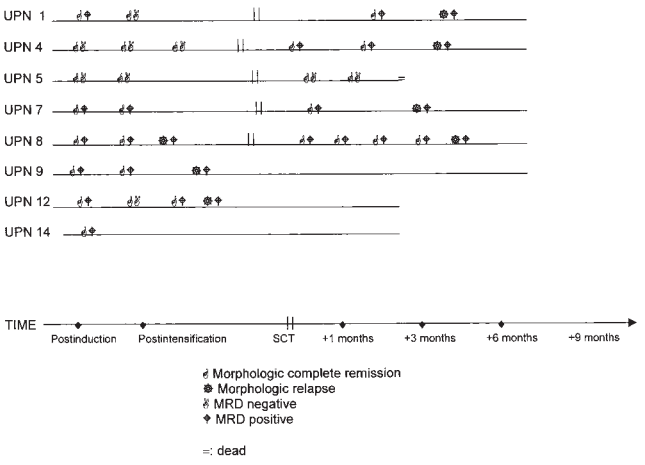

Figure 3 MRD analysis in AML patients with MLL rearrangements.

after induction and/or intensification therapy was detected in six out of eight patients and in all these samples the myeloid/lymphoid CD34+ ratio was abnormal. Four of these MRD+ patients received a SCT in first CR. In three of these, residual leukemic cells with the same aberrant phenotype as at diagnosis and an abnormal myeloid/lymphoid CD34+ ratio were detected after SCT. The remaining patient relapsed after intensification chemotherapy and received an allo-SCT in early relapse. Thereafter, the myeloid/lymphoid CD34+ ratio was abnormal in all the samples tested, although no aberrant cells were detected and the patient relapsed 7 months after SCT. All but one patient with residual leukemic cells detected by flow cytometry after induction and/or intensification relapsed. In these patients MRD was also detected after SCT. An abnormal CD34 ratio was the exclusive immunophenotypic alteration in only one patient who relapsed 5 months after SCT, being negative for aberrant immunophenotypes. One patient without residual leukemic cells died in CR and the remaining patient with a short follow-up is currently in CR despite MRD+ detection.

Discussion

Molecular lesions represent one of the most important prognostic markers in AML and are increasingly used in therapeutic stratification. MLL rearrangements are one of the most frequent molecular alterations detected in a variety of hematological malignancies.[3–5]In this series, MLL rearrangements were detected in 15% of adults with *de novo* AML. In accordance with previous reports,[27] this molecular lesion was most commonly associated with monocytic leukemias, although we found six patients with MLL rearrangements classified as M1, M2 or M4 categories. All the non-M5 or M4 cases corresponded to MLL tandem duplications. In adult AML, the partial tandem duplication of MLL has been described as a frequent molecular defect ranging between 4% and 21%.[8,9] This high variability depends on the inclusion criteria of the patients and the methods used for MLL rearrangement detection. Southern blot is the best screening technique since all the MLL rearrangements can be detected using this method.[28]

In contrast to 11q23 translocations in ALL, there are few reports about the immunophenotype characteristics of AML patients with MLL rearrangements. The European 11q23 Workshop on Hematological Malignancies[18] showed a predominance of these cases in the myelomonocytic phenotype with a frequent expression of lymphoid markers especially in the t(9;11) translocation. We compared the phenotype characteristics of MLL+ and MLL– patients in an attempt to detect a specific pattern in MLL rearrangements. A consistent myeloid immunophenotype with monocytic differentiation was found in the MLL+ group. Nevertheless, no statistically significant differences were found in either group. These results show that the lack of a specific immunologic pattern in 11q23 rearrangements is in part due to the heterogeneity of the cases. Our data suggest that patients with PTD of MLL gene would have a fairly typical, although not specific immunophenotype pattern characterized by the coexpression of mature monocytic antigens with immature markers.

Another interesting finding in the MLL+ group was the high incidence of aberrant phenotypes present at diagnosis. In contrast to the MLL– cases, all the MLL+ patients showed al least one aberrant immunophenotype. Asynchronous maturation was present in all the MLL+ cases. This observation would suggest that MLL rearrangement could occur in an uncommitted cell with a multilineage potential. The alteration of this gene could result in abnormal control of proliferation and differentiation in monocytic progenitor cells.[29]The high incidence of aberrant phenotypes in these patients could be applied to the MRD study. Of the techniques suitable for MRD detection, immunophenotyping and PCR are the most commonly used. Few studies have been performed on MRD in AML patients with MLL rearrangements. Most of them included a low number of patients with 11q23 chromosomal translocations.[29,30] Only one of these studies investigated MRD in a patient with PTD.[19] In the light of our findings, flow cytometry could be applied to MRD detection in most MLL+ patients given the high incidence of aberrant phenotypes. This technique is especially suitable for patients with partial tandem duplications in whom RT-PCR methods cannot easily be employed.[31] To the best of our knowledge, the monitoring of MRD by flow cytometry in AML cases with MLL rearrangements has not been reported. The frequent detection of MRD in patients in morphologic CR could explain the poor outcome of MLL+ patients. The residual leukemic cells observed in the majority of MLL+ patients after the induction treatment could account for the higher frequency of relapse and the shorter remission when compared with patients without MLL rearrangements. This is especially interesting in patients with a normal karyotype. The prognostic relevance of complex, as well as the 'favorable' karyotypes has been reported by a number of groups. However, adverse risk factors in patients with AML and a normal karyotype are scarce. Our data are in agreement with the findings of other groups who suggest that MLL partial duplications are associated with a poor outcome in AML patients with a normal karyotype.[8,9]

In conclusion, MLL rearrangements assessment by molecular methods can identify a subgroup of adult AML patients with a poor prognosis.[32,33] Flow cytometry can provide ancillary data on MLL status, establishing the abnormal immunophenotype which will be used in the MRD study. This method could be employed in the different molecular types of MLL rearrangements.

Wide and prospective studies are warranted to define the prognostic significance of MLL rearrangements. Our diagnostic efforts should be aimed at identifying all patients with MLL rearrangements so that they can be readily treated with novel therapies.

Acknowledgements

FIS 00/0352; L Muñoz is recipient of a Grant FIJC-99/ESP-GLAXO from the José Carreras International Leukemia Foundation. We thank the following centers and physicians for their participation in the CETLAM LMA-99 protocol: Institut Calalá d'Oncologia, Hospitalet (JJ Berlanga); Hospital de la Santa Creu i San Pau, Barcelona (L Muñoz, JF Nomdedéu, A Aventín, S Brunet, J Sierra); Hospital Josep Trueta, Girona (R Guardia, C Fernández); Hospital Joan XXIII, Tarragona (A Llorente); Hospital Verge de la Cinta, Tortosa (L Font); Hospital Clínic, Barcelona (N Villamor, D Colomer, J Esteve); Hospital Vall d'Hebrón, Barcelona (G Acebedo, J Bueno); Hospital Son Dureta, Mallorca (J Bargay); Hospital del Mar, Barcelona (C Pedro); Hospital Clínico, Valencia (M Tormo); Hospital Juan Canalejo, A Coruña (JP Torres); Hospital German Trias i Pujol, Badalona (JM Ribera); Hospital Arnau de Vilanova, Lleida (JM Sánchez); Hospital Clínico, Málaga (MP Queipo de Llano); Mútua de Terrassa, Terrassa (JM Martí); Clínica Teknon, Barcelona (P Vivancos).

References

1 Mrózek K, Heinonen K, de la Chapel A, Bloomfield C. Clinical significance of cytogenetics in acute myeloid leukemia. *Semin Oncol* 1997; **24**: 17–31.
2 Rabbitts TH. Chromosomal translocations in human cancer. *Nature* 1994; **372**: 143–149.
3 Ziemin-van der Poel S, McCabe NR, Gill HJ, Espinosa R III, Patel Y, Harden A, Rubinelli P, Smith SD, LeBeau MM, Rowley JD, Diaz MO. Identification of a gene, MLL, that spans the breakpoint in 11q23 translocations associated with human leukemias. *Proc Natl Acad Sci USA* 1991; **88**: 10735–10739.
4 Rowley JD. Rearrangements involving chromosome band 11q23 in acute leukemia. *Semin Cancer* 1993; **4**: 377–385.
5 Lo Coco F, Mandelli F, Breccia M, Annino L, Guglielmi C, Petti MC, Testi AM, Alimena G, Croce CM, Canaani E, Cimino G. Southern blot analysis of ALL–1 rearrangements at chromosome 11q23 in acute leukemia. *Cancer Res* 1993; **53**: 3800–3803.
6 Cimino G, Rapanotti MC, Elia L, Biondi A, Fizzotti M, Testi AM, Tosti S, Croce CM, Canaani E, Mandelli F, Lo Coco F. ALL-1 gene rearrangements in acute myeloid leukemia: association with M4-M5 French–American–British classification subtypes and young age. *Cancer Res* 1995; **55**: 1625–1628.
7 Sorensen P, Chen C, Smith F, Arthur D, Domer P, Bernstein I, Korsmeyer S, Hammond G, Kersey J. Molecular rearrangements of the MLL gene are present in most cases of infant acute myeloid leukemia and are strongly correlated with monocytic or myelomonocytic phenotypes. *J Clin Invest 1994;* **93**: 429–437.
8 Caligiuri MA, Strout MP, Lawrence D, Arthur DC, Baer MR, Yu F, Knuutila S, Mrózek K, Oberkircher AR, Marcucci G, de la Chapelle A, Elonen E, Block AM, Rao PN, Herzig GP, Powell BL, Ruutu T, Schiffer CA, Bloomfield C. Rearrangement of ALL1 (MLL) in acute myeloid leukemia with normal cytogenetics. *Cancer Res* 1998; **58**: 55–59.
9 Schnittger S, Kinkelin U, Schoch C, Heinecke A, Haase D, Haferlach T, Büchener T, Wörmann B, Hiddermann W, Griesinger F. Screening for MLL tandem duplication in 387 unselected patients with AML identify a prognostically unfavourable subset of AML. *Leukemia* 2000; **14**: 796–804.
10 Di Martino J, Cleary ML. MLL rearrangements in haematological malignancies: lessons from clinical and biological studies. *Br J Haematol* 1999; **106**: 614–626.
11 Rubnitz JE, Behm FG, Downing JR. 11q23 rearrangements in acute leukemia. *Leukemia* 1996; **10**: 4–82.
12 Mrózek K, Heinonen K, Lawrence D, Carroll AJ, Koduru P, Rao K, Strout M, Hutchinson R, Moore J, Mayer R, Schiffer C, Bloomfield C. Adult patients with *de novo* acute myeloid leukemia and t(9;11)(p22;q23) have superior outcome to patients with other translocations involving band 11q23: a cancer and leukemia Group B study. *Blood* 1997; **11**: 4532–4538.
13 Orfao A, Chillón MC, Bortoluci AM, López-Berges MC, García-Sanz R, Gonzalez M, Tabernero MD, García-Marcos MA, Rasillo AI, Hernández-Rivas J, San Miguel JF. The flow cytometric pattern of CD34, CD15 and CD13 expression in acute myeloblastic leukemia is highly characteristic of the presence of PML/RARα gene rearrangements. *Haematologica* 1999; **84**: 405–412.
14 Borowitz MJ, Rubnitz J, Nash M, Pullen DJ, Cemitta B. Surface antigen phenotype can predict TEL-AML1 rearrangement in childhood B-precursor ALL: a Pediatric Oncology Group study. *Leukemia* 1998; **12**: 1764–1770.
15 Tabernero MD, Bortoluci AM, Alaejos I, López-Berges MC, Rasillo A, García-Sanz R, Sayagués JM, González M, San Miguel JF, Orfao A. Adult precursor B-ALL with BCR/ABL gene rearrangements displays a unique pattern of CD10, CD34, CD13 and CD38 expression that can be used to screen for this translocation. *Leukemia* 2001; **15**: 406–414.
16 Tien HF, Hsiao CH, Tang JL, Tsay W, Hu CH, Kuo YY, Wang CH, Chen YC, Shen MC, Lin DT, Lin KH, Lin KS. Characterization of acute myeloid leukemia with MLL rearrangements – no increase in the incidence of coexpression of lymphoid-associated antigens on leukemic blasts. *Leukemia* 2000; **14**: 1025–1030.
17 Baer MR, Stewart CC, Lawrence D, Arthur DC, Mrózek K, Strout MP, Davey FR, Schiffer CA, Bloomfield CD. Acute myeloid leukemia with 11q23 translocations: myelomonocytic immunophenotype by multiparameter flow cytometry. *Leukemia* 1998; **12**: 317–325.
18 Swansbury GJ, Slater R, Bain BJ, Moorman AV, Secker-Walker LM on behalf of the European 11q23 Workshop participants. Hematological malignancies with t(9;11)(p21-22;q23) – a laboratory and clinical study of 125 cases. *Leukemia* 1998; **12**: 792–800.
19 Satake N, Sakashita A, Kobayashi H, Maseki N, Sakurai M, Kaneko Y. Minimal residual disease in acute monocytic leukemia patient with trisomy 11 and partial tandem duplication of MLL. *Cancer Genet Cytogenet* 1997; **96**: 26–29.
20 San Miguel JF, Martínez A, Macedo A, Vidriales MB, López-Berges C, González M, Caballero D, García-Marcos MA, Ramos F, Fernández-Calvo J, Calmuntia MJ, Diaz-Mediavilla, Orfao A. Immunophenotyping investigation of minimal residual disease is a useful approach for predicting relapse in acute myeloid leukemia patients. *Blood* 1997; **90**: 2465–2470.
21 Martinez A, San Miguel JF, Vidriales MB, Ciudad J, Caballero MD, López-Berges MC, Moro MJ, Calmuntia MJ, Ortega F, Orfao A. An abnormal $CD34^+$ myeloid/$CD34^+$ lymphoid ratio at the end of chemotherapy predicts relapse in patients with acute myeloid leukemia. *Cytometry* 1999; **38**: 70–75.
22 Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick H, Sultan C. Proposed revised criteria for the classification of acute myeloid leukemia: a report of the French–American–British cooperative group. *Ann Intern Med* 1985; **103**: 620–626.
23 Cheson BD, Cassileth PA, Head DR, Schiffer CA, Bennet JM, Bloomfield CD, Brunning R, Gale RP, Grever MR, Keating MJ, Sawitsky A, Stass S, Weinstein H, Woods WG. Report of the National Cancer Institute-sponsored workshop on definitions of diagnosis and response in acute myeloid leukemia. *J Clin Oncol* 1990; **8**: 813–819.
24 Mitelman F. *ISCN (1995): An International System for Human Cytogenetic Nomenclature*. S Karger: Basel, 1995.
25 Bene MC, Castoldi G, Knapp W, Ludwing WD, Matutes E, Orfao A, van't Veer MB European Group for the Immunological Classification of Acute Leukemias. Proposals for the immunological classification of acute leukemias. *Leukemia* 1995; **9**: 1783–1786.
26 Muñoz L, López O, Martino R, Brunet S, Bellido M, Sierra J, Nomdedéu J. Minimal residual disease detection with immunophenotype and RT-PCR in Philadelphia positive acute lymphoblastic leukemia. *Hematologica* 2000; **85**; 40–46.
27 Bernard OA, Berger R. Molecular basis of 11q23 rearrangements in hematopoietic malignant proliferations. *Genes Chromos Cancer* 1995; **13**: 75–85.
28 Cimino G, Rapanotti MC, Sprovieri T, Elia L. ALL1 gene alterations in acute leukemia: biological and clinical aspects. *Haematologica* 1998; **83**: 350–357.
29 Mitterbauer G, Zimmer C, Fonatsch C, Haas OA, Thalhammer-Scherrer R, Schwarzinger I, Kalhs P, Jaeger U, Lechner K,

Maannhalter C. Monitoring of minimal residual leukaemia in patients with MLL-AF9 positive acute myeloid leukaemia by RT-PCR. *Leukemia* 1999; **13**: 1519–1524.

30 Cimino G, Elia L, Rapanotti MC, Sprovieri T, Mancini M, Cuneo A, Mecucci C, Fioritoni G, Carotenuto M, Morra E, Liso V, Annino L, Saglio G, De Rossi G, Foá R, Mandelli F. A prospective study of residual-disease monitoring of the ALL1/AF4 transcript in patients with t(4;11) acute lymphoblastic leukemia. *Blood* 2000; **95**: 96–101.

31 Schnittger S, Wörmann B, Hiddermann W, Griesinger F. Partial tandem duplications of the MLL gene are detectable in peripheral blood and bone marrow of nearly all healthy donors. *Blood* 1998; **92**: 1728–1734.

32 Macintyre E, Bourquelot P, Leboeuf D. MLL cleavage occurs in approximately 5% of *de novo* acute myeloid leukemia, including in patients analyzed before treatment induction. *Blood* 1997; **89**: 2224–2226.

33 Poirel H, Rack K, Delabesse E, Radford-Weiss I, Troussard X, Debert C, Leboeuf D, Bastard C, Picard F, Veil-Buzyn A, Flandrin G, Bernard O, Macintyre E. Incidence and characterization of MLL gene (11q23) rearrangements in acute myeloid leukemia M1 and M5. *Blood* 1996; **87**: 2496–2505.