HOX11L2 expression defines a clinical subtype of pediatric T-ALL associated with poor prognosis

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The most frequent oncogenic activation events characterized in childhood T acute lymphoblastic leukemia (T-ALL) result in the transcriptional activation of genes coding for transcription factors. The main genes are TAL1/SCL, a member of the basic region helix-loop-helix gene family, and HOX11L2, a member of the homeobox-containing protein family. To gain insight into the pathogenesis of this type of hematologic malignancy, we analyzed 28 T-ALL samples. SIL-TAL1/SCL fusion was detected in 6 patients; expression of HOX11L2 was observed in 6 patients and of HOX11 in 3 patients. With one exception, these activations did not occur simultaneously in the same patients, and they allowed the subclassification of 50% of the patients. SIL-TAL1 fusion was detected in association with HOX11 expression in one patient and with a t(8;14) (q24;q11) in another. High expression of LYL1, LMO2, or TAL1 was observed mainly in samples negative for HOX11L2 expression. HOX11L1 and HOX11 expression were observed in one instance each, in the absence of detectable chromosomal abnormality of their respective loci, on chromosomes 2 and 10, respectively. HOX11L2 expression was associated with a chromosome 5q abnormality, the location of the HOX11L2 locus in each case tested. Finally, our data show that HOX11L2 expression was a suitable marker for minimal residual disease follow-up and was significantly associated with relapse (P = .02).

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Patients and methods

Patients

Patients were children with T-cell malignancies diagnosed at Trousseau Hospital (Paris, France) from March 1996 to September 2001. Diagnoses were based on standard morphologic and histochemical parameters of leukemic cells and on the expression the T-cell antigens, cytoplasmic CD3, CD2, CD5, CD7, and the absence of B-cell antigens. Thirty-two patients were given diagnoses of T-ALL during that time, and samples for 28 were available. Informed consent was obtained from patients and their parents according to the Declaration of Helsinki. Clinical data of T-ALL patients are summarized in Table 1. Samples from children with T-lymphoblastic lymphoma (5 patients), B-ALL (52 patients), and acute myeloblastic leukemia (AML, 15 patients) diagnosed during the same period of time were also used. Bone marrow (BM) or peripheral blood (PB) samples were obtained at the time of diagnosis and during cytoligic remission or at relapse and were cryopreserved.

Patients were treated according to the French multicenter risk-adapted protocol FRALLE. T-ALL patients were assigned to the high-risk arm. Corticosteroid—defined as the persistence of more than 25% blasts in BM aspiration at day 21—were indications that patients should be moved to the very high-risk arm and should undergo allogeneic or...
buffer (1 mM each dNTP, 3 mM MgCl$_2$, 75 mM KCl, 50 mM Tris-HCl pH 8.3), from healthy donor samples, and 5 normal bone marrow RNA samples were extracted (Eurobio, Les Ulis, France). Normal peripheral blood lymphocyte RNA was extracted according to the manufacturer instructions. RNA from normal fetal spleen, fetal thymus, and adult spleen were obtained by direct nucleotide sequence analysis.

Follow-up time, median 28 mo

Overall survival rate 82.1%

Number of events 6 of 28

2-year EFS rate 64.7%

M3 at day 21 5 of 28

Corticoresistant patients 11 of 27

Risk-adapted treatment arms

1 of 28

†Bulky disease* 14 of 28

‡Without mediastinal enlargement 6 of 28

With mediastinal enlargement 8 of 28

Induction failure 2 of 28

2-year EFS rate 64.7%

Number of events 6 of 28

Overall survival rate 82.1%

Follow-up time, median 28 mo

*Defined by the presence of hepatomegaly or splenomegaly reaching the umbilical or lymphoadenopathy of more than 5 cm or mediastinal enlargement.
†One patient (UPNT26) had 67% blasts at diagnosis.‡Patient UPNT7 is a 1-year-old child treated in INFANT protocol.

autologous bone marrow transplantation during the first complete remission (CR). Clinical data of the patients are presented in Table 1.

### Immunophenotyping

Mononuclear cells isolated from bone marrow aspirates were stained with a standard panel of antibodies against CD2, CD5, CD7, CD3, CD1a, TCR $\alpha\beta$, TCR $\gamma$δ, CD19, CD20, CD22, CD24, CD79a, IgM, CD10, CD34, HLA-DR, CD45, CD13, CD15, and CD33. Results were classified as positive when a given monoclonal antibody stained more than 20% of leukemic cells. At least 5000 cells per sample were analyzed using a FACSort cytomter and CELLQuest software (both from Becton Dickinson, Le Pont de Claix, France).

### RNA and DNA methods

Total RNA was extracted from frozen patient samples using RNAble (Eurobio, Les Ulis, France) according to the manufacturer’s instructions. RNA from normal fetal spleen, fetal thymus, and adult spleen were obtained from commercial sources. Adult thymus RNA was a kind gift from Karen Leroy (Hôpital Henri Mondor, Créteil, France), and 4 normal fetal thymi were a kind gift from Jelena Martinovic (Hôpital Necker–Enfants Malades, Paris, France). Normal peripheral blood lymphocyte RNA was extracted from healthy donor samples, and 5 normal bone marrow RNA samples were obtained from B-ALL patients in long-term remission (CR longer than 5 years). Genomic DNA was extracted using standard methods.

RNA was reverse transcribed from 1 µg total RNA in a final volume of 20 µL containing reverse transcription–polymerase chain reaction (RT-PCR) buffer (1 mM each dNTP, 3 mM MgCl$_2$, 75 mM KCl, 50 mM Tris-HCl pH 8.3), 10 U RNASin (Promega, Madison, WI), 100 mM dithiothreitol, 100 U SuperScript II (Gibco-BRL, Cergy Pontoise, France), and 25 µM random hexamers.

One hundred nanograms cDNA equivalent of RNA was analyzed in each PCR experiment. PCR was carried out in a final volume of 50 µL with 0.5 U AmpliGold polymerase (PE Applied Biosystems, Foster City, CA), 200 µM each dATP, dCTP, dGTP, 400 µM dUTP, 25 pmol each primer (see below), and 2.5 mM MgCl$_2$. Cycle parameters were set for 10 minutes at 95°C and for 15 seconds at 95°C, 40 seconds at 60°C, and 40 seconds at 72°C for 35 cycles. Specificity of each HOX11, HOX11L1, and HOX11L2 RT-PCR product was confirmed by direct nucleotide sequence analysis.

### Primers and probes

Screening for HOX11L2, HOX11L1, and HOX11 expression was carried out by standard RT-PCR using the following primers: HOX11L2 F0, GCCGATCGGC- CACCCCTACCA; HOX11L2 R2, CCGCTCGGCTTCGGCTTCCT; HOX11L1-286F0, AGACCATGTTGACGGAGAAG; HOX11L1-413Rw, GYGCTTGGCCCTCGGTAGTTG; HOX11L1-712F0, CTGGCAAGGCGCTCAAATG; and HOX11L1-810Rw, GGCTCTCGGTTCCTCCGACGTC.

Primers and probe sequences used for real-time quantitative PCR amplification (RQ-PCR) of HOX11L2 and HOX11 were selected with the assistance of the computer program Primer Express (PE Applied Biosystem). They were designed on different exons to avoid amplification from residual genomic RQ-HOX11L2-3, CAAGACCTTGTGTTCAAAACC; RQ-HOX11L2-4, AGGCTGTATGGAGTCTGGTGA; probe, FAM-CAGCTGCAACAC-GACGCCTTCCTCA-TAMRA; RQ-HOX11-1F, AAATGACCGAGCG-CAGGT; RQ-HOX11-2R, GTTCCGTTGCTGCCTTCG; and probe, FAM-AACCGCGGACAAATGGGACG-TAMRA. Expression analyses of TAL1, LYL1, and LMO2 were performed using the primers described by Ferrando et al.14

The CDKN2A/CDKN2D copy number was estimated using an RQ-PCR assay starting from genomic DNA (adapted from15). SIL-TAL1 fusion RNA was detected as recommended by the BIOMED consortium for minimal residual studies in acute leukemia.16 Detection of the ABL transcript was performed as described.17

### Real-time polymerase chain reaction

Theoretical and practical aspects of RQ-PCR have already been described.18 Figure 1A shows changes in reporter fluorescence during PCR reactions starting from the known copy number (10$^8$ to 10$^2$) of the HOX11L2 cDNA. Fluorescence was expressed as normalized F1/F2 against background fluorescence accumulated during the first 15 PCR cycles, whereas cycle number indicated the point of the reaction at which the fluorescence generated by the cleavage of the probe crossed a fixed threshold above the baseline (crossing point or Cp). The standard curve was obtained by correlation of the standard concentration versus the Cp value, as shown in Figure 1B. A strong correlation between the Cp and the HOX11L2 copy number ($r > 0.99$) was found over a range of at least 6 orders of magnitude, with a PCR efficiency value of 90%. Similar results were obtained with HOX11 standard and the endogenous control ABL (data not shown).

For each patient and control sample, the quality and quantity of RNA were assessed by the amplification of ABL gene transcripts in independent

![Figure 1](https://www.bloodjournal.org/content/100/3/992/F1.large.jpg)
RQ-PCR runs. Samples were considered eligible for testing only when the 
Cp of the internal reference ABL was lower than 30. Quantitative results 
were thus expressed as 1000 times the normalized copy number of the target 
gene against the copy number of the endogenous ABL gene.

PCR reactions were performed using the Light Cycler System (Hoffman-
LaRoche, Grenoble, France). For each reaction, 100 ng reverse-transcribed 
RNA sample was added to 15 µl of PCR mix containing 1X LC master 
mix, 5 mM MgCl2, 300 µM each primer, and 200 µM probe. Thermal 
cycling conditions consisted of an initial denaturation step at 95°C for 10 
minutes followed by 50 cycles at 95°C for 15 seconds and 60°C for 1 
minute. Experiments were performed in duplicate for each data point. Each 
PCR run included the standard curve, a control without reverse transcrip-
tase, and a control without template.

Cyto genetic s and fluorescence in situ hybridization
Cytogenetic studies were performed in Hôpital Saint Antoine on bone 
marrow, blood cells, or both after short-term cultur es for 17 and 24 hours. 
RHG banding technique was applied. Karyotypes are summarized in Table 
2. Fluorescence in situ hybridization (FISH) was carried out using the usual 
techniques. In addition to whole chromosome 5 and 14 painting probes 
(INSERM U301 and Appligene Oncor [Ilkirch, France, respectively), 
chromosome 5 Y AC clone (885a6) and BAC clones (already described or 
selected from available human sequence data bases) were used as probes as 
follows: HOX11L2 (5g35)—593F7, 22A8N14, 45L16, 1q42—2576L4, 
1082A3; CDKN2A/CDKN2D (9p21)—145E5, 70L8; HOX11 (10q24)— 
31L3; and HOX11L1 (2p13)—140K4.

Table 2. Gene status, expression, and chromosome studies in 28 children with T-ALL

<table>
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<tr>
<th>Patient</th>
<th>HOX11L2</th>
<th>HOX11</th>
<th>LYL1</th>
<th>LMO2</th>
<th>TAL1</th>
<th>CDKN2A/CDKN2D</th>
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<td>13 120</td>
<td>112 000</td>
<td>64 366</td>
<td>136 084</td>
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<td>ND</td>
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<td>Failure</td>
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<td>43 521</td>
<td>30 139</td>
<td>3 500</td>
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<td>22 463</td>
<td>1 329</td>
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<td>743</td>
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<td>1 429</td>
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<td>1 211</td>
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<td>310</td>
<td>66 377</td>
<td>±</td>
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<td>8 864</td>
<td>2 224</td>
<td>1 932</td>
<td>±</td>
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<td>8 204</td>
<td>2 208</td>
<td>+/+</td>
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<td>580</td>
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<tr>
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<td>7 695</td>
<td>2 251</td>
<td>717</td>
<td>+/+</td>
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<td>1 124</td>
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<td>46,XY,inv[7]d[22]</td>
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</tr>
<tr>
<td>UPNT26</td>
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<td>15 456</td>
<td>14 965</td>
<td>±</td>
<td>Failure</td>
<td></td>
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<td>34 388</td>
<td>19 939</td>
<td>321</td>
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<td>3 956</td>
<td>+/+</td>
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<td>250</td>
<td>191</td>
<td>436</td>
<td>—</td>
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<tr>
<td>Bone marrow</td>
<td>—</td>
<td>10 792</td>
<td>13 202</td>
<td>27 197</td>
<td>—</td>
<td>—</td>
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</table>

HOX11 and HOX11L2 normalized copy numbers are indicated in positive samples. 
+ indicates presence of the SIL-TAL1 fusion or 1 copy of the CDKN2A/CDKN2D locus; —, absence of the SIL-TAL1 fusion or 1 CDKN2A/CDKN2D locus; ND, not done. 
Copy numbers of samples considered positive for LYL1, TAL1, and LMO2 are in bold numbers. 
Thymus and bone marrow expression level is the mean of 5 normal samples.

Statistical methods
Qualitative and categorized quantitative variables were compared to each 
other using χ2 analysis with Yates correction. For event-free survival (EFS) 
time, the period taken into account was the interval between the diagnosis 
and an event or the last examination if no events occurred. Survival curves 
were calculated according to the Kaplan-Meier method with Statview 4.5 
software (Abacus Concept, Berkeley, CA), and differences were assessed 
using the log-rank test.

Results

HOX11 gene family expression analysis
We used RT-PCR to detect HOX11L2 expression in a panel of 28 
pediatric patients with T-ALL, 5 with T-lymphoblastic lymphoma, 
52 with B-ALL, and 15 with AML. No specific fragment could be 
amplified from the T-lymphoblastic lymphoma, B-ALL, or AML 
samples, whereas a single 244-nucleotide fragment was observed 
in 6 of 28 (21.4%) T-ALL samples (Figure 2; Table 2).

To evaluate the expression level of the HOX11L2 gene, we 
developed a real-time quantitative RQ-PCR assay that was used to 
analyze the patients expressing HOX11L2. In these samples, the 
expression was homogeneously high, with HOX11L2 normalized 
copy number ranging from 3984 to 21 680. In contrast, when
investigated in normal hematopoietic tissue, HOX11L2 expression was not detected in adult spleen, adult peripheral blood lymphocytes, or control bone marrow. Using the same assay, HOX11L2 was found to be expressed at a very low level in fetal thymus (1.2 normalized copy number), fetal spleen (0.57 normalized copy number), and adult thymus (0.7 normalized copy number). Taken together, these results are consistent with a restricted expression pattern of the HOX11L2 gene and a specific association between high HOX11L2 expression and T-ALL within leukemic samples.

The expression of HOXII was observed in 3 of 28 (10.7%) samples, though 1 (UNPT5) exhibited a very low level of expression when compared to the 2 others. Virtually no expression was detected in thymus or bone marrow controls. Results are summarized in Table 2. All patients except UNPT2 were searched for the expression of HOXII, the third member of the HOXI family. One (UPNT1) exhibited low expression of HOXII in addition to HOX11L2 (data not shown).

Expression of other T-ALL oncogenes

We next searched for the expression of other T-cell oncogenes and for TAL1, LYL1, and LMO2 genes. Because those genes are expressed during normal hematopoietic differentiation, we used RQ-PCR analyses to accurately estimate the level of gene expression. For LYL1 and LMO2, samples expressing more than the bone marrow controls were considered positive.

For TAL1, samples expressing more than the weaker expressing SIL-TAL1-positive patient (UNPT8, see below) were considered positive. As shown in Table 2, using these thresholds, 14 patients were found positive for LYL1, 9 for LMO2, and 14 for TAL1. Of note, $\chi^2$ analysis suggests a significant association between LYL1 and LMO2 expression ($P = .002$).

Oncogenic lesions

We next examined the T-ALL samples for the presence of other known T-ALL frequent, specific oncogenic events. The SIL-TAL1 fusion is specific for T-ALL and is known to be due to an infra-microscopic (approximately 80-kb) deletion that leads to the transcription of the first exon of SIL fused to the coding sequences of TAL1. In our T-ALL patients, the SIL-TAL1 fusion transcript was detected in 6 of 28 (21.4%) samples (Table 2), in keeping with previous reports.8

In one instance (UPNT5), HOXII expression was observed in a patient with SIL-TAL1 fusion. No co-expression of HOXII with SIL-TAL1 or HOXII was observed in any patient.

A frequent but nonspecific event observed in T-ALL is the inactivation of the CDKN2A/CDKN2D genes, occurring mainly through the deletion of 1 or 2 gene copies.11 When genomic DNA was available, the number of CDKN2A/CDKN2D copy was estimated by RQ-PCR according to the described procedure.15 The status of this locus was also investigated by FISH analysis using BAC clones specific for the CDKN2A/CDKN2D locus on 9 patients. Taken together, homozygous deletion of CDKN2A/CDKN2D was found in 13 of 24 samples (Table 2), in keeping with previous reports,11 but does not appear to be obviously associated with the other specific lesions investigated here—HOXII, HOXII, and SIL-TAL1 expression.

Cytogenetic and FISH studies

We next wanted to compare our molecular results with cytogenetic data. Karyotype analysis of the patient samples is summarized in Table 2. Two patients exhibited a recognized chromosomal translocation: UPNT3 exhibited t(10;11)(p14;q14), expected to result in a CALM-AF10 fusion gene, and UNPT9 showed t(8;14)(q24;q11), expected to lead to transcriptional activation of the c-MYC gene.21 No cytogenetic abnormality of the LYL1(19p13), TAL1 (1p32), or LMO2 (11p13) loci was observed in these samples.

Because it detects an SIL-TAL1 fusion transcript, the RT-PCR assay allows direct detection of the rearranged TAL1 copy, which can be considered a bona fide oncogenic event. In contrast, because the known abnormalities of HOXII and HOXII genes result in transcriptional activation of these genes without any kind of fusion at the RNA level, RT-PCR analysis demonstrated only the expression of these genes, without any clue to the underlying molecular reasons. To establish whether the expression of these 2 genes was associated with a structural abnormality of the corresponding locus, we investigated the structure of chromosome 10, on which the HOXII gene lies, and of chromosome 5, on which the HOXII gene lies, using classical cytogenetic and FISH analyses.

Three samples show HOXII expression. No material for cytogenetic studies was available for UNPT5 or UNPT6. No 10q24 abnormality could be uncovered in UNPT7 through FISH using a specific BAC clone (data not shown). Based on our RQ-PCR data, HOXII was not detectably expressed during normal bone marrow differentiation, and the molecular reasons for its expression in patient samples without 10q24 abnormalities remain to be established. Similarly, no abnormality of the HOXII locus could be detected through FISH analysis using a specific BAC clone on UNPT1 material (data not shown).

HOXII expression was observed in 6 patients, but material for cytogenetic studies was available for further investigation in only 4 patients. In 2 of them (UNPT11 and UNPT24), FISH analysis uncovered t(5;14)(q35;q32), known to be associated with HOXII expression. The involvement of the HOXII locus was further established using specific BAC probes (data not shown).
the 2 other patients, conventional karyotype was normal in UNPT1 and showed a der(5) in UNPT8 (Table 2). The involvement of the HOX11L2 locus was assessed in these 2 patients using samples obtained in relapse through the use of chromosome 5 YAC and BAC probes. These probes gave a split signal on each patient’s metaphase chromosomes, between a der(5) and a submetacentric chromosome identified by whole chromosome painting as chromosome 7 in each instance (data not shown).

**HOX11L2 is a marker for minimal residual disease monitoring**

Because HOX11L2 expression is barely detected in normal hematopoietic tissues, we used RQ-PCR to monitor HOX11L2 expression and to follow the disease course in 2 patients. HOX11L2 level of expression was estimated in bone marrow samples from UNPT18 collected at complete remission (CR1) and 4 months after diagnosis. Samples were also analyzed at the time of patient UNPT18 relapse, which occurred 10 months after diagnosis, and were collected 13 and 14 months after diagnosis during CR2. A second relapse occurred 1 month after the last sample analyzed.

Similar analysis was performed on samples from UNPT24, collected at diagnosis and at CR1. Two samples, collected 4 and 9 months after diagnosis while the patient was still in CR1, were also investigated. As shown in Figure 3, samples from UNPT18 showed low but consistently detectable levels of HOX11L2 expression, even in samples obtained after remission appeared as complete, based on cytologic and immunologic data. This high level of HOX11L2 expression is likely to reflect the persistence of leukemic cells. The patient had a relapse 9 months after CR1, and again HOX11L2 expression remained detectable in CR2. Furthermore, a dramatic increase of HOX11L2 expression was observed 1 month before the occurrence of a second relapse. In contrast, in UNPT24 patient samples in CR1, HOX11L2 expression rapidly decreased and remained at very low levels. These data illustrate the potential use of HOX11L2 expression as a marker for minimal residual disease monitoring.

**Relation between specific oncogenic events, immunophenotype, and clinical data**

We next searched for an association between a specific immunophenotype and one of the T-ALL subgroup defined by the expression of HOX11, HOX11L2, or the presence of the SIL-TAL1 fusion. Data are summarized in Table 3. As previously described, the SIL-TAL1-positive samples were not associated with a precise phenotype but are in keeping with a late cortical phenotype (CD5+/CD8+).

All the HOX11L2-positive samples were positive for CD1a and CD4; the association was close to significance with CD4 ($P = .055$). Most of the samples were also positive for CD10 (4 of 6; 66.6%), CD8 (4 of 6; 66.6%), and CD34 (4 of 6; 66.6%). When compared with HOX11-positive samples, the prominent differences are a less frequent expression of CD10 and CD34 in the latter samples. Taken together, these data indicate a slightly different immunophenotype for HOX11- and HOX11L2-expressing samples. Nevertheless, each reflected an early cortical stage of thymocyte differentiation.

Six patients in this series had relapses. Among them, 4 had been shown to express HOX11L2 at diagnosis. Only one patient with SIL-TAL1 fusion (and t(8;14)) and none with HOX11 expression had relapses. Association of the clinical outcome (24-month EFS) with clinical and genetic data were submitted to statistical analysis. Selected results are shown in Table 4. WBC and HOX11L2 expression were shown to be significantly associated with a relapse event ($P = .05$ and $P = .02$, respectively). Interestingly, as shown in Figure 4, when adjusted for WBC, HOX11L2 expression remained significantly predictive of relapse in this series ($P = .03$).

**Discussion**

The high frequency of ectopic expression of the HOX11L2 gene in childhood T-ALL prompted us to draw a broad molecular picture of this disease. We investigated a panel of 28 pediatric patients with T-ALL for the presence and association of the frequent known oncogenic activation, expression of the related HOX11 and HOX11L2 genes, SIL-TAL1 fusion, and gross alteration of the CDKN2A/CDKN2D locus.

A surprisingly high number of samples was found to express at least 1 of 3 other T-cell oncogenes tested—TAL1, LYL1, and LMO2—in the absence of obvious abnormalities of their loci. In this series, the LMO2-positive samples also expressed LYL1, a feature of immature hematopoietic cells. TAL1 expression in the

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![Figure 4. Event-free survival curves of patients stratified by WBC and expressing or not HOX11L2. Note that curves of patients expressing and not expressing HOX11L2 are superimposed in the WBC < 50 x 10^9/L group.](image)

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**Table 3. Immunophenotype according to gene expression or genetic status of T-ALL samples**

<table>
<thead>
<tr>
<th>Sample</th>
<th>CD3</th>
<th>CD10</th>
<th>CD2</th>
<th>CD1a</th>
<th>CD4</th>
<th>CD8</th>
<th>CD3</th>
<th>TCR αβ</th>
<th>TCR γδ</th>
<th>My</th>
</tr>
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<tbody>
<tr>
<td>HOX11L2+</td>
<td>4 of 6</td>
<td>4 of 6</td>
<td>5 of 6</td>
<td>5 of 5</td>
<td>6 of 6</td>
<td>4 of 6</td>
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<td>1 of 5</td>
<td>3 of 6</td>
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<tr>
<td>HOX11L2−</td>
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<td>5 of 22</td>
<td>18 of 20</td>
<td>8 of 14</td>
<td>11 of 22</td>
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<td>11 of 21</td>
<td>9 of 17</td>
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<td>8 of 21</td>
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<tr>
<td>SIL-TAL1+</td>
<td>1 of 6</td>
<td>1 of 6</td>
<td>6 of 6</td>
<td>3 of 5</td>
<td>3 of 6</td>
<td>6 of 6</td>
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<tr>
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<td>8 of 22</td>
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<td>10 of 14</td>
<td>14 of 22</td>
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<td>9 of 20</td>
<td>7 of 17</td>
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<td>8 of 14</td>
<td>5 of 7</td>
<td>9 of 14</td>
<td>7 of 14</td>
<td>3 of 12</td>
<td>3 of 10</td>
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<td>7 of 12</td>
</tr>
<tr>
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<td>10 of 11</td>
<td>6 of 8</td>
<td>6 of 11</td>
<td>6 of 11</td>
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<td>10 of 16</td>
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<td>5 of 12</td>
<td>2 of 13</td>
<td>6 of 15</td>
</tr>
</tbody>
</table>

My indicates the presence of CD13, CD15, or CD33.
absence of obvious genomic alteration of its locus has been
reported in T-ALL samples, though the proportions are still
low.10–12 Whether expression of those genes reflects bona
fide oncogenic activation of a transcriptional regulatory
cascade or merely a block in differentiation remains to
be established.

The expression of HOX11 and HOX11L2 and the presence of
SIL-TAL1 fusion was observed in 3 (10%), 6 (21%), and 6 (21%)
patients, respectively, in keeping with previous reports.2,8 As
expected, a high frequency of CDKN2A/CDKN2D
mutations was observed in the group of patients with high
WBC counts (greater than 50 × 109/L) at diagnosis. Interestingly,
all patients of this group who expressed HOX11L2 had relapses.
Although the small size of this series prevents any de-
finite conclusion, this observation indicates that HOX11L2
patients have poor prognoses. Additional studies are needed
for these conclusions for clinical application.

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However, HOX11 was expressed at a very low level in UPNT5
in comparison with the 2 other patients expressing HOX11L2,
challenging a direct role in leukemogenesis. In addition, HOX11
and HOX11L1 were expressed in 2 samples in the absence of any
obvious chromosomal abnormality of the corresponding locus, a
situation already reported for HOX11.14,23 The mechanism and
the meaning of these expressions are yet to be established.

HOX11L2 expression was detected in T-ALL but not in a series of
B-ALL and AML patients, underscoring the specificity of the
HOX11L2 ectopic expression. Cytogenetic and FISH analyses
could be performed in 4 of 6 T-ALL patients expressing HOX11L2
and unraveled a t(5;14) translocation in 2 patients and a t(5;7)
translocation in the 2 others. These data show that HOX11L2
expression in T-ALL is dependent on a molecular abnormality
of 5q35. In addition, FISH analysis of 14 samples did not identify
additional patients with a 5q35 abnormality (data not shown),
confirming a tight relation between 5q35 abnormality and expres-
sion of HOX11L2. Because no or very low expression of HOX11L2
is detected in normal samples, we applied this quantitative assay to
follow the outcome of 2 patients. Interestingly, the expression level
of HOX11L2 was similar at diagnosis in both patients but differed
markedly in the early times of treatment. One patient who
maintained a relatively high level of HOX11L2 expression had a
relapse, whereas the other patient showed a quick drop in the
expression level of HOX11L2 and did not have a relapse for 10
months. These data suggest that monitoring HOX11L2 expression
could be useful to follow the clearance of leukemic cells during
the early phases of treatment, which is thought to be important for
risk assessment.26,27

SIL-TAL1 fusion has not been associated with a distinct T-ALL
clinical subgroup.8 On the contrary, HOX11L2-expressing samples
might define a T-ALL subgroup which shows a constant expression
of CD1a and CD4 because all patients analyzed to date express
both antigens.6,7

Successful treatment is achieved in approximately 60% to 75% of
T-ALL patients. Interestingly, 4 of 6 patients in this series who
had relapses were HOX11L2-expressing patients, whereas only
one exhibited SIL-TAL1 fusion and none expressed HOX11L2. All
relapse events were observed in the group of patients with high
WBC counts (greater than 50 × 109/L) at diagnosis. Interestingly,
all patients of this group who expressed HOX11L2 had relapses.
Although the small size of this series prevents any definitive
conclusion, this observation indicates that HOX11L2 patients have
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conclusions for clinical application.

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HOX11L2 expression defines a clinical subtype of pediatric T-ALL associated with poor prognosis

Paola Ballerini, Annick Blaise, Maryvonne Busson-Le Coniat, Xin Ying Su, Jessica Zucman-Rossi, Mircea Adam, Jacqueline van den Akker, Christine Perot, Beatrice Pellegrino, Judith Landman-Parker, Luc Douay, Roland Berger and Olivier A. Bernard