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Partial deletions of long arm of chromosome 6: biologic and clinical implications in adult acute lymphoblastic leukemia

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Within 285 adult acute lymphoblastic leukemias (ALL) included in the multicenter GIMEMA 0496 trial and prospectively studied by conventional cytogenetics, 18 cases (6%) with long arm deletion of chromosome 6 (6q) were identified. These cases were divided into: (i) del(6q) only (*n* = 6); (ii) del(6q) plus other numerical and/or structural abnormalities (*n* = 8); (iii) del(6q) and other 'specific' translocations (*n* = 4). The biologic and clinical features of the patients carrying this anomaly, as well as their outcome, were compared with those of 267 patients without del(6q). A T cell phenotype was more frequently associated with del(6q) cases in general (*P* = 0.001) and particularly with cases presenting del(6q) as the isolated abnormality (*P* = 0.0027). No significant difference with respect to multidrug resistance (MDR)/P glycoprotein expression was observed between the two groups of patients (21% *vs* 28% of MDR-positive cases, respectively). A *BCR-ABL* fusion transcript was less frequently detected in cases with del(6q) (11%) compared with those without the anomaly (29%). p15 and p16 deletions were identified by Southern blot analysis in 21% of cases with del(6q) and in 26% of cases without del(6q). In this latter group, a T cell phenotype was less frequently associated with p15 and/or p16 deletion than in the group carrying del(6q) (36% *vs* 100% of cases, *P* = 0.011). Overall, patients with ALL and del(6q) had a high complete remission (CR) rate (83%); however, they had a lower 18 month event-free survival (31% *vs* 41%) and a higher relapse rate (70% *vs* 37%, *P* = 0.02) compared with patients without del(6q). To date, this is the largest series of adult ALL cases reported with del(6q) homogeneously treated, which have also been prospectively studied for MDR expression and for the detection of known fusion genes. This anomaly, as an isolated change, identifies a subset of cases with hyperleukocytosis (median WBC count 52 × 10⁹/l) and a strict correlation with a T cell phenotype. Overall, del(6q) seems to be associated with an unfavorable clinical outcome, although this finding will need to be confirmed by extended FISH analysis.
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Introduction

Cytogenetic abnormalities are frequently detected in patients with acute lymphoblastic leukemia (ALL). Within the different specific structural changes identified, some, eg t(9;22) and t(4;11) in B-lineage ALL, depict biologically distinct prognostic entities, while for others, less frequently observed, a relevant prognostic impact is not well defined.[1]

Deletions of the long arm of chromosome 6 (6q) have been described mainly in childhood ALL, where they account for 5–15% of cases and identify a subset of patients showing a T or B cell immunophenotype and a prognostic likelihood similar to that of cases with no detectable cytogenetic abnormality.[2–4] The detection of a del(6q), which is usually associated with other karyotypic abnormalities and is, less frequently, the sole chromosomal abnormality, is probably underestimated by conventional chromosome banding if the loss of material involves a small region. In adult ALL, del(6q) occurs somewhat less frequently than in children and seems to preferentially correlate with a T cell phenotype; this abnormality does not appear to be associated with an unfavorable prognosis.[5–7] In this category of patients, however, too few cases have been reported to date to allow definitive clinical and biologic correlations.

We hereby report 18 cases with del(6q) identified by conventional cytogenetics in a series of 285 adult ALL included in the GIMEMA (Gruppo Italiano Malattie EMatologiche dell'Adulto) 0496 trial and prospectively studied. The biologic and prognostic implications of this abnormality are discussed and correlated with other karyotypic abnormalities and phenotypic parameters.

Materials and methods

Patients

Of 285 adult patients with *de novo* ALL successfully analyzed by cytogenetics as part of a prospective GIMEMA study (ALL 0496) between October 1996 and September 2000, 18 patients (6%) were found to have del(6q) either as an isolated cytogenetic anomaly or associated with other structural and/or numerical changes. The multicenter study ALL 0496 protocol required a central handling and analysis of bone marrow (BM) samples at presentation, that also included molecular and multidrug resistance (MDR) studies. All samples were sent overnight to the laboratories of the Department of Cellular Biotechnologies and Hematology, University 'La Sapienza' of Rome; the specific investigations were performed at the same center, at the Department of Clinical and Biological Sciences, 'SL Gonzaga' Hospital, Orbassano, University of Turin, at the Hematology and Bone Marrow Transplantation Unit, Univer-

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sity of Perugia, at the Department of Biochemistry and Medical Biotechnologies, University 'Federico II' of Naples and at the Department of Biomedical Science, Hematology Unit, University of Ferrara.

All patients were treated according to the GIMEMA ALL 0496 protocol, which is derived from the ALLVR589 regimen[8] and included patients aged 16–60 years with a diagnosis of ALL, with the exclusion of L3 B-ALL. The protocol design is based on an induction period (daily prednisone (PDN) for 1 month, high-dose daunorubicin (DNM) (90 mg/m^2 for three doses), weekly vincristine (VCR) (five doses), asparaginase (L-ASE) (10 doses)); consolidation (cytarabine (Ara-C) (2 g/m^2/12 h for 2 days) and etoposide (VP-16) (150 mg/m^2/day for 3 days)); chemo- (intrathecal methotrexate (i.t. MTX)) and radio- (18 Gy) prophylaxis of CNS involvement; maintenance over a 3 year period (daily 6 mercaptopurine (6-MP), weekly i.m. MTX plus periodical re-inductions (monthly for 6 months, then at months 9, 12, 18, 24, 30, 36) alternating cycles A (DNM, VCR and PDN) and B (cyclophosphamide (CTX), VCR and PDN)). The GIMEMA 0496 protocol also provided a centralized collection of BM samples of the relapsed cases.

Cytology and immunology

The diagnosis of ALL was established according to standard morphocytochemical and immunophenotypic criteria,[9,10] and the cases were grouped in B- and T-lineage ALL.

Cytogenetic analysis

Cytogenetic analyses were performed on direct and 24 h cultured preparations of bone marrow cells without stimulation. GTG banding chromosomes were classified according to the International System for Human Cytogenetic Nomenclature (ISCN, 1995).[11] At least 10 metaphases were required to consider the case evaluable. A central karyotype review was also set up.

DNA analysis

High molecular weight DNA was extracted from Ficoll–Hypaque isolated mononuclear cells, digested to completion with *Eco*RI, *Hin*dIII, or *Bgl*II restriction endonucleases, size-fractioned by electrophoresis through 0.8% agarose gels, denatured and transferred to nitrocellulose membranes. Prehybridization and hybridization with p15 and p16 denatured probes were performed as already reported.[12,13] Filters were washed at room temperature for 10 min in 0.2 × SSC/0.1% sodium dodecyl sulfate (SDS) and at 50°C for 15 to 20 min in the same solution. The filters were then exposed for 2 to 8 days at –80°C for autoradiography using intensifying screens.

RT-PCR

Total RNA was extracted from BM or peripheral blood mononuclear cells collected at diagnosis and cryopreserved in guanidium isothiocyanate, according to the method of Chomczynski and Sacchi.[14]

Multiplex RT-PCR

We used a multiplex RT-PCR system, derived by the method described by Pallisgaard *et al*.[15] This method has been modified to identify the most frequent fusion genes in ALL (ie *MLL-AF4, MLL-ENL, BCR-ABL* p190 (e2a2) and p210 (b2a2,b3a2) isoforms, *E2A-PBX1, TEL-AML1*). To achieve maximal sensitivity, the method consisted of a nested PCR protocol in which mRNA was retrotranscribed using oligonucleotides specific to all the above cited chimeric genes. In brief, 1 μg of total RNA was incubated at 65°C for 5 min with specific c-DNA primers (2.5 pmol of each) and then reverse transcribed by incubation at 37°C for 45 min in a total volume of 25 μl containing 20 U RNase inhibitor (Boehringer, Mannheim, Germany), 1 mmol/l of each dNTP, 10 mmol/l dithiothreitol, 50 mmol/l Tris-HCl, pH 8.3, 75 mmol/l KCl, 3 mmol/l $MgCl_2$, and 400 U Moloney murine leukemia virus reverse transcriptase (BRL, Bethesda, MD, USA). After the incubation, the cDNA reaction mixture was diluted with ddH_2O to 50 μl. As a first step of our RT-PCR multiplex assay, two reaction tubes were set up to amplify the *BCR-ABL* p190 (e2a2) and p210 (b2a2,b3a2) isoforms, *ALL1-AF4*, *ALL1-ENL*, (tube No. 1) and *E2A-PBX1*, *TEL-AML1* (tube No. 2). Five μl of diluted cDNA reaction were added to each of the two 20 μl multiplex mixtures, containing 11 mmol/l Tris-HCl pH 8.3, 55 mmol/l KCL, 1.65 mmol/l $MgCl_2$, 0.2 mmol/l of each DNTP a mixture of oligonucleotide primers (5 pmol of each primer) and 1.5 U AmpliTaq-Gold polymerase (Applied Biosystem, Branchburg, NJ, USA). After an initial activation of the polymerase at 95°C for 15 min, 25 cycles of PCR amplification were done followed by 20 cycles of PCR amplification of nested RT-PCR, as previously reported.[15] Fifteen μl of each PCR reaction were electrophoresed in a 1.5% agarose gel for 60 min at 100 V and stained with ethidium bromide. Negative controls without DNA template were included for all PCR reaction mixtures.

Split-out PCR

Because each multiplex reaction identifies a number of translocations, to determine and verify a fusion gene in a positive multiplex reaction we therefore performed split-out analysis using individual primer sets. The split-out was performed using the same reaction conditions as for the multiplex PCR.

Negative controls without DNA template were included for all PCR reaction mixture.

Multidrug resistance (MDR)

MDR1 expression was measured by flow cytometric test detecting P-gp expression, as already reported.[16] Flow cytometric analysis was conducted using a FACScan (Becton Dickinson, Mountain View, CA, USA) operated at 488 nm which detects red (PE-conjugated streptavidin) fluorescence. Data acquisition and analyses were performed using a specific software (Becton Dickinson).

Expression of the P-gp (MDR-1) was performed on gated leukemic blasts by using the Kolmogorov–Smirnov statistic method (D-value), which measures the differences between two distribution functions and generates a value ranging from 0 to 1.0. A value of 0 was recorded in cases in which the fluorescence of the control cells was brighter than that of the antibody-stained cells.

Statistical methods

Clinical and laboratory features of cases divided into two different groups (i and ii+iii) were compared using the Student's *t*-test or the chi-square test, when appropriate. Event-free survival (EFS) was defined from the date of diagnosis to the first event or last follow-up. Events included induction failure (non-response to therapy or death during induction), leukemic relapse at any site, death of any causes, whichever occurred first. Continuous complete remission (CCR) was calculated from the date of CR achievement to leukemic relapse at any site or last follow-up. EFS and CCR probabilities were calculated using the Kaplan–Meier method.

Results

The 18 patients with del(6q) were grouped into three categories: (i) isolated del(6q) (six cases); (ii) del(6q) associated with other numerical and/or structural chromosomal abnormalities (eight cases); (iii) del(6q) combined with another ALL 'specific' chromosomal abnormality, such as t(9;22), t(4;11), t(1;19) and t(10;14) (four cases). The del(6q) breakpoint, defined in 17/18 cases, encompassed the 6q21-q23 region in 11, 6q25-q27 in four and was in a more centromeric region in two. Among del(6q) cases with additional karyotypic aberrations (groups ii+iii), these were of structural and numerical type in six instances and of structural type in only the remaining six. In 3/12 cases these consisted of B-lineage specific changes, namely t(9;22), t(1;19) and t(4;11), and in 1/12 cases of a T-lineage-specific translocation, namely t(10;14).

The biologic and clinical features of the three patient groups at presentation and the overall outcome are reported in Table 1; the biologic and clinical features of del(6q) patients compared with cases without del(6q) are summarized in Table 2. The median age of del(6q) patients (24.5 years, range 16–47) was younger than that of patients without del(6q) (30.7 years, range 14–60.1). The median WBC count of the 18 patients was 14×10^9/l (range 0.5–274); patients in group i had higher WBC counts than those in groups ii+iii (median 52×10^9/l *vs* 14×10^9/l). The immunophenotypic characterization showed a T-lineage ALL in 6/6 cases of group i and in 3/12 cases in groups ii+iii. While the WBC count did not differ significantly between group i and groups ii+iii ($P = 0.34$), the T cell phenotype resulted in being significantly associated with group i patients ($P = 0.0027$). Overall, patients with del(6q) had a greater likelihood of having a T-lineage ALL than patients lacking this anomaly ($P = 0.001$).

DNA analysis of the p16 and/or p15 locus were investigated in 14/18 cases; homozygous and/or hemizygous deletions were observed in 2/5 cases in group i and in 1/8 case in group ii. RT-PCR analysis revealed a *BCR-ABL, E2A-PBX1, MLL-AF4* fusion transcript in the three cases of group iii which also carried the corresponding chromosome translocations. A BCR/ABL rearrangement was also detected in one case of group ii. No fusion gene was detected in the remaining 14 cases.

Detection of MDR1/Pgp was investigated in 14/18 and was found to be overexpressed in three.

Overall, 15/18 patients (83%) achieved CR after induction therapy. Two patients, one in group i and one in group ii, were resistant to induction treatment and one patient in group i died during induction. Of the 15 CR patients, in two it is too soon for follow-up (Nos 6 and 15) and 13 have an adequate follow-up. Two patients in group i and two in group ii (30%) remain in CR at 46, 26, 49 and 28 months from diagnosis, while nine patients (70%), one in group i, five in group ii and three in group iii, have relapsed at a median time of 5 months from diagnosis. Overall, patients with deletion of chromosome arm 6q had a significantly higher likelihood of relapse than patients without del(6q) ($P = 0.02$). Among patients with del(6q), the 18 months EFS and CCR probabilities were 31% (95% CL: 8.5–54%) and 38% (95% CL: 12–64.9%), respectively (Figure 1). Among patients without del(6q), the 18 months CCR and DFS probabilities were 41% and 53%, respectively (Table 2).

Chromosome analysis was also performed at the time of first relapse in a series of 50 patients and proved to be evaluable in 40 (80%). A del(6q) was detected in six cases (15%); of these three (Nos 3, 16 and 18) already carried the abnormality at presentation, while the remaining three cases displayed the anomaly only at the time of relapse (Table 3).

Discussion

In this prospective study of 285 adults with cytogenetically characterized *de novo* ALL, 18 patients presented del(6q), either as the only clonal chromosome abnormality or in conjunction with other changes. To our knowledge, this is the first report in which cases with this anomaly were also studied for MDR expression and molecular abnormalities, with the aim of better defining the biological and prognostic significance of del(6q) in adult ALL. The frequency of del(6q) in our series (6%) is similar to that (4–6%) observed in previous studies.[5,6] However, these data might not reflect the true frequency of this anomaly, because poor morphology of ALL chromosomes, on the one hand, and occurrence of small interstitial deletions beyond the sensitivity of conventional cytogenetics, on the other hand, may cause underestimation. Molecular and FISH analyses performed mostly in childhood ALL have overcome these drawbacks, showing 6q deletions in up to 30% of investigated cases.[17] At least two regions, 6q21–23 and 6q25–27, have been identified by molecular tools as being frequently deleted, the former in ALL and the latter in non-Hodgkin's lymphoma, and a number of candidate tumor suppressor genes have been localized in these areas.[17–20] In our series, of the 17 cases in which we determined the breakpoint, 11 (65%) showed deletions that encompassed the 6q21-q23 region, while four showed a breakpoint distal to 6q23. This finding is in agreement with that by Zhang *et al*,[20] who showed that deletions in 6q21 occur preferentially in ALL and high grade non-Hodgkin lymphomas. In childhood ALL, recurrent breakpoints appeared less clustered than in adults; nonetheless, most reported deletions include the region 6q21.[4]

Previous published studies have suggested that del(6q) have a preferential association with a T cell phenotype in adult patients; this correlation strongly emerges from the present study, in which half of del(6q) patients had T-lineage ALL, as compared with 20% for patients without a del(6q) ($P = 0.001$).[5,6] No differences have emerged in previous reports between cases carrying the anomaly as the isolated change and cases with del(6q) plus other cytogenetic aberrations. According to the presence of additional karyotypic abnormalities, we subdivided our 18 cases into three categories, which allowed different peculiarities to be highlighted. As reported in pediatric series, cases with isolated del(6q) (group i) tend to have a higher median WBC count compared to groups ii+iii (52 *vs* 14×10^9/l). Notably, cases in group i showed a stringent

Table 1 Biological and clinical data of 18 patients with del(6q)

Patient	*Age*	*WBC (10^9/l)*	*Karyotype [No. abnormal metaphases]*	*Chimeric transcript type*	*p15/p16 status*	*Immuno phenotype*	*MDR*	*Response to induction*	*Rel*	*Outcome*
Group i										
1	17	143.0	46,XY,del(6)(q21) [16]	none	wt	T	no	res	—	died
2	23	0.5	46,XY,del(6)(q23) [10]	none	wt	T	ND	CR	no	CCR (+46 mo)
3	38	13.7	46,XY,del(6)(q21q26) [5]	none	wt	T	no	CR	yes	alive in II CR
4	18	90.0	46,XY,del(6)(q21q24) [4]	none	p15 p16	T	no	NE	no	died
5	35	135.0	46,XY,del(6)(q15) [5]	none	p16	T	no	CR	no	CCR (+26 mo)
6	26	13.0	46,XY,del(6)(q21q23) [6]	none	wt	T	pos	CR	no	too early
Group ii										
7	31	2.0	46,XY,del(6)(q12q15),del(7)(q33q35) [19]	*BCR-ABL*	ND	B	pos	CR	yes	died
8	21	35.0	46,XY,+1,t(1;16)(q32p11),del(6)(q21),i(14)(q10),–22 [10]	none	wt	B	no	CR	yes	died
9	31	86.0	46,XY,del(6)(q12q14),del(8)(q11q13) [6]	none	p15p16	T	no	CR	yes	died
10	35	5.0	43,XX,del(6)(q?q?),–10,–13,–14 [15]	none	ND	B	no	CR	yes	alive in II CR
11	38	5.5	46,XY,del(6)(q24)/48,idem,+2m [6]	none	ND	B	ND	CR	no	CCR (+49 mo)
12	23	13.0	47,XY,del(6)(q24),i(7)(q11),add(14)(q31),+m [11]	none	ND	T	ND	res	—	died
13	47	33.0	46,XY,del(5)(p13),del(6)(q24) [8]	none	wt	B	no	CR	yes	died
14	21	15.0	55–62,XXX,add(1)(q34),add(4)(q35),+5,del(6)(q15q21),+7, +8,+9,+10,+11,+12,add13(q34),+15,+16,+17,+17,+18,+19, +20,+21,+22,+m [cp 9]	none	wt	B	no	CR	no	CCR (+28mo)
Group iii										
15	19	14.8	46,XY,del(6)(q15q23),t(10;14)(q24;q11),del(6)(q24) [14]	none	wt	T	ND	CR	no	too early
16	38	274.0	46,XY,t(4;11)(q21;q23),del(6)(q24) [10]	*MLL-AF4*	wt	B	pos	CR	yes	died
17	23	2.3	47,XY,del(6)(q15q21),t(9;22)(q34;q11),+der(22)t(9;22) [5]	*BCR-ABL*	wt	B	no	CR	yes	died
18	16	8.8	46,XY,add(6)(q21),del(6)(q13),der(19)t(1;19)(q23;p13) [14]	*E2A-PBX1*	wt	B	no	CR	yes	alive in II CR

wt, wild type; ND, not done; CR, complete remission; MDR, multidrug resistance; res, resistant; NE, not evaluable; CCR, continuous complete remission.

Table 2 Biologic and clinical features of adult patients with and without del(6q)

	Median age	Median WBC ($\times 10^9$/l)	Immunophenotype			p15/p16 % del	BCR/ABL % pos	MDR % pos	RC	Relapse		EFS (18 mos)	CCR (18 mos)
			T	B	P value					%	P value		
Patients with del (6q) (18 cases)	24.5	14.0	9	9	0.001	21	11	21%	83%	70%	0.02	31%	38%
Patients without del(6q) (267 cases)	30.7	15.0	48	219		26	29	28%	76%	37%		41%	53%

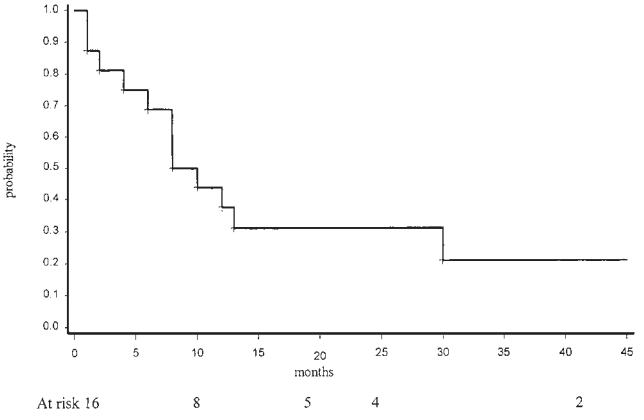

Figure 1 Event-free survival (EFS) for adult ALL patients with del(6q) at presentation.

association with a T cell phenotype (6/6 cases), while cases in groups ii+iii preferentially showed a B-lineage ALL (9/12 cases) ($P = 0.0027$). Therefore, the association of del(6q) with additional cytogenetic and genetic changes seems to drive the leukemic proliferation towards a B immunophenotype, especially if they consist of B-lineage specific translocations such as t(4;11) or t(9;22) (no T-lineage ALL among the three cases in group iii). No differences in terms of type of deletion emerged comparing the group with isolated del(6q) and those with the other cytogenetic categories. RT-PCR identified *BCR-ABL* (two cases), *E2A-PBX1* (one case), *AF4-MLL* (one case) fusion transcripts in groups ii+iii only; overall, a *BCR-ABL* fusion transcript was less frequently detected in cases with del(6q) compared with those without this anomaly (11% *vs* 29%). Molecular analysis revealed one case with *TEL-AML1* fusion gene among patients without del(6q).

Southern blot detected p15 and/or p16 deletions in 3/14 (21%) investigated cases with del(6q) and in 42/163 (26%) cases without 6q deletion. All three p15/p16 deleted cases in the del(6q) group showed a T cell phenotype, while T-lineage ALL was associated with p15/p16 loss in 36% of cases without del(6q) ($P = 0.011$).

Prognosis of adult ALL patients with del(6q) is still debated. Two large studies on cytogenetics of adult ALL did not associate del(6q) with an unfavorable prognosis compared with cases without 6q changes. The Groupe Français de Cytogénétique Hématologique identified 27 cases (6%) with del(6q) among 443 ALL patients treated with an induction regimen including an association of standard doses of anthracyclin, vincristine, prednisone plus cyclophosphamide or L-asparaginase[5]. Allogeneic or autologous BMT in first CR was performed as consolidation therapy on 40% of the cases. Patients with del(6q) had a CR rate of 88%, a median EFS of 11 months and a 3-year EFS of 47%. The MRC UKALL XA[6] identified 15 cases (6%) with del(6q) among 350 cases receiving as induction therapy standard doses of daunorubicin, vincristine, L-asparaginase, prednisone and one or two additional intensification therapies (5-day course of cytarabine, etoposide and thioguanine with two doses of daunorubicin). A CR rate of 87%, a median disease-free survival (DFS) of 23 months and a 3-year DFS of 27% were observed. Different data emerge from the study of Walters *et al*,[21] in which 105 patients with ALL were treated with VAD (vincristine, adriamycin and

Table 3 Biologic data of six patients with del(6q) at relapse, three with the abnormality at diagnosis and three without

	Karyotype		Phenotype
	Diagnosis	Relapse	
Cases with del(6q) at diagnosis			
3	46,XY,del(6)(q21q26)	46,XY/46,XY,del(6)(q21q26)	T
16	46,XY,t(4;11)(q21;q23), del(6)(q24)	48,XXY,t(4;11)(q21;q23),+del(6)(q21)/ 48,idem,del(5)(q22)	B
18	46,XY,add(6)(q21),del(6)(q13), der(19)t(1;19)(q23;p13)	49,XY,del(3)(p22),–4,add(6)(q21),del(6)(q13), +del(6)(q13),+7,add(9)(q34),–10,add(11)(p15), der(19)t(1;19)(q23;p13),+3m/49,idem,add(1)(q44)	B
Cases without del(6q) at diagnosis			
	46,XY	46,XY,dup(1)(p12p22),del(6)(q15),del(10)(p12)	T
	47,XY,+13	47,XY,+13/47,XY,idem,del(6)(q21q23)/ 47,XY,idem,add(17)(q21q23)	B
	46,XY	47,XY,+8/47,XY,idem,del(6)(q21q?)	B

dexamethasone) and VAD plus cyclophosphamide as induction therapy, followed by a 2-year rotating maintenance program including adriamycin and high-dose cytarabine and a late intensification with cyclophosphamide, carmustine, and etoposide followed by autologous BMT. Cases with del(6q) (4%) were included, together with those having t(9;22) and 14q+ abnormalities, in an unfavorable prognostic group (CR rate of 65%, median response duration of 7 months, 3-year survival rate of less than 10%). Consistent with the findings of Walters *et al*,[21] our data, from a cohort of patients treated with an identical therapeutic regimen, indicate that patients with del(6q), in spite of the high percentage of CR (83%), tended to have lower EFS and CCR probabilities (31% and 38% *vs* 41% and 53%, respectively) (Figure 1) than patients without del(6q). Moreover, the relapse rate of del(6q) patients was significantly different to that of patients without this anomaly (70% *vs* 37%, $P = 0.02$) and it remained higher, although not significantly, even excluding from the del(6q) patient group the four cases with poor prognosis rearrangements (55% *vs* 38%). Although derived from a limited number of cases, these data, should require a reassessment of the risk associated with this karyotypic change.

Karyotypic analysis could be re-assessed in three cases at first relapse (Nos 3, 16, 18) and in all the abnormality was found; interestingly, while cases 16 and 18 (group iii and a B cell phenotype) presented a clonal evolution, the karyotype of case 3 (group i and a T cell phenotype) did not change, further supporting the strict correlation between isolated del(6q) and T cell phenotype.

In the framework of a centralized re-evaluation of the biologic features of all relapsing cases entered in the ALL 0496 protocol, a del(6q) could be identified in 6/40 (15%) cases studied at first relapse, further suggesting that this anomaly may indeed be more frequent than detected by conventional cytogenetic.

In conclusion, del(6q) when present as isolated change, has to be considered as a specific anomaly that defines a subset of adult ALL patients with peculiar features (T cell phenotype, high WBC count). Futhermore, in light of the results of the present study based on the largest series of adult ALL patients with del(6q) treated homogeneously so far reported, the prognostic significance of this anomaly should be reassessed. The exact incidence and clinical impact of del(6q) will be conclusively defined through extended FISH analysis.

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