

Fluorescence *in situ* hybridization study of TEL/AML1 fusion and other abnormalities involving TEL and AML1 genes. Correlation with cytogenetic findings and prognostic value in children with acute lymphoblastic leukemia

ANGEL MARTÍNEZ-RAMÍREZ,* MIGUEL URIOSTE,*
TRINIDAD CONTRA,^o ANGELES CANTALEJO,[#] AMANDIO TAVARES,^o
JOSÉ ANTONIO PORTERO,[^] BLANCA LÓPEZ-IBOR,[§]
MERCEDES BERNACER,^{**} CARLOS SOTO,^{**}
JUAN CRUZ CIGUDOSA,* JAVIER BENÍTEZ*

Correspondence: Angel Martínez-Ramírez, MD, Dpto. de Genética Humana, Programa de Patología Molecular, Centro Nacional de Investigaciones Oncológicas (CNIO), Ctra. Majadahonda-Pozuelo, Km. 2, 28220 Majadahonda, Madrid, Spain. Fax: international +34.9.15097055. E-mail: angmartinez@cnio.es

Background and Objectives. The *TEL/AML1* fusion is the most common genetic abnormality found in childhood acute lymphoblastic leukemias (ALL). Although it is very difficult to identify by conventional cytogenetic techniques it can be readily detected using fluorescence *in situ* hybridization (FISH). We carried out cytogenetic and FISH studies on 42 children with ALL in order to know the frequency of this translocation in our population, the incidence of *TEL* and/or *AML1* gene alterations, and their correlation with clinical evolution and prognosis. In addition, we performed reverse transcription polymerase chain reaction (RT-PCR) in some cases, confirming the feasibility of FISH techniques in the detection of this translocation.

Design and Methods. Bone marrow samples were obtained from 42 childhood ALL patients. The copy number of *AML1* and *TEL* genes were studied using fluorescent *in situ* hybridization with a dual color DNA probe specific for the *AML1* and *TEL* genes.

Results. We found a frequency of *TEL/AML1* fusion of 17% in our sample. Double *TEL/AML1* fusion, lack of *TEL* signal and extra *AML1* signals were frequent additional FISH abnormalities. Duplication of a chromosomal complement, deletion of chromosome 12p arm, and polysomies of chromosome 21 are plausible explanations for these additional FISH findings. However, a relatively high proportion of our cases (9.5%) presented specific amplification of *AML1*. A statistically significant difference in prognosis was found between patients with and without

haematologica 2001; 86:1245-1253

http://www.haematologica.it/2001_12/1245.htm

*Departamento de Genética Humana, Programa de Patología Molecular, Centro Nacional de Investigaciones Oncológicas (CNIO), Instituto de Salud Carlos III, Madrid: Spain. ^oDepartamento de Oncología, Hospital Niño Jesús, Madrid: Spain. [#]Servicio de Hematología, Hospital Infantil Gregorio Marañón, Madrid: Spain. ^oCentro Genética Clínica, Porto; Portugal. [^]Servicio de Hematología, Hospital Virgen de la Concha, Zamora; Spain. [§]Servicio de Hematología, Hospital de San Rafael, Madrid: Spain. ^{**}Servicio de Hematología, Fundación Jiménez Díaz (FJD), Madrid: Spain.

these additional *AML1* or *TEL* FISH alterations ($p < 0.02$), which could be related to the presence of specific karyotypes.

Interpretations and Conclusions. The frequency of *TEL/AML1* fusion is similar to that found in other populations (17%). We found that FISH analysis of *AML* and *TEL* is related to the evolution of the disease. The absence of alterations in these genes revealed by FISH could be indicative of bad prognosis, while the presence of alterations is related to a good evolution. Our results suggest that inter-phase FISH analysis to search for alterations in *AML* and *TEL* genes could be extremely useful for complementing cytogenetic studies and for providing additional information about the possible outcome of the disease in patients with ALL.

©2001, Ferrata Storti Foundation

Key words: acute lymphoblastic leukemia, *TEL/AML1* fusion gene, *AML1* amplification, t(12;21), fluorescence *in situ* hybridization.

The translocation 12;21, which involves the *TEL* gene on chromosome 12p13 and the *AML1* gene on chromosome 21q22, is associated with acute lymphoblastic leukemias (ALL), especially in children. Although it is difficult to identify by conventional cytogenetic techniques, *TEL/AML1* fusion can be readily detected using fluorescence *in situ* hybridization (FISH).^{1,2} FISH analysis has shown that *TEL/AML1* fusion is the

most common genetic abnormality in childhood ALL, being found in about 25% of cases. It also occurs in 3-4% of adult ALL cases.^{3,4,5} The translocation is associated with an early onset of the disease, a B-lineage immunophenotype, and a good prognosis.⁶

Extra cytogenetic abnormalities, associated with the t(12;21), are frequently found. The most common structural and numerical aberrations are 12p rearrangements and trisomy 21, respectively.⁷ A double fusion signal, detected by FISH, is another common finding in patients with t(12;21).^{8,9} Deletions involving the untranslocated TEL allele, detected by cytogenetic and/or FISH techniques, are also often found in patients with TEL/AML1 fusion.^{3,10,11,12,13} Although deletions on the short arm of chromosome 12 are recurrent alterations found in a wide range of hematologic neoplasias, the leukemogenic role of the genes located there, TEL and p27, is unknown.¹⁴

The AML1 gene is also involved in many chromosomal aberrations associated with hematologic disorders. More than 40 different patterns of translocations or rearrangements involving the 21q22 region have been described,¹⁵ and amplification of the 21q22 region in pediatric patients with ALL has also been detected by comparative genomic hybridization (CGH).¹⁶ Thus, the AML1 gene could have an important role in the 21q22 amplicon in childhood ALL.^{16,17}

The incidence of TEL-AML1 fusion ranges between 18 and 30% in B-lineage childhood ALL, and is similar in different populations.^{6,11,18-24} In marked contrast to these findings, a recent report has revealed a very low frequency (3%) of TEL-AML1 fusion in a Spanish population,²⁵ thereby suggesting that geographic differences exist.

Here we present cytogenetic and FISH studies carried out on a series of 42 pediatric patients with ALL at presentation. In our Spanish series, we found a frequency of TEL/AML1 fusion similar to that previously described in American, European and Oriental populations. Furthermore, we identified a high frequency of additional abnormalities of AML1 and/or TEL genes that can be correlated with the prognosis of the disease.

Design and Methods

Patients

Bone marrow samples were obtained from 42 children diagnosed as having acute lymphoblastic leukemia between 1995 and 2001. All patients were classified according to the French-American-British (FAB) criteria and treated by standard protocols (most

of them with the BFM95 protocol, the remaining with the BFM90 protocol).²⁶

Immunophenotypic studies

Flow cytometry immunophenotypic analysis was performed in all cases, using a variable panel of a large number of antibodies specific for the CD2, CD3, CD4, CD7, CD8, CD10, CD19, CD20, CD22, CD34, CD79, tdt and HLA-DR antigens and immunoglobulin κ light chains (Ig M and Ig S) (Dako A/S, Copenhagen, Denmark).

Conventional cytogenetics

Bone marrow samples were incubated in RPMI 1640 with 20% fetal calf serum for 1 day at 37°C. Cells were exposed to colcemid (0.1 μ g/mL) for 1.5 h at 37°C and harvested routinely. Metaphase chromosomes were GTG-banded by a conventional trypsin-Giemsa technique and karyotyped according to the International System for Human Cytogenetic Nomenclature.²⁷

Fluorescence in situ hybridization (FISH)

Chromosome spreads were prepared directly from the same samples used for conventional analysis and left overnight at room temperature. Slides were placed on a plate at 90°C for 10 min, dehydrated through a series of ethanol washes and denatured in the presence of the probe on a plate at 75°C for 1 min. The TEL/AML1 dual-color translocation FISH probe (Vysis, London, UK) was used for the detection of the TEL/AML1 rearrangement. At least 200 interphase nuclei were analyzed for each case. Positive and negative controls from the REH cell-line and five normal individuals, respectively, were used. Cell images were captured using a CCD camera (Photometrics SenSys camera) connected to a personal computer running the Chromofluor image analysis system (Applied Imaging Ltd.).

RNA preparation

Total RNA was prepared either by the guanidinium thiocyanate phenol-chloroform extraction method or by using the TRI REAGENT Kit (Molecular Research Centre, INC) according to the manufacturer's recommendations. One microgram of cell line RNA or 1-2 mg of patient RNA were reverse transcribed with 1.5 units of AMV Reverse Transcriptase (Promega Madison, USA). Following denaturation at 80°C for 5 min, the cDNA synthesis was carried out at 42°C for 60 min using random hexamers (Amersham Pharmacia Biotech Inc.) in a total volume of 40 μ L. Subsequently, the cDNA was heated to 94°C for 10 min to inactivate the reverse transcriptase and was then stored at -20°C.

Table 1. Clinical and cytogenetic data from 42 children with ALL at diagnosis.

No.	Clinical data							Cytogenetic data				
	Immunophenotype		Type	Sex/Age	Dx	CR	Survival	Conventional cytogenetics	FISH			
	B markers	T markers							TEL/AML1	TEL	AML1	
1	+	-	pre-B	F/13	09/83	Yes	159m	46,XX,t(9;22)(q34;q11)/46,XXn	n	n		
2	+	-	pre-B	M/13	05/96	No	18m	ND	n	n	n	
3	+	-	pre-B	F/10	05/96	Yes	18m	46, XX,t(9;22)(q34;q11)	n	n	n	
4	+	-	pre-B	F/6	06/96	Yes	44m +	46,XX	n	n	n	
5	+	-	pre-B	M/4	09/98	Yes	19m	46,XY,t(9;22)(q34;q11)/48-49,XY,t(9;22)(q34;q11),t(9;22)(q34;q11),+3-4mar	n	n	n	
6	+	-	pre-B	F/6	05/00	Yes	10m	+46,XX	n	n	n	
7	+	-	pre-B	M/5.9	09/00	Yes	12m +	46,XY	n	n	n	
8	+	-	pre-B	M/4	09/00	Yes	12m +	46,XY	n	n	n	
9	+	-	pre-B	M/6	09/00	Yes	12m +	46,XY	n	n	n	
10	+	-	pre-B	M/13	09/00	Yes	12m +	46,XY	n	n	n	
11	+	-	B	F/7	10/00	ND	ND	46,XX	n	n	n	
12	+	-	B	M/3.6	01/97	Yes	51m +	46,XY	n	n	n	
13	+	-	pre-B	M/3.3	03/01	Yes	7m +	46, XY,Bp-	n	n	n	
14	+	-	pre-B	F/4.8	03/01	Yes	12m +	46,XX	n	n	n	
15	-	+	T	M/10	01/95	Yes	10m	46,XY,+1,-5,-7,inv(3)(?/?),del(12)(p?),+mar/46,XY	n	n	n	
16	-	+	T	M/13	12/92	Yes	165m	50,XY,MAKA	n	n	n	
17	-	+	T	F/9	10/96	Yes	119m	46,XX,-19,+der(19)add(19)(p?)/46, XX	n	n	n	
18	-	+	T	M/5	11/00	Yes	11m +	46,X,-Y,-5,+der(1),+mar/46, XY	n	n	n	
19	+	-	pre-B	F/3.5	03/00	Yes	12m +	46,XX,t(X;3)(p?:q?)/46,XX	f	n	n	
20	+	-	pre-B	F/5.5	05/96	Yes	58m +	ND	f	-	n	
21	+	-	pre-B	M/13	04/97	Yes	19m	46,XY	f	-	n	
22	+	-	pre-B	M/4.6	11/98	Yes	14m +	45,XY,-D/46,XY	f	-	n	
23	+	-	pre-B	M/3.9	04/00	Yes	11m +	46,XY	f, f	+	n	
24	+	-	pre-B	M/5.3	08/00	Yes	9m +	46,XY	f, f	+	n	
25	+	-	pre-B	M/12	02/01	Yes	8m +	47,XY,t(2;14)(p11;q32),del(3)(q26),del(6)(q23),-10,11p+,12q+,-18,+21,+mar/46,XY	f, f	n	n	
26	+	-	pre-B	F/2	05/96	Yes	46m	+52-55,XX, ... /46, XX	n	n	+	
27	+	-	pre-B	F/6	09/96	Yes	42m+	ND	n	+	+	
28	+	-	pre-B	M/9	09/98	Yes	30m+	53-56,XY,+X,+5,+17,+4-7mar/46,XY	n	n	+	
29	+	-	pre-B	M/7	10/99	Yes	17m +	47,XY,+21/46,XY	n	n	+	
30	+	-	pre-B	M/2.9	05/00	Yes	10m +	54-57,XY,MAKA/46,XY	n	n	+	
31	+	-	pre-B	M/9	05/99	Yes	22m +	47,XY,+21/47,XY,+21,del(5)(p?)/46,XY	n	n	+	
32	+	-	B	M/8	05/00	ND	ND	70-80,XY, ... /46,XY	n	+	+	
33	+	-	B	M/4	10/00	ND	ND	ND	n	n	+	
34	+	-	B	M/2.6	11/00	ND	ND	55,XY,+X,+4,+6,+10,+11,+14,+18,+21,+21/46,XY	n	n	+	
35	+	-	pre-B	M/3	01/01	Yes	10m +	47,XY,+21/54-55,XY, ...	n	n	+	
36	+	-	pre-B	F/6	03/01	Yes	7m +	53-57,XY, ... /46,XX	n	-	+	
37	-	+	T	F/11.6	02/01	No	13m	46,XX,del(2)(p?)/47,XX,+?/46,XX	n	n	+	
38	+	-	pre-B	M/2.9	01/00	Yes	14m +	46,XY	n	n	+	
39	+	-	pre-B	F/3.4	07/00	Yes	8m +	46,XY	n	n	+	
40	+	-	pre-B	F/15	10/00	Yes	13m+	46,XX,add(1)(p?)/46,XX,add(1)(p?)del(6)(q25)/46,XX,del(6)(q25)/46,XX	n	n	+	
41	+	-	pre-B	M/15	12/95	Yes	14m	46,XY,Bq+	n	-	n	
42	-	+	T	F/13.7	01/99	Yes	26m +	45, XX,-D,Bq+/46,XX,Bq+	n	-	n	

ND: no data; n: normal; f: fusion; +: extra signals; -: lack of signals; MAKA: major karyotype abnormalities.

Molecular detection of *t(12;21)*

After cDNA synthesis a PCR was performed to detect chimeric transcripts derived from the translocation *t(12;21)*. All samples were analyzed using standardized primers, protocols and criteria. To verify the integrity of the isolated RNA and the correct synthesis of the cDNA, the ubiquitously expressed ABL gene was amplified in a separate PCR reaction. All assays were carried out using appropriate positive (cDNA from the REH cell line) and negative controls (cDNA from a healthy donor and H₂O). The PCR was carried out in two steps. The first round of the PCR was performed with the external primers, and the second one with reverse internal primers. Amplification was performed with a 9700 Perkin Elmer Thermocycler (Perkin Elmer, Germany).

In the first round of PCR, 2 μ L of cDNA were used. The PCR was carried out in a final volume of 20 μ L with 15 PCR-Buffer (Reaction Buffer 10 \times -Biotools B & M labs, S.A. Spain), 1.5 mM MgCl₂ (MgCl₂ 50 mM-Biotools B & M labs, S.A. Spain), 0.2 mM of each dNTP (Promega Madison, USA), 5 pmol of each primer (Gibco BRL), and 1 unit of DNA polymerase (Biotools B & M labs S.A., Spain). After an initial melting step (5 min at 94°C), 30 amplification cycles of 30 s at 94°C, 30 s at 60°C and 50 s at 72°C were performed, followed by an extension step of 7 min at 72°C. One microliter of the first round PCR product was subjected to the second round of PCR, differing in the final volume (25 μ L) and in the extension time (1 min at 72°C). Twenty microliters of the final PCR products were analyzed on a 3% agarose gel (MetaPhor agarose) and visualized by ethidium bromide staining.

Comparative genomic hybridization (CGH)

CGH was performed according to Kallioniemi *et al.*,²⁸ with some modifications. The tumor (test) and normal (reference) DNAs were labeled using the *nick translation* kit from Vysis. Six matched references were used to perform CGH for the patients and the normal and altered controls. Briefly, 200 ng of each labeled DNA were hybridized to normal male metaphase spreads in the presence of 15 mg of Cot-1 DNA, for 3 days. After washes, chromosomes were counterstained with DAPI in an antifade solution.

Digital image analysis and interpretation of CGH results

Slides were analyzed with an Olympus AX60 epifluorescence microscope equipped with a CCD camera (Photometrics SenSys camera). A minimum of 15 metaphases per hybridization per case were

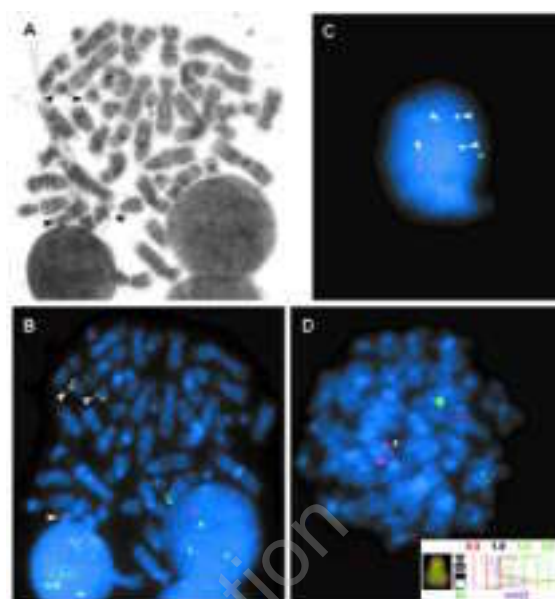


Figure 1. A representative metaphase from case #25; (A) GTG-banded and (B) DAPI-stained showing two fusion signals on derivative chromosome 21 and the residual signal from AML1 probe (red) on derivative chromosome 12. (C) Interphase nucleus with one copy of TEL (green signal), one copy of AML1 (large red signal), two fusion signals (yellow), and the two residual signals from the AML1 probe (small red signal). (D) Fluorescence *in situ* hybridization results on a metaphase from case 38 showing multiple signals for AML1 probe on one of the chromosomes 21 (red signals) (arrow), a single signal on the other chromosome 21 and two signals for TEL on both chromosomes 12. The insert image shows the comparative genomic hybridization profile of chromosome 21 from the same case, with the amplification threshold (1.5) exceeded.

analyzed using an image analysis system (Chromofluor image analysis system, Applied Imaging Ltd.) that could process three-color images (green for tumor DNA hybridization, red for normal reference DNA hybridization and blue for DNA counterstain). The green and red fluorescence intensities were calculated and the green-to-red ratio profiles along the chromosome axis were displayed. Ratios greater than 1.20 and less than 0.80 were taken to represent chromosomal gain and loss, respectively. Telomeric and heterochromatic regions were excluded from the analysis.²⁹

Statistical analysis

We used the two-tailed Fisher's exact test with a confidence interval of 95% to detect any significant associations between presence of abnormal FISH patterns and survival at the end of the report.

Table 2. Summary of FISH results obtained with TEL/AML1 probe and survival.

FISH AML1/TEL	Children	Survival ^a
normal FISH	18/42 (43%)	n=17; 59% alive
abnormal FISH	24/42 (57%)	n=20; 90% alive ^e
aml1/tel fusion:	7/42 (17%)	n=7; 85% alive ^e
tel deletion	3/42	
double fusion	3 ^b /42	
aml1 gains:	15/42 (36%)	n=12; 100% alive
polysomy	11/42	
gains	4/42	
tel alterations:	5/42 (12%)	n=4; 75% alive
deletion	3 ^c /42	
gains	2 ^d /42	

^aStatistical analysis normal/abnormal FISH ($p < 0.02$). ^bOne out of three also presented +21. ^cOne out of three also showed +21. ^dboth also presented +21. ^eCase #21 died of causes not related to the disease.

Results

Clinical data from the 42 pediatric patients are shown in Table 1. Thirty-six out of 42 samples had a B-immunophenotype and the remaining cases showed T-markers. The sex ratio was 1.6:1 (26 males and 16 females) and the patients' age ranged between 2 and 15 years (mean 7 years). All patients achieved complete clinical remission, except cases 2 and 37. Currently, 13 patients remain in complete remission, 10 are being treated, 9 have died and 6 cases have been recently diagnosed (2001). We have no data for four cases.

A conventional cytogenetic study was carried out in 38 patients. The cytogenetic results are given in Table 1. Fourteen patients (37%) showed a normal karyotype, while 24 cases (63%) presented different numerical and/or structural abnormalities. The t(12;21) was not observed in any case. A complex karyotype, with three or more alterations, was detected in 29% (7/24) of the patients; moreover, hyperdiploid karyotypes without structural alterations accounted for 16.5% (4/24). In addition, a partial deletion of 12p was detected in case 15. A chromosome Ph', t(9;22)(q34;q11), was identified in three patients (cases 1, 3 and 5).

FISH and RT-PCR studies

The FISH study with TEL/AML1 probe was carried out on all 42 samples. The results are shown in Tables 1 and 2. Eighteen cases (43%) showed a normal pattern of hybridization (two red and two green signals). TEL/AML1 fusion was detected in 7 patients (17%) but only one case had TEL/AML1 fusion as the only FISH abnormality (case 19); 3 other cases showed additional deletion of the nor-

mal TEL allele (cases #20, 21 and 22) and 3 cases presented double TEL/AML1 fusion (cases #23, 24 and 25).

Seventeen out of 42 samples (40%) had no TEL/AML1 fusion but presented other FISH anomalies involving AML1 or TEL signals. The most common abnormality was extra AML1 signals observed in 15 patients; 12 samples showed only extra AML1 signals and the other 3 had extra AML1 copies and an additional TEL signal (cases #27 and 32), or lacked a TEL signal (case #36). The increase of AML1 was associated with the gain of a chromosome 21 in 8 cases, but in the other four cases the patients presented a normal karyotype. The lack of a TEL signal without AML1 abnormalities was detected in 2 samples (cases #41 and 42). The correlation between cytogenetic and FISH results is shown in Table 1.

We were able to perform a RT-PCR specific for the amplification of the TEL/AML1 fusion transcript in eight samples. The results were positive in two patients (cases #24 and 25) and negative in the other six patients (cases #6, 7, 12, 18, 30 and 40). These results are in agreement with those obtained by FISH.

CGH results

CGH study was performed in one patient for whom the cytogenetic and FISH results were not in concordance. This case had a normal karyotype but presented extra (4 or 5) signals of the AML1 gene as revealed by FISH; CGH studies showed a specific amplification of the 21q22 region (case #38) (Figure 1D).

Clinical correlation

All patients with TEL/AML1 fusion achieved complete clinical remission; 3 cases presented a normal karyotype, 2 cases had single alterations and one had a complex karyotype (we obtained no cytogenetic results from case #20). In addition, one child died during the treatment of causes not related to the disease (case #21) and was excluded from the statistical analysis. On the other hand, we observed 17 cases with AML1 and TEL alterations as revealed by FISH. All cases achieved complete clinical remission, except one child (case #37). Thirteen cases showed an abnormal karyotype with numerical and structural alterations; two presented a normal karyotype and two were without cytogenetic results.

So, 24 patients had abnormalities as revealed by FISH and only 2 of them (8.5%) have died during the course of their disease (cases 37 and 41) (Table 2). Eighteen cases showed a normal pattern of FISH hybridization. All these cases achieved complete

clinical remission, except one child (case #2). Of these patients, 55% remain in complete remission, while the other 7 children have died (39%). These were patients whose karyotype showed either the Ph' chromosome, numerical and structural alterations and/or complex karyotypes. We have no data about one case (case #11).

Association of FISH results with clinical features

We compared the clinical evolution of two groups of patients selected on the basis of normal or abnormal FISH pattern for the TEL and AML1 genes. There was a statistically significant association between presence/absence of these alterations as revealed by FISH and the children's survival ($p < 0.02$).

Discussion

The t(12;21) is an alteration commonly associated with childhood ALL and is correlated with a good prognosis. It results in a fusion of the TEL and AML1 genes.^{2,10} Both genes are usually involved in other translocations in different types of leukemias,^{30,31} suggesting that they are deeply involved in the leukemogenesis process.

We performed a combined cytogenetic and FISH study on a group of ALL patients (42 children) in order to analyze the incidence of this translocation in our population and the correlation between the FISH and cytogenetic results. We found an incidence of TEL/AML1 fusion of 17% in our childhood cases. These results are consistent with those obtained by other groups in series of childhood ALL from USA,⁶ most European countries,^{8,11,18,21,23} Japan,^{19,24} Taiwan,²⁰ and Brazil.³² Our results differ from those obtained by Garcia-Sanz *et al.*²⁵ from another Spanish population, who found a low frequency (3%) of TEL/AML1 fusion in their series of 38 childhood ALL using FISH and RT-PCR. These different results could be better explained by technical rather than by true geographic differences.

In addition to these results, we found a high frequency of additional abnormalities involving TEL and/or AML1 in our series which can be explained in accordance with the cytogenetic results.

Patients with t(12;21)

We found 7 of the 42 children to be positive for the t(12;21). Conventional cytogenetic techniques revealed 3 cases with a normal karyotype (cases #21, 23 and 24), 2 cases with a single alteration (cases #19 and 22) and 1 case with a complex karyotype (case #25). One case yielded no cytogenetic data (case #20). Three of these 7 TEL/AML1 fusion-positive cases also showed deletions of the

TEL allele that had not been detected by conventional cytogenetic techniques (cases #20, 21 and 22). Deletion of the TEL gene has frequently been found by other authors.^{3,10,11,12,13} McClean *et al.*³³ suggested that this might result in a reduction of the presumed oncogenetic potential of the fusion gene, which could be abolished by loss of the normal TEL allele, providing a growth advantage to the cells containing the t(12;21). This could explain the high frequency of TEL allele deletions.

We also found three samples with double fusion signals. Two of these patients (cases #23 and 24) (Figure 1C) showed two fusion signals and two residual signals. These results could be explained by duplication of the der(12) and der(21) harboring the TEL/AML1 fusion or by triploidy following duplication of the abnormal complement.^{8,9} As the cytogenetic results of cases #23 and 24 were normal, it is probable that the altered line had a different division rate and, for this reason, all the analyzed metaphases were normal and the alterations were undetectable. In the third (case #25), the double fusion was the result of a duplication of the der(21)t(12;21) in a complex karyotype (Figures 1A-B). This was the only positive case in which a fusion with a complex karyotype was revealed by conventional cytogenetic techniques.

In two of the seven positive cases for which RNA was available, molecular study for the detection of the translocation t(12;21) confirmed the FISH results. In addition, six other cases were negative for the translocation by RT-PCR also in agreement with the FISH results. These results show a good correlation between both techniques for the diagnosis of t(12;21).

Patients with AML1 alterations

Fifteen out of the 42 children (35%) had extra copies of AML1. The most common origin of these gains is polysomy of chromosome 21, as we have demonstrated by conventional cytogenetics in 11 children and as has also been suggested by other authors.^{7,34-36} However, four of our patients had more than four copies of the AML1 gene without polysomy of chromosome 21; two patients (cases #38 and 39) had a normal karyotype and two patients (cases #37 and 40) had structural alterations different from t(12;21). In sample #38 the extra copies were located tandemly in a derivative chromosome 21 by a metaphase FISH study (Figure 1D). A CGH study of this case showed specific amplification of the 21q22 region, confirming the results obtained by the FISH study. Thus, the increase in copy number of AML1 in these patients

without polysomy of chromosome 21 could have occurred by intrachromosomal amplification. The increased number of copies of AML1, either as polysomies or as amplifications, indicates the importance of this gene in the leukemogenesis process.^{16,37}

Patients with TEL alterations

We observed gains and deletions of TEL signals in five cases. Two of these cases presented gains of TEL and each one additionally also had an extra AML1 signal (cases 27 and 32). In case #32 this finding could be explained by the presence of hyperploidy with polysomy in chromosomes 12 and 21. Cytogenetic results were not obtained in the other case. On the other hand, we had three cases with deletions of TEL signal. These deletions had not been observed by conventional cytogenetics, which highlights the importance of FISH for complementing conventional studies.

Clinical implications

Some studies have suggested that the t(12;21) is a marker related to a good prognosis,^{6,33} although other authors have obtained different results.^{3,38} Harbott *et al.*³⁹ published data suggesting that relapses occur later in t(12;21) positive patients, who have a longer median remission duration of up to 2 years after diagnosis. However, in all larger studies, a good prognosis could be demonstrated, whereas the percentage of treatment failures is higher in smaller studies.

In our series, most cases with the translocation t(12;21) remain alive (only one child positive for the fusion has died from complications arising from his treatment). The same occurred when the patients presented amplifications of AML1 or alterations of the TEL gene by FISH; they are currently alive and free of disease (91% of cases). In these cases, we usually observed normal cytogenetics, or single or numerical alterations. The identification of this type of alteration by interphase FISH could, therefore, be associated with a good evolution and prognosis. Conversely, 39% (7/18) of the patients with normal FISH died while they had the disease: three presented a Ph' chromosome and three others showed complex karyotypes with structural abnormalities. Thus, the absence of TEL and AML1 alterations revealed by FISH could be associated with a poor prognosis in childhood ALL because of the correlation with karyotypes linked to bad prognosis. A statistical analysis of normal FISH and abnormal groups showed significant differences ($p < 0.02$).

In summary, we found a frequency of t(12;21) in

children (17%) similar to that observed in other series. Our study comparing cytogenetic and FISH results showed that AML1 amplification is a frequent finding, related to polysomy of chromosome 21 in most cases; however, in 26.5% of children with AML1 amplification it is due to specific amplifications of this gene. Finally, we found that FISH analysis of AML and TEL is a characteristic that is closely related to the evolution of the disease. The absence of alterations in these genes revealed by FISH could be indicative of bad prognosis, while the presence of alterations is related to a good evolution. This is due to the type of cytogenetic alteration associated with these FISH results: Ph' chromosome and complex karyotypes in the case of normal FISH results, versus normal karyotypes, single or numerical alterations in the case of abnormal FISH results. Thus, our results suggest that interphase FISH analysis to search for alterations in AML and TEL genes could be extremely useful, a) for complementing cytogenetic studies, and b) for providing additional information about the possible outcome of the disease in patients with ALL.

Contributions and Acknowledgments

AMR: conception, design, FISH studies, literature search, analysis and interpretation of data, and drafting the article and revision; MU: conception, design, chromosome studies, interpretation of data and drafting the article; TC, AC, AT, JAP, BLI, MB and CS collection and clinical data; JCC: statistical analysis, interpretation of data and drafting the article; JB: conception and design, analysis and interpretation of data, revising the paper and final approval of the version to be submitted. The order for the authors reflects their intellectual contribution to the project.

We thank Blanca Fernández Martínez and Teresa López Jiménez for their technical assistance. Angel Martínez-Ramírez is a fellow of the Instituto de Salud Carlos III.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Prof. Giuseppe Saglio, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Prof. Saglio and the Editors. Manuscript received July 23, 2001; accepted October 12, 2001.

Potential implications for clinical practice

The availability of molecular methods to detect the most prognostic relevant chromosomal translocations in childhood ALL has suggested that genetic features of leukemic cells are used to define risk classification of the individual patient.⁴⁰ Interphase FISH analysis of AML1 and TEL gene abnormalities may be a very useful tool for this purpose.

References

- Romana SP, Le Coniat M, Berger R. t(12;21): a new recurrent translocation in acute lymphoblastic leukemia. *Genes Chromosomes Cancer* 1994; 9:186-91.
- Golub TR, Barker GF, Bohlander SK, et al. Fusion of the TEL gene on 12p13 to the AML1 gene on 21q22 in acute lymphoblastic leukemia. *Proc Natl Acad Sci USA* 1995; 92:4917-21.
- Raynaud S, Mauvieux L, Cayuela JM, et al. TEL/AML1 fusion gene is a rare event in adult acute lymphoblastic leukemia. *Leukemia* 1996; 10:1529-30.
- Aguiar RC, Sohal J, van Rhee F, et al. TEL/AML1 fusion in acute lymphoblastic leukaemia of adults. M.R.C. Adult Leukaemia Working Party. *Br J Haematol* 1996; 95:673-7.
- Kwong YL, Wong KF. Low frequency of TEL/AML1 in adult acute lymphoblastic leukemia. *Cancer Genet Cytogenet* 1997; 98:137-8.
- Shurtleff SA, Buijs A, Behm FG, et al. TEL/AML1 fusion resulting from a cryptic t(12;21) is the most common genetic lesion in pediatric ALL and defines a subgroup of patients with an excellent prognosis. *Leukemia* 1995; 9:1985-9.
- Raynaud SD, Dastugue N, Zoccola D, Shurtleff SA, Mathew S, Raimondi SC. Cytogenetic abnormalities associated with the t(12;21): a collaborative study of 169 children with t(12;21)-positive acute lymphoblastic leukemia. *Leukemia* 1999; 13:1325-30.
- Aguiar RC, Sohal J, van Rhee F, et al. TEL-AML1 fusion in acute lymphoblastic leukaemia of adults. M.R.C. Adult Leukaemia Working Party. *Br J Haematol* 1996; 95:673-7.
- Andreasson P, Hoglund M, Bekassy AN, et al. Cytogenetic and FISH studies of a single center consecutive series of 152 childhood acute lymphoblastic leukemias. *Eur J Haematol* 2000; 65:40-51.
- Romana SP, Mauchauffe M, Le Coniat M, et al. The t(12;21) of acute lymphoblastic leukemia results in a tel-AML1 gene fusion. *Blood* 1995; 85:3662-70.
- Cavé H, Cacheux U, Raynaud S, et al. ETV6 is the target of chromosome 12p deletions in t(12;21) childhood acute lymphocytic leukemia. *Leukemia* 1997; 11:1459-64.
- Kempski H, Chalker J, Chessells J, et al. An investigation of the t(12;21) rearrangement in children with B-precursor acute lymphoblastic leukaemia using cytogenetic and molecular methods. *Br J Haematol* 1999; 105:684-9.
- Kempski HM, Sturt NT. The TEL-AML1 fusion accompanied by loss of the untranslocated TEL allele in B-precursor acute lymphoblastic leukaemia of childhood. *Leuk Lymphoma* 2000; 40:39-47.
- Aissani B, Bonan C, Baccichet A, Sinnott D. Childhood acute lymphoblastic leukemia: is there a tumor suppressor gene in chromosome 12p12.3? *Leuk Lymphoma* 1999; 34:231-9.
- Mitelman F, Johansson B, Mertens F. eds. *Mitelman Database of Chromosome Aberrations in Cancer*; 2001. Available at URL: <http://cgap.nci.nih.gov/Chromosome/Mitelman>.
- Niini T, Kanerva J, Vetterranta K, Saarinen-Pihkala UM, Knuutila S. AML1 gene amplification: a novel finding in childhood acute lymphoblastic leukemia. *Haematologica* 2000; 82:362-6.
- Larramendy L, Huhta T, Vetterranta K, et al. Comparative genomic hybridization in childhood acute lymphoblastic leukemia. *Leukemia* 1998; 12:1638-44.
- Romana SP, Poirel H, Leconiat M, et al. High frequency of t(12;21) in childhood B-lineage acute lymphoblastic leukemia. *Blood* 1995; 86:4263-9.
- Nakao M, Yokota S, Horiike S, et al. Detection and quantification of TEL/AML1 fusion transcripts by polymerase chain reaction in childhood acute lymphoblastic leukemia. *Leukemia* 1996; 10:1463-70.
- Liang DC, Chou TB, Chen JS, et al. High incidence of TEL/AML1 fusion resulting from a cryptic t(12; 21) in childhood B-lineage acute lymphoblastic leukemia in Taiwan. *Leukemia* 1996; 10:991-3.
- Borkhardt A, Cazzaniga G, Viehmann S, et al. Incidence and clinical relevance of TEL/AML1 fusion gene in children with acute lymphoblastic leukemia enrolled in the German and Italian multicenter therapy trials. *Associazione Italiana Ematologia Oncologia Pediatrica and the Berlin-Frankfurt-Münster Study Group. Blood* 1997; 90:571-7.
- Spathas DH, Stewart J, Singer IO, Theriault A, Bovey M, Connor JM. Detection of t(12;21) in childhood acute lymphoblastic leukemia by fluorescence in situ hybridization. *Cancer Genet Cytogenet* 1999; 110:7-13.
- Trka J, Zuna J, Hrusak O, et al. Impact of TEL/AML1-positive patients on age distribution of childhood acute lymphoblastic leukemia in Czech Republic. *Pediatric Hematology Working Group in Czech Republic. Leukemia* 1998; 12:996-1007.
- Takahashi Y, Horibe K, Kiyoi H, et al. Prognosis significance of TEL/AML1 fusion transcript in childhood B-precursor acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 1998; 20:190-5.
- García-Sanz R, Alaejos I, Orfao JA, et al. Low frequency of the TEL/AML1 fusion gene in acute lymphoblastic leukaemia in Spain. *Br J Haematol* 1999; 107:667-9.
- Schrappé M, Reiter A, Zimmermann M, et al. Long-term results of four consecutive trials in childhood ALL performed by the ALL-BFM study group from 1981 to 1995. *Berlin-Frankfurt-Münster. Leukemia* 2000; 14:2205-22.
- ISCN. *Guidelines for cancer cytogenetics*. In: Supplement to an International System for Human Cytogenetic Nomenclature (F. Mitelman, ed.) Karger, Basel; 1995.
- Kallioniemi A, Kallioniemi OP, Sudar D, et al.

- Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. *Science* 1992; 258:818-21.
29. Kallioniemi OP, Kallioniemi A, Piper J, et al. Optimizing comparative genomic hybridization for analysis of DNA sequence copy number changes in solid tumors. *Genes Chromosomes Cancer* 1994; 10:231-43.
 30. Nucifora G, Rowley JD. AML1 and the 8;21 and 3;21 translocations in acute and chronic myeloid leukemia. *Blood* 1995; 86:1-14.
 31. Wlodarska I, La Starza R, Baens M, et al. Fluorescence in situ hybridization characterization of new translocations involving TEL(ETV6) in a wide spectrum of hematologic malignancies. *Blood* 1998; 91:1399-406.
 32. Magalhaes IQ, Pombo-de-Oliveira MS, Bennett CA, et al. TEL/AML1 fusion gene frequency in paediatric acute lymphoblastic leukaemia in Brazil. *Br J Haematol* 2000; 111:204-7.
 33. McClean TW, Ringold S, Neuberg D, et al. TEL/AML1 dimerizes and is associated with a favorable outcome in childhood acute lymphoblastic leukemia. *Blood* 1996; 88:4252-8.
 34. Berger R. Acute lymphoblastic leukemia and chromosome 21. *Cancer Genet Cytogenet* 1997; 94:8-12.
 35. Loncarevic IF, Roitzheim B, Ritterbach J, et al. Trisomy 21 is a recurrent secondary aberration in childhood acute lymphoblastic leukemia with TEL/AML1 gene fusion. *Genes Chromosomes Cancer* 1999; 24:272-7.
 36. Elghezal H, Le Guyader G, Radford-Weiss I, et al. Reassessment of childhood B-lineage lymphoblastic leukemia karyotypes using spectral analysis. *Genes Chromosomes Cancer* 2001; 30:383-92.
 37. Dal Cin P, Atkins L, Ford C, et al. Amplification of AML1 in childhood acute lymphoblastic leukemias. *Genes Chromosomes Cancer* 2001; 30:407-9.
 38. Lanza C, Volpe G, Basso G, et al. Outcome and lineage involvement in t(12;21) childhood acute lymphoblastic leukemia. *Br J Haematol* 1997; 97:460-2.
 39. Harbott J, Viehmann S, Borkhardt A, Henze G, Lampert F. Incidence of TEL/AML1 fusion gene analyzed consecutively in children with acute lymphoblastic leukemia in relapse. *Blood* 1997; 90:4933-7.
 40. Biondi A. Molecular diagnosis and monitoring of ALL: applications and limitations. *Haematologica* 2000, EHA 5 Educational Book, p. 98. Available from URL: http://www.haematologica.it/free/eha5_edu_biondi.pdf

©Ferrata Storti Foundation