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Deletions of the long arm of chromosome 5 define subgroups of T-cell acute lymphoblastic leukemia

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Haematologica 2016
Volume 101(8):951-958

ABSTRACT

Recurrent deletions of the long arm of chromosome 5 were detected in 23/200 cases of T-cell acute lymphoblastic leukemia. Genomic studies identified two types of deletions: interstitial and terminal. Interstitial 5q deletions, found in five cases, were present in both adults and children with a female predominance (chi-square, *P*=0.012). Interestingly, these cases resembled immature/early T-cell precursor acute lymphoblastic leukemia showing significant down-regulation of five out of the ten top differentially expressed genes in this leukemia group, including *TCF7* which maps within the 5q31 common deleted region. Mutations of genes known to be associated with immature/early T-cell precursor acute lymphoblastic leukemia, i.e. *WT1*, *ETV6*, *JAK1*, *JAK3*, and *RUNX1*, were present, while *CDKN2A/B* deletions/mutations were never detected. All patients had relapsed/resistant disease and blasts showed an early differentiation arrest with expression of myeloid markers. Terminal 5q deletions, found in 18 of patients, were more prevalent in adults (chi-square, *P*=0.010) and defined a subgroup of *HOXA*-positive T-cell acute lymphoblastic leukemia characterized by 130 up- and 197 down-regulated genes. Down-regulated genes included *TRIM41*, *ZFP62*, *MAPK9*, *MGAT1*, and *CNOT6*, all mapping within the 1.4 Mb common deleted region at 5q35.3. Of interest, besides *CNOT6* down-regulation, these cases also showed low *BTG1* expression and a high incidence of *CNOT3* mutations, suggesting that the *CCR4-NOT* complex plays a crucial role in the pathogenesis of *HOXA*-positive T-cell acute lymphoblastic leukemia with terminal 5q deletions. In conclusion, interstitial and terminal 5q deletions are recurrent genomic losses identifying distinct subtypes of T-cell acute lymphoblastic leukemia.

Introduction

Deletion of the long arm of chromosome 5, del(5q), is the most frequent genomic loss in myeloid diseases.[1] Two distinct common deleted regions (CDR) were identified in myelodysplastic syndromes and acute myeloid leukemia. Del(5q), as the sole cytogenetic abnormality, occurs in 10-15% of myelodysplastic syndromes and is known as "the 5q- syndrome".[1] It is characterized by a 1.5 Mb CDR (5q32-q33) where the putative oncosuppressor *RPS14* is mapped.[2] Del(5q) associated with other cytogenetic changes, often within a complex karyotype, is prevalent in

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Received: February 5, 2016
Accepted: April 29, 2016
Pre-published: May 5, 2016.

doi:10.3324/haematol.2016.143875

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/101/8/951

acute myeloid leukemia and in high-risk myelodysplastic syndromes.[3] Here it is characterized by a ~1 Mb CDR (5q31) and haploinsufficiency of the oncosuppressor *EGR1*.[4] Conversely, del(5q) has been rarely reported in B- and T- acute lymphoblastic leukemia (ALL).[5-8]

T-lineage ALL (T-ALL), a heterogeneous group of leukemias, is characterized by co-occurrence of multiple genetic lesions.[9] Interestingly, cooperative genetic defects have been described in T-ALL, suggesting that perturbation of specific cell processes are needed for the development of overt leukemia.[10,11] Translocations causing *TAL/LMO*, *TLX1*, *TLX3*, *MYB*, *MEF2C*, *NKX2-1/2*, or *HOXA* over-expression define distinct gene expression signatures and are known as "type A" abnormalities.[6,12-14] These rearrangements co-occur with multiple mutations and imbalances, named "type B" abnormalities, which activate oncogenic signaling cascades, including *JAK/STAT*, *PI3K/AKT*, and *RAS/MEK/ERK*. The most prevalent type B abnormalities are loss-of-function mutations and genomic losses, indicating that tumor suppressor genes, such as *CDKN2A/B*, *PHF6*, *LEF1*, *PTEN*, *WT1*, *ETV6*, and *PTPN2*, play pivotal roles in the initiation of T-*ALL*.[9,15]

To provide insights into the prevalence and specific features of del(5q), in T-ALL, we screened a series of 200 T-ALL cases by fluorescence *in situ* hybridization (FISH). We found two distinct types of recurrent 5q deletions: interstitial (I-5q) and terminal (T-5q). I-5q was identified in 2.5% of cases with a genomic profile closely resembling immature/early T-cell precursor (ETP) ALL. These findings indicate that I-5q is a recurrent cytogenetic event in T-ALL with early differentiation arrest of blasts. T-5q, identified in 9% of cases, clustered within the *HOXA* category and was characterized by a high incidence of abnormalities involving the *CCR4-NOT* complex and its closely related transcription factor *BTG1*. These observations indicate that *HOXA* over-expression, haplo-insufficiency of genes at T-5q, and deregulation of the CCR4-NOT complex are characteristic of this subgroup of leukemia.

Methods

We investigated the incidence of del(5q) in a cohort of 66 adult (≥18 years) and 134 pediatric T-ALL patients from the Italian (GIMEMA LAL-0496 and LAL-0904 and AIEOP LLA-2000) and UK clinical trials (MRC, ALL2003 and ALL97) (*Online Supplementary Table S1*, *Online Supplementary Information*).[10,16,17] All patients or their parents/guardians gave informed consent to sample collection and molecular analyses, in agreement with the Declaration of Helsinki. The study was approved by the local bio-ethical committee (research project 2014-025).

Combined interphase FISH identified T-ALL-associated

Table 1. Clinical, hematologic and molecular-cytogenetic features of 5 cases of T-ALL with interstitial 5q deletion.

N.	Sex/Age	Immunophenotype	Karyotype	ETP-ALL related mutations	Genetic category	CI-FISH	CNV	Follow-up (months)
1	F/9	**undefined**: CD34+, CD33+	n.a.	***DNM2, WT1, LPHN2***	***HOXA***	***SET-NUP214*** [80%] del(5)(q31q33) [87%] del(6)(q15q21) [82%] del *WT1* [85%]	**LOSS**: *CREBBP*/16p13.3, *JAKMIP2*/5q32, SYNCRIP/6q14.3	64†
2	F/16	**ETP**	46,XX[15]	***RUNX1, JAK3, ETV6, PHF6***	***HOXA***	***HOXA*-translocation** [60%] del(5)(q14q31) [55%] del *ETV6/CDKN1B* [59%]	**LOSS**: 5q14.3-q32,12p13.2-p12.3, 14q32.11-q32.12,16q22.2-q24.3 **GAIN**: 1q32.1-q44,19q13.2-q13.4 **CN-LOH**: 6q12-q27	63†
3	F/27	**pro-T**	n.a.	***WT1, PHF6***	***HOXA***	***SET-NUP214*** [93%] del(5)(q31) [99%] del(6)(q21) [98%] del *WT1* [85%] del *RB1* [96%] del *ATM* [99%] del *TP53* [99%]	**LOSS**: 2p14-p13.3,3q26.31-q26.33,5q11.1-q12.2,5q21.1-q22.1,5q23.2,5q31.1-q31.3,5q31.3-q32,6q21,6q22.31,6q22.33,7p12.2-p11.2,9q34.11-q34.13,11p14.1-p13,11p11.2-p11.12,11q14.1-q23.3,12q14.1-q23.3,13q13.3-q14.3,13q21.33-q31.1,14q12,16q21-q22.1,17p13.2-p13.1,19q13.32 **CN-LOH**: 1p36.33-p36.11	12†
4	F/49	**pro-T**	46,XX[15]	***JAK3,JAK1, PHF6***	***MEF2C***	***RUNX1*-translocation** [82%] del(5)(q31) [84%] gain *DDX3X* [46%]	**LOSS**: 5q31.1 **GAIN**: Xp22.33-p11.23 **CN-LOH**: 9q33.2-q34.3	7†
5	M/37	**ETP**	48,XY,del(5)(q31q33),+8, del(9q),der(12),del(13q), +21[10]	**n.a.**	**unclassified**	del(5)(q31) [93%] del(6)(q16q21) [93%] del(9)(q32/TAL2 [97%] del *RB1* [99%] del(20)(p11) [92%] trisomy 21 [95%]	**LOSS**: 1q25.1, 2p13.2, 3q13.1-q13.3, 5q21.3-33.2, 6q15-q22.3, 9q22.3-q33.2, 11q14.2-q23.2, 12q13.1, 13q13.3-q21.3, 16p13.3, 20p11.2 **GAIN**: 21q11.2-q22.3	1†

N.: patient number; F: female; M: male; CI-FISH: combined interphase fluorescence in situ *hybridization (the percentage of cells with abnormal CI-FISH is indicated between brackets); CNV: copy number variations were determined by haloplex analysis (case n.1) or single nucleotide polymorphism array (cases n. 2-5); n.a.: not available; ETP: early T-cell precursor; †dead. Only mutations typically associated with immature/ETP-ALL were reported. In patient n. 1 the panel of antibodies tested did not allow to stage of differentiation of leukemic blasts to be determined. Patients n. 2 and 3 were studied by transcriptome sequencing and gene expression profiling.*

genomic rearrangements which classified cases into defined subgroups (*Online Supplementary Table S2*).[10,16,17] Deletions of 5q were detected by FISH with LSI *EGR1*/D5S23, D5S721 Dual Colour probe, RP11-182E4/RP11-453D13 and CTB-31E20/RP11-266N12 for rearrangements of *TLX3*, RP11-117L6 for *NPM1* abnormalities, and RP1-240G13 for deletions of the subtelomeric 5q region. Cases with del(5q) were further characterized with clones for 5p13-qter (*Online Supplementary Table S3*). Single nucleotide polymorphism array, denaturing high performance liquid chromatography, Sanger sequencing, haloplex polymerase chain reaction,[11] transcriptome sequencing,[18] and gene expression profiling were performed in cases with available material (*Online Supplementary Information*).

Results and Discussion

Incidence and distribution of del(5q) in T-cell acute lymphoblastic leukemia

FISH and single nucleotide polymorphism array revealed two types of recurrent del(5q) in 23/200 T-ALL patients: I-5q (5 cases) (Table 1) and T-5q (18 cases) (Table 2). In 11/18 cases with T-5q, the deletion was very large and included the CDR of I-5q cases (Figure 1A). Overall, del(5q) was mainly associated with the *HOXA* category (16/23; 69.5%). It was found at diagnosis in 22 cases while in the remaining case it was detected only during

Table 2. Clinical, hematologic, and molecular-cytogenetic features of 18 cases of T-ALL with terminal 5q deletion

N.	Sex/Age	Immunophenotype	Karyotype	Gene mutations	Genetic category	CI-FISH	CNV	Follow-up (months)
CHILDREN								
1	F/11	pre-T	n.a.	*NOTCH1, CNOT3*	***HOXA***	***TCRB-HOXA*** [65%] del(5)(q35)[96%] del CDKN2A/B[95%]	**LOSS**: 5q34-q35.3, 9p21.3 **GAIN**: 13q21-q34 **CN-LOH**: 9p24.3-p21.3, 9p21.3-p21.1	48
2	F/9	pre-T	46,XX,del(5)(q31q35),inv(7)(p15q?)[8] 46,XX[6]	*NOTCH1*	***HOXA***	***HOXA*-translocation**[80%] del(5)(q31)[94%]	**LOSS**: 5q14.3-q35.3 **GAIN**: 1q32.1-q42.13, 1q42.3-q44	20
3	M/10	n.a.	n.a.	*NOTCH1, ETV6*	***HOXA***	***NUP98-RAP1GDS1***[90%] del(5)(q31)[98%] *TCRB*-rearrangement[80%] del *HOXA*[99%] del *EZH2*[99%]	**LOSS**: 5q23.2-q35.3, 7q22.2-q31.1, 7q34-q36.3 **GAIN**: 7p14.1, 7q22.1-q22.2, 7q31.1-q34	98
4	M/18	ETP	n.a.		***HOXA***	***SET-NUP214***[96%] del(5)(q31)[96%]	**LOSS**: 5q31-q35.3, 16q11.2-q24.2, 9q34.11-q34.13	24†
5	F/4	n.a.	46,XX[20]		**UNCLASSIFIED**	del(5)(q35)[50%] del *FBXW7*[13%] del *CDKN2AB*[50%]	n.a.	88
6	M/17	cortical	46,XY,del(6)(q25)[5] 46,XY[19]	*NOTCH1*, *NRAS*	**UNCLASSIFIED**	del(5)(q31)[90%] gain 11p13-15[79%] del *NF1*[79%]	**LOSS**: 1p36.33-p36.22, 1q43, 5q31.1-q35.3, 17q11.2, 17q11.2 **GAIN**: 7q21.11, 11p15.5-p11.2	9†
7	F/7	cortical	n.a.	*NOTCH1*	**UNCLASSIFIED**	del(5)(q31)[42%] del(6q15q16)[20%] del *HOXA*[26%] gain *MYB*[23%] gain *MYC*[46%] del *CDKN2AB*[45%] gain *RB1*[12%]	**LOSS**: 2q34-q37.3, 5q15-q35.3, 6q12-q16.3, 7p22.2-p11.2, 8p23.2-p12, 9p21.3 **GAIN**: Xp22.33-q28, 2q32.3-q34, 6q21-q27, 7q11.21-q11.22, 8q21.3-q24.3, 15q11.2-q26.3	10†
N.	**Sex/Age**	**Immunophenotype**	**Karyotype**	**Gene mutations**	**Genetic category**	**CI-FISH**	**CNV**	**Follow-up (months)**
ADULTS								
8	M/22	ETP	46,XY,del(6)(q15q16),del(12)(p13)[1] 46,XY[10]	*NOTCH1*, *NRAS*, *PIK3R1*	***HOXA***	***TCRB-HOXA***[24%] del(5)(q31)[27%] del(6)(q15-21)[27%] del *ETV6*[53%] del *TP53*[20%] del *NF1*[32%]	**LOSS**: 4q32.3, 5q21.3-q35.3, 6q13-q22.1, 7q34, 12p13.31-p11.22, 13q21.33, 17p13.3-q12, 17q22 **GAIN**: 18p11.32	21†
9	M/32	pre-T	46,XY[15]	*NOTCH1*	***HOXA***	***TCRB-HOXA***[80%] del(5)(q14)[83%] del(6)(q15q16)[85%] del *CDKN2AB*[56%] trisomy 8[67%]	**LOSS**: 1p36.3-36.2, 4q26,5q11.1-q35.3, 6p22.3-22.2,6p12.1, 6q14.1-16.1, 9p21.3,9q21.3-21.3, 9q32-q33.2,9q33.3-q34.1,19p13.2 **GAIN**: 5p15.3-q11.1,7p21.1-p15.2,7q11.2-q21.1, 7q22.1-22.3,7q34,7q36.1,8p23.3-q24.3 **CN-LOH**: 7p22.3-p21.1,7p15.2-p11.1,7q22.1-q22.3,7q22.2-q34	10†

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disease progression (13 months after diagnosis); it belonged to the major abnormal clone in all cases but one. Cytogenetically, both types of del(5q) were always associated with additional chromosome abnormalities and had significantly more DNA copy number abnormalities than cases without del(5q) [median 7 (range, 3-28) *versus* 3.5 (1-14), *P*=0.003] (*Online Supplementary Table S4*).

Deletions of 5q define two independent subgroups of T-cell acute lymphoblastic leukemia

We have data to show that I-5q and T-5q are cytogenetic markers of two subgroups of T-ALL, with different age and gender distributions and distinct genomic backgrounds.

Interstitial 5q T-cell acute lymphoblastic leukemia

I-5q was detected in five patients (2.5%) (Table 1). Four of these five patients were females (Pearson χ^2 test, *P*=0.012) but there was no distinctive age distribution. Cytogenetically, three cases carried *HOXA*-activating rearrangements (2 carried *SET-NUP214* and 1 carried *TCRB-HOXA*); patient n. 4 had a translocation involving *RUNX1*, suggesting likely membership of the *MEF2C* category;[14] patient n. 5 was unclassified (*Online Supplementary Information* and *Online Supplementary Figure S1*). The cases of I-5q T-ALL had a higher incidence of genomic losses than cases of T-ALL without del(5q) [mean 9.5 (range, 1-22) *versus* 3.1 (1-11)], a difference which did not, however, reach statistical significance likely due to the small sample size (*Online Supplementary*

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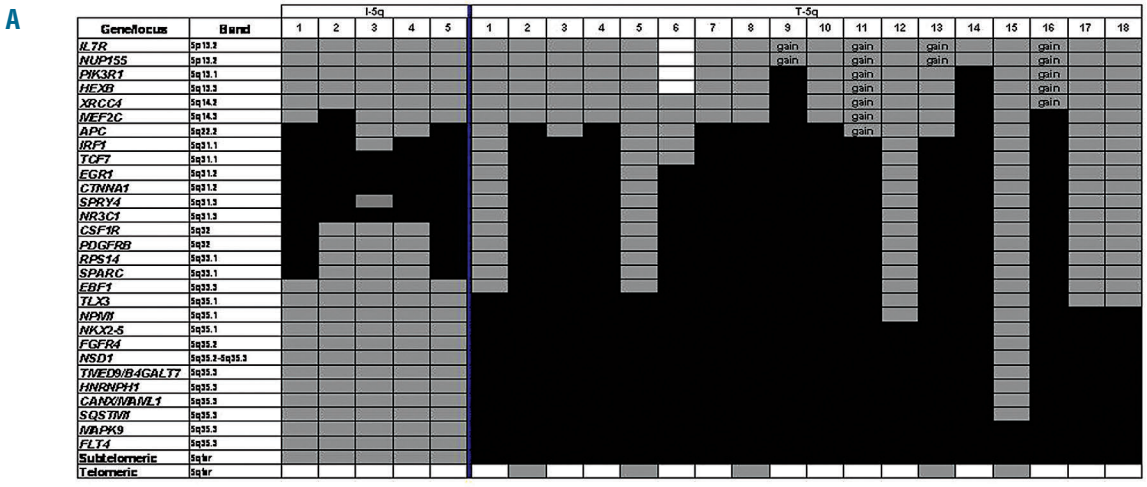
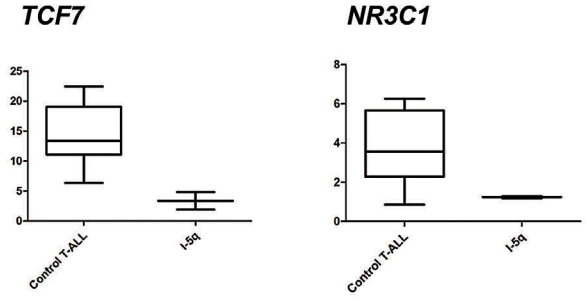
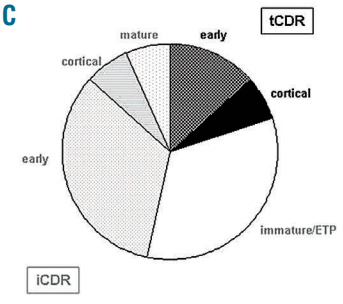
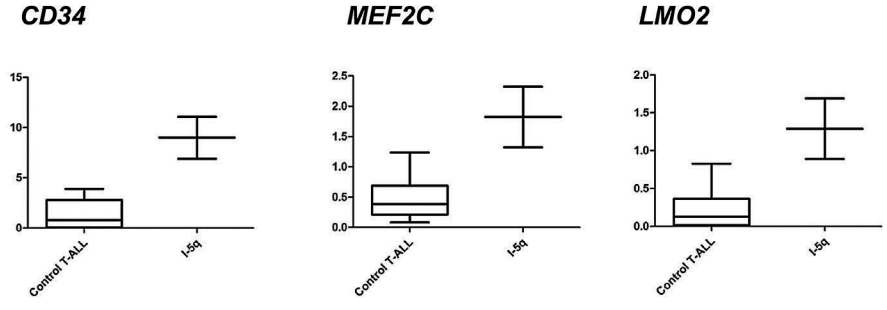
10	M/51	pre-T	46,XY[15]	*NOTCH1*	***HOXA***	***TCRB-HOXA*** [91%] del(5)(q31)[97%] del *CDKN2AB*[90%] gain *BCL11B*[80%]	**LOSS**: 5q21.1, 5q21.3-q35.3, 9p24.3-p21.1, 15q25.2-26.3 **GAIN**: 9p21.1-p13.2, 14q31.3-q32.3 **CN-LOH**: 1q31.2-q32.2, 2q22.1-q31.3, 3q23-q26.3, 6q22.3-q24.3, 10q24.3-q26.2	26†
11	M/37	pre-T	n.a.	*PTEN*	***HOXA***	***CALM-MLLT10***[98%] del(5)(q31)[96%] del(6)(q15q16)[92%] del *CDKN2AB*[92%] gain *MYC*[90%]	**LOSS**: 2p16.1, 2q22.1, 2q24.2-q24.3, 2q31.1, 2q31.3, 2q32.1-q32.2, 2q33.3-q34, 2q34-q35, 2q35-q37.3, 3p24.3, 3p12.2-p12.1, 5q22.3-q35.3, p25.1-p24.3, 6p24.3-p24.2, 6p22.3, 6p22.2-p21.33, 6 6p21.2-p21.1, 6p21.1-q21,9p24.1-p23, 9p21.3, 9q31.1, 11q14.1-q23.3, 21q11.2-q22.3 **GAIN**: 5p15.33-q22.3, 6q21, 8q21.12-q24.3, 19p13.3-q13.43 **CN-LOH**: 6q21-q27	1†
12	M/26	cortical	46,Y,t(X;10)(p12;p13), add(1)(p36), del(9)(p11p24)[10] 46,idem,del(6q15)[1] 46,XY[11]	*NOTCH1*, *CNOT3*	***HOXA***	***DDX3X-MLLT10***[85%] del(5)(q35)[90%] del(6)(q15-21)[77%] del *CDKN2AB*[87%]	n.a.	84
13	M/25	cortical	46,XY,del(1)(q42), t(4;11)(q21;p15), del(5)(q31q35),del(7)(p21), add(16)(p13)[10]	*NOTCH1*	***HOXA***	***NUP98-RAP1GDS1***[90%] del(5)(q31)[80%] *MYB* tandem dup[26%]	**LOSS**: 5q23.2-q35.3, 16p13.3-p13.1 **GAIN**: Xp22.33-p11.3, 5p15.3-p13.3, 6q23.3	42
14	F/19	pre-T	45,XX,t(4;11)(q13;p15),der(5;17)(p10;q10)[15]	*TP53*	***HOXA***	***NUP98*-translocation** [16%] del(5)(q13) [17%] del *TP53* [20%]	**LOSS**: 1p36.33-p36.23, 5q11.1-q35.3, 17p13.3-p11.1 **GAIN**: 1q32.3-q44	72
15	M/20	pre-T	46,XY[15]		***HOXA***	***SQSTM1-NUP214***[85%] del *CDKN2AB*[81%] del *WT*[85%] del *NF1*[85%]	**LOSS**: 5q35.3, 9p23-p22.3, 9p21.3, 9q32-q33.3, 10p12.1-p11.2, 11p13, 13q13.1, 13q13.2-13.3, 13q32.2-q32.3, 13q32.3-q33.1, 13q33.1-q33.2, 16q24.1-q24.3, 17q11.2, 21q22.2 **GAIN**: 7q31.3, 9q34.1-q34.3	16†
16	F/39	mature	46,XX[10]	*PTEN*, *CNOT3*	***HOXA***	***MLL*-translocation**[60%] del(5)(q35)[17%] del(6)(q16q21)[60%] *MYB* tandem dup[59%] del *PTEN*[50%]	**LOSS**: 5q14.3-q35.3, 6q11.1-q22.33, 10q23.2-q23.31 **GAIN**: 5p15.33, 6q23.3, 13q31.1-q34	25
17	M/22	cortical	n.a.	*NOTCH1*, *KRAS*	***TLX3***	***TLX3*-translocation**[92%] del(5)(q35)[92%] del *CDKN2AB*[96%] del *LEF1*[38%] gain *ETV6*[97%]	n.a.	16
18	M/25	n.a.	46,XY,add(2)(q36)[5]/46,XY[18]	*NOTCH1*	***TLX3***	***TLX3*-translocation**[98%] del(5)(q35)[98%] del *BCL11B*[98%]	**LOSS**: 5q35.1-q35.3 **GAIN**: 14q32.2-q32.3 **CN-LOH**: 4q11-q35.1	38†

N.: patient number; CI-FISH: combined interphase fluorescence in situ *hybridization (the percentage of cells with abnormal CI-FISH is indicated between brackets); CNV: copy number variation (detected by single nucleotide polymorphism array); n.a.: not available; nl: normal; del: deletion; †: dead. Patient n.3 was studied by haloplex; n. 12, 16, and 17 by transcriptome sequencing; n. 5 was not studied for gene mutations. Patients n. 4 and 15 were wild type for hotspot mutations of* NOTCH1, FBXW7, FLT3, TP53, CNOT3, *and* K/NRAS. *Gene expression profiling was performed in patients n. 4, 6, 12, 15-18. In patient n. 8 the T-5q was detected 13 months after diagnosis, while the patient was still receiving treatment.*









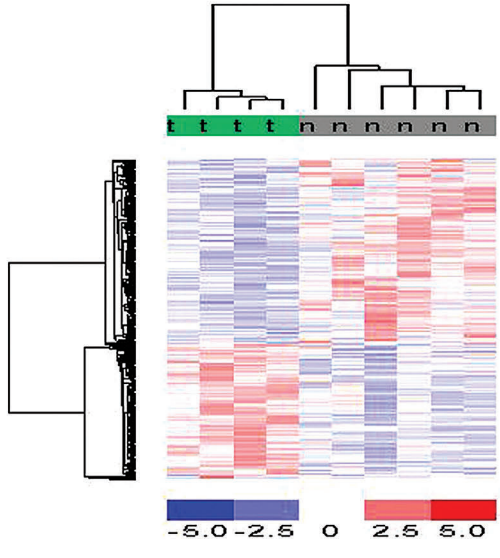


Figure 1. Genomic characteristics of I-5q and T-5q T-ALL. (A) Schematic representation of 5q deletions in our 23 T-ALL cases. Patients' numbers refer to Table 1 for cases with I-5q, and to Table 2 for cases with T-5q. Black boxes indicate monoallelic deletion; gray boxes, diploidy; white boxes, not tested; gain, presence of three copies; (B) *TCF7* and *NR3C1* expression in two T-ALL with I-5q *vs.* control [7 cases without del(5q) and 3 cases with T-5q]; (C) Distribution of terminal CDR (tCDR) (black) (3 cases) and interstitial CDR (iCDR) (gray) (3 cases with I-5q and 9 cases with large T-5q including the I-5q CDR) within the HOXA-category: all five cases with an immature/ETP phenotype as well as five of seven cases of early T-ALL had I-5q or large T-5q including the iCDR; (D) Expression of CD34, MEF2C, and LMO2 in two cases with I-5q *vs.* ten controls [7 cases without del(5q) and 3 cases with T-5q]; (E) Supervised gene expression profiling analysis of four *HOXA* positive cases with T-5q (t) and six without (n), identified 327 differentially expressed genes.

Table S4). They were significantly associated with *WT1* deletions (n=2) (χ^2, p=0.002) and del(6q) (n=3) (χ^2, *P*=0.002); these del(6q) shared a CDR at band 6q21, encompassing the putative onco-suppressor genes *SEC63* and *FOXO3* (*Online Supplementary Table S5*).[7,19]

All I-5q had a CDR at band 5q31 (Figure 1A), encompassing the 1 Mb CDR of high-risk myelodysplastic syndrome/acute myeloid leukemia del(5q).[3,4,20] When we compared I-5q- positive cases with T-5q and T-ALL without del(5q), we found that the expression levels of *IRF1*, *EGR1*, *CTNNA1*, *HNRNPAO*, *TIFAB*, and *CXXC5*, known putative onco-suppressors in *in vitro* and/or *in vivo* models[20-24] mapping within the CDR, did not differ between the three groups; while the presence of I-5q was associated with significant down-regulation of *NR3C1* and *TCF7* genes (Figure 1B, *Online Supplementary Information* and *Online Supplementary Figures S2* and *S3*).

NR3C1 belongs to the nuclear hormone receptor superfamily that includes the mineral-corticoid and estrogen receptors. Once NR3C1 binds to steroid hormones, it acts as a direct transcriptional regulator.[25] *NR3C1* deletion has been associated with relapse in pediatric and adult B-cell ALL and predicted corticosteroid resistance in T-ALL, thus influencing response to treatment.[26,27] *TCF7* is an essential transcriptional regulator of T-cell specification, commitment, and lineage determination.[28] In mouse models, *Tcf7*$^{-/-}$ induced a T-cell malignancy that was similar to human ETP-ALL as 117 deregulated genes were common to both.[29] *In vivo* studies suggested that *TCF7* haplo-insufficiency rendered pre-malignant thymocytes susceptible to later lesions which subsequently transformed them into leukemic blasts. In fact, low *Tcf7* expression predisposed murine thymocytes to acquire Notch1-activating mutations which were invariably found in *Tcf7*$^{-/-}$ lymphomas.[29,30] Interestingly, *NOTCH1* mutations were present in four out of five cases of T-ALL with I-5q. Together these data suggest strong similarities between mouse Tcf7$^{-/-}$ T-cell malignancies and human T-ALL with I-5q assigning a role to *TCF7* haplo-insufficiency in this subgroup of leukemia.

Finally, among *HOXA*-positive T-ALL, ten of 12 cases with immature/ETP or early phenotype lost the I-5q CDR (*Online Supplementary Table S6* and Figure 1C). Among them five cases had the typical features of the high-risk subgroup recently named *HOXA*-positive ETP ALL.[31,32] As far as we know, loss of genes at the I-5q CDR is the first recurrent cytogenetic change so far described in this subgroup.

Terminal 5q T-cell acute lymphoblastic leukemia

T-5q T-ALL was detected in 18 patients (9%), who were mainly adults (χ^2, *P*=0.010) but there was no association with gender (12 males; 6 females), stage of blast differentiation (7 pre-T, 5 cortical, 2 ETP, 1 mature, and 3 undefined), or white blood cell count (Table 2). Cytogenetically, 13 cases belonged to the *HOXA* category [with rearrangements of: *HOXA* (n=5), *NUP98* (n=3), *NUP214* (n=2), *MLLT10* (n=2), and *MLL* (n=1)], two cases to the *TLX3* category (*Online Supplementary Information* and *Online Supplementary Figure S4*), while three cases remained unclassified. T-5q ALL were significantly associated with del(6)(q14q15) (χ^2, *P*=0.002), and genomic gains (*P*=0.0038) (*Online Supplementary Table S4*), of which the most frequent was gain of chromosome 5p arm, found in four out of 17 (23.5%) fully characterized cases. We also found a high incidence of *NF1* deletions (4 cases)(χ^2, *P*=0.002) and recurrent *N/KRAS* mutations (3 cases), consistent with RAS/MEK pathway involvement in ~23% of cases (*Online Supplementary Information*).[33-35]

Although T-5q deletions varied greatly in size, they all had a common 1.4 Mb CDR (chr5:179257527-180719789, GRCh37) telomeric of *SQSTM1* (Figure 1A). The T-5q CDR contained one *LIN* gene (long intergenic non-protein coding), five olfactory receptor genes, three microRNA, eight LOC non-coding RNA, and 37 genes. Supervised gene expression profiling analysis showed that only eight out of the 26 genes with probe-sets available at the CDR, i.e. *MAPK9*, *TBC1D9B*, *RFP130*, *TRIM52*, *TRIM52-AS1*, *HEIH*, *ZFP62*, and *CNOT6*, were significantly down-regulated in six cases with T-5q T-ALL compared to 22 T-ALL without.

Genetic profile links interstitial 5q with immature/early T-cell precursor acute lymphoblastic leukemia

ETP-ALL, a distinct subgroup of T-ALL, is defined by a typical immunophenotype which is negative for CD1a and CD8, negative or weakly positive for CD5, and positive for at least one of the following markers: CD34, CD117, HLA-DR, CD13, CD33, CD11b, and CD65.[36] ETP-ALL also shows a distinct genetic profile with high expression of two bHLH transcription factors, *LYL1* and *LMO2*, a high incidence of mutations typically associated with the pathogenesis of acute myeloid leukemia, and a low frequency of typical T-ALL lesions, such as *CDKN2A/B* deletions and *NOTCH1* mutations.[37] ETP-ALL has been associated with a dismal outcome due to poor response to chemotherapy and a high rate of resistance/early relapse, namely in cases with genomic rearrangements associated with *HOXA* deregulation.[31,32,38] On the other hand, a recent clinical trial demonstrated a high rate of continuous complete remission in children.[39]

In our study, all I-5q T-ALL were characterized by early differentiation arrest of leukemic blasts with expression of at least one stem cell/myeloid antigen (Table 1). In fact patients n. 2 and n. 5 satisfied all diagnostic criteria for ETP-ALL.[36] *RB1*/13q14 deletions co-occurred in two of five cases. A critical analysis of previous studies showed that del(5q) had already been found in immature/ETP ALL.[6,34] Indeed, del(5q) together with del(13q) were the two cytogenetic changes most frequently associated with immature/ETP ALL as they were both detected in 23% of cases (4/17), and co-occurred in 11% (2/17).[6,36] Additional evidence that I-5q T-ALL are closely related to the immature/ETP subtype of T-ALL came from identification of *PHF6*, *JAK3*, *JAK1*, *DNM2*, *WT1*, *ETV6*, and/or *RUNX1* mutations and lack of *CDKN2AB* deletion/mutation in all analyzed cases.[37,40,41]

It is noteworthy that in addition to *TCF7*, other significantly down-regulated genes in I-5q T-ALL were *TDRKH*, *PCGF5*, *HDAC4*, and *MTA3*, so that our I-5q patients carried five out ten of the most differentially expressed (down-regulated) genes seen in human ETP-ALL (*Online Supplementary Table S7* and *Online Supplementary Figures S2, S3, S5-8*).[29,36] Moreover, among the 22 genes which have been reported to be significantly over-expressed in ETP-ALL,[6,14,36,37,40-42] *MEF2C*, *LMO2*, and *CD34* were significantly up-regulated in I-5q (Figure 1D). Overall, our data highlight two informative aspects of I-5q. First, genomic profiles link I-5q and immature/ETP ALL; furthermore all

five cases with I-5q were poor responders to standard therapy. In fact, patient n. 1 was a late remitter despite being assigned to the most intensive arm of the ALL2003 MRC trial. She received a bone marrow transplant from an unrelated donor in second remission, relapsed shortly afterwards and died. Patient n. 2 had an early relapse and also received a bone marrow transplant from an unrelated donor while she was in second remission. She had a second relapse after the transplant and died of her disease. The other three patients had resistant disease and died within 1 year. Although there were too few patients in the present series to draw any definitive conclusions, I-5q marks a particularly high-risk subgroup of immature/ETP ALL for which alternative targeted therapies should be developed. Among them, JAK/STAT inhibitors, which are highly effective in ETP-ALL xenograft models,[43] and the BCL2 inhibitor ABT-737, which restores the sensitivity to steroids in cell lines with high MEF2C expression,[44,45] might be considered in the treatment of these refractory leukemias.

Terminal 5q is a HOXA*-positive cytogenetic subgroup*

T-5q was found in 27% of patients belonging to the *HOXA* group (13/48 cases) (χ^2; P<0.001) in which it behaved as a type B event (Table 2). Supervised gene expression profiling analysis compared four *HOXA*-positive cases with T-5q and six without to determine whether T-5q defined specific pathways within the *HOXA* category: t-test analysis (P≤0.005) identified 327 genes (Figure 1E). Of the 130 over-expressed genes in T-5q cases, functional annotation analysis revealed enrichment of genes involved in nuclear lumen, DNA replication and mRNA metabolic processes, such as *CDC45*, *CDC5L*, *CHEK1*, *E2F3*, and *FANCD2*, suggesting specific deregulation of these pathways. Among the down-regulated genes, *FYN* and *LCK* tyrosine kinases, *IL7R*, *ZAP70*, *ADD3*, and the adaptor protein, *BTG1*, are known oncogenes/tumor suppressors in ALL.

Within the T-5q CDR we observed down-regulation of *TRIM41*, *ZFP62*, *MAPK9*, *MGAT1*, and interestingly, *CNOT6*. This is the first report of *CNOT6* involvement in human cancer and its down-regulation is consistent with it being a putative onco-suppressor gene as indicated by *in vitro* data.[46] Besides *CNOT6* down-regulation, our T-5q T-ALL cases were associated with a high rate of *CNOT3* mutations (18%). Notably, *CNOT3* loss-of-function mutations were found in ~7% of adult T-ALL but no specific association with any major molecular subgroups has been reported.[47] Both *CNOT6* and *CNOT3* encode for members of the CCR4-NOT complex, which consists of two major modules: the deadenylase module composed of two subunits with deadenylation enzymatic activity (CNOT6 or CNOT6L and CNOT7 or CNOT8) and the NOT module (CNOT1, CNOT2, and CNOT3). The CCR4-NOT complex is involved in chromatin modification, cellular response to DNA-damage, transcription elongation, RNA export, nuclear RNA surveillance, and miRNA-mediated deadenylation of mRNA.[48-50] Although, CCR4-NOT involvement in human tumors has been rarely reported, a recurrent hotspot P131L mutation of *RQCD1* (formerly known as *CNOT9*) has recently been identified in ~4% of cutaneous melanomas.[51] Our findings of a high rate of deletion/loss-of-function mutations and down-regulation of members of the CCR4-NOT complex as well as low expression of *BTG1*, a directly interacting adaptor protein of CCR4-NOT, suggest that this complex plays a role in the subset of *HOXA*-positive T-ALL with T-5q.

In conclusion, the present study has identified distinct recurrent 5q deletions in T-ALL, corresponding to different genomic landscapes and defining two cytogenetic subgroups. I-5q identified a subgroup of immature T-ALL, found predominantly in females, with an ETP-like genetic profile and poor response to current treatments. T-5q designated a subgroup of *HOXA*-positive T-ALL, mainly found in adults and associated with a high rate of CCR4-NOT complex abnormalities. In I-5q, two putative onco-suppressors, *NR3C1* and *TCF7*, mapping to the 5q31 CDR, were down-regulated. In T-5q *CNOT6*, a member of the CCR4-NOT complex, mapping to the 5q35 CDR, was significantly down-regulated. Due to the heterogeneity of treatment and age of our 18 patients, the clinical impact of T-5q could not be established, while the unresponsive T-ALL with I-5q should be considered for new experimental therapies.

Acknowledgments

Associazione Italiana per la Ricerca sul Cancro (AIRC, IG-15525), Fondo per gli Investimenti della Ricerca di Base (FIRB 2011 RBAP11TF7Z_005), Programmi di Ricerca scientifica di rilevante Interesse Nazionale (PRIN Cod. 2010NYKNS7_003), Associazione Sergio Luciani, Fabriano, Italy, and AIRC 5x1000; Bloodwise, formerly Leukaemia and Lymphoma Research.

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