The Journal of Molecular Diagnostics, Vol. 22, No. 5, May 2020

ELSEVIER

the Journal of Molecular Diagnostics

jmd.amjpathol.org

Design of a Comprehensive Fluorescence *in Situ* Hybridization Assay for Genetic Classification of T-Cell Acute Lymphoblastic Leukemia

Roberta La Starza,* Valentina Pierini,* Tiziana Pierini,* Valeria Nofrini,* Caterina Matteucci,* Silvia Arniani,* Martina Moretti,* Anair G. Lema Fernandez,* Fabrizia Pellanera,* Danika Di Giacomo,* Tiziana C. Storlazzi,† Antonella Vitale,‡ Paolo Gorello,* Gabriella Sammarelli,§ Giovanni Roti,§ Giuseppe Basso,¶ Sabina Chiaretti,‡ Robin Foà,‡ Claire Schwab,‖ Christine J. Harrison,‖ Pieter Van Vlierberghe,** and Cristina Mecucci*

From the Hematology and Bone Marrow Transplantation Unit, Department of Medicine, Molecular Medicine Laboratory, University of Perugia, Perugia, Italy; the Department of Biology,† University of Bari Aldo Moro, Bari, Italy; the Division of Hematology,‡ Department of Translational and Precision Medicine, Sapienza University, Rome, Italy; the Hematology and Bone Marrow Transplantation Unit,§ Department of Medicine and Surgery, University of Parma, Parma, Italy; the Division of Pediatric Hemato-Oncology,¶ Department of Pediatrics Salus Pueri, University of Padova, Padova, Italy; the Leukaemia Research Cytogenetic Group,‖ Northern Institute for Cancer Research, Newcastle University, Newcastle-upon-Tyne, United Kingdom; and the Department of Biomolecular Medicine,** Ghent University, Ghent, Belgium*

Accepted for publication February 3, 2020.

Address correspondence to Cristina Mecucci, M.D., Ph.D., or Roberta La Starza, M.D., Ph.D., Hematology and Bone Marrow Transplantation Unit, Department of Medicine, Molecular Medicine Laboratory, CREO, P.le Menghini 9, 06132 Perugia, Italy. E-mail: cristina.mecucci@unipg.it or roberta.lastarza@unipg.it.

T-cell acute lymphoblastic leukemia (T-ALL) results from deregulation of a number of genes via multiple genomic mechanisms. We designed a comprehensive fluorescence *in situ* hybridization (CI-FISH) assay that consists of genomic probes to simultaneously investigate oncogenes and oncosuppressors recurrently involved in chromosome rearrangements in T-ALL, which was applied to 338 T-ALL cases. CI-FISH provided genetic classification into one of the well-defined genetic subgroups (ie, *TAL/LMO*, *HOXA*, *TLX3*, *TLX1*, *NKX2-1/2-2*, or *MEF2C*) in 80% of cases. Two patients with translocations of the *LMO3* transcription factor were identified, suggesting that *LMO3* activation may serve as an alternative to *LMO1/LMO2* activation in the pathogenesis of this disease. Moreover, intrachromosomal rearrangements that involved the 10q24 locus were found as a new mechanism of *TLX1* activation. An unequal distribution of cooperating genetic defects was found among the six genetic subgroups. Interestingly, deletions that targeted *TCF7* or *TP53* were exclusively found in *HOXA* T-ALL, *LEF1* defects were prevalent in *NKX2-1* rearranged patients, *CASP8AP2* and *PTEN* alterations were significantly enriched in *TAL/LMO* leukemias, and *PTPN2* and *NUP214-ABL1* abnormalities occurred in *TLX1/TLX3*. This work convincingly shows that CI-FISH is a powerful tool to define genetic heterogeneity of T-ALL, which may be applied as a rapid and accurate diagnostic test. *(J Mol Diagn 2020, 22: 629—639; https://doi.org/10.1016/j.jmoldx.2020.02.004)*

T-cell acute lymphoblastic leukemia (T-ALL) is a rare leukemia subtype, accounting for 15% of pediatric and 25% of adult ALL cases.[1] The disease is heterogeneous at the clinical and biological level and, mainly in adults, is characterized by a poor response to chemotherapy.[2] In the updated World Health Organization classification,[3] T-ALL is defined only by morphologic features and immunophenotype, without inclusion of molecular-cytogenetic criteria to classify patients with T-ALL into specific genetic entities. However, gene

R.L.S. and V.P. contributed equally to this work.

Supported by Associazione Italiana Ricerca sul Cancro (AIRC) Investigator grant 15525 (C.Me.) and Start-up Investigator grant 17107 (G.R.); Progetti di Ricerca di Rilevanza Interesse Nazionale (PRIN) 2017 code 2017PPS2X4 (C.Me.); the Sergio Luciani Association, Fabriano (C.Me.); Fondazione Cassa di Risparmio Perugia grants 2016.0113.021 (C.Me.) and 2018.0418.021 (R.L.S.); a Società Italiana di Ematologia Sperimentale (SIES) 2018 A. Maiolo specializzata in citogenetica grant (T.P.); European Research Council starting grant 639784 (P.V.V.); and Bloodwise, UK (C.S.).

Disclosures: None declared.

https://doi.org/10.1016/j.jmoldx.2020.02.004

Table 1 Clinical and Hematologic Characteristics of Patients With T-Cell Precursor Acute Lymphoblastic Leukemia

Characteristic	Children (n = 225)	Adults (n = 113)	Total cohort (n = 338)	P value
Age, years*				
Range	3–18	19–78	3–78	—
Median	10	35	15	—
Median	10	35	35	—
Sex				
Male	168	82	250	—
Female	57	31	88	—
Phenotype	127	96	223	—
Typical	110	68	178	—
ETP-ALL	17	28	45	—
Total WBC count $\times$ 10^9/L	63	92	155	—
$<100 \times 10^9$/L	32	72	104	—
$\geq 100 \times 10^9$/L	31	20	51	—
Classification	213	113	326	—
TAL/LMO	71	18	89	0.0007
HOXA	40	36	76	0.0061
TLX3	41	9	50	0.0092
TLX1	9	18	27	0.0005
NKX2-1/2-2	16	1	17	0.0083
MEF2C	1	1	2	—
Unclassified	35	30	65	—
Failed	12	0	12	—
Numerical changes	22	15	37	—
Imbalances	180	92	272	—
Triploidy or tetra-ploidy	3	1	4	—
Normal CI-FISH	10	3	13	—

*Fisher exact tests (SPSS version 20; IBM) were used to evaluate distribution of the main genetic groups according to age.

—, not applicable; CI-FISH, comprehensive fluorescence *in situ* hybridization; ETP-ALL, early precursor T-cell acute lymphoblastic leukemia; WBC, white blood cell.

expression profiling has found that T-ALL comprises distinct subgroups, according to the level of expression of (onco) genes coding for transcription factors critical in hematopoiesis and/or T-cell development, maturation, and differentiation [ie, *TAL/LMO*, *HOXA*, *TLX3*, *TLX1*, *NKX2-1/2*-2, and *MEF2C* (type A abnormalities)].[4–7] Furthermore, gene expression profiling has also identified an immature gene expression signature, which characterizes a specific subtype of T-ALL, termed early T-cell precursor ALL (ETP-ALL). In these cases, the leukemic blasts express myeloid or stem cell antigens (ie, CD34/CD117, CD13, and/or CD33), whereas they are negative for CD1a, CD4, and CD8, and negative or weakly positive for CD5.[8] Although ETP-ALL has common immunophenotypic and expression markers, its genomic background appears largely heterogeneous, with involvement of multiple T-lymphoid and myeloid genes.[8,9]

In addition to type A abnormalities, integrated genomic analysis has uncovered a number of additional aberrations, including activating and inactivating mutations, chromosomal gains and losses, and balanced or unbalanced translocations. These events, herein referred to as type B, involve epigenetic factors, ribosomal proteins, and proteins that belong to signaling pathways, such as JAK/STAT, RAS, WNT, and PI3K/AKT.[1,5,6,9–11] They occur nonrandomly in close association with the primary genetic changes, indicating that specific concurrent events are required for leukemic development and expansion within each group.[10–15]

Because of the large number of genes and the variability of molecular mechanisms underlying their deregulation, more than one technological approach is usually required for complete genetic characterization of T-ALL. Thus, translation of biomolecular information into routine diagnostics and clinical practice has remained challenging, and as a result, comprehensive, prospective studies within clinical trials are lacking. Nevertheless, genetic markers, especially if combined with minimal residual disease quantification, may fine tune individual risk stratification and assist in predicting sensitivity to new drugs.[16–25] The French group FRALLE[20] found that persistence of minimal residual disease after induction, together with unfavorable genetic characteristics, including *N/K-RAS* mutations and *PTEN* alterations, improved risk assessment. Other examples include *MYC* translocations[12] and the absence of *CDKN2A* deletions,[21] which both appeared to identify high-risk T-ALL subgroups. Moreover, preclinical studies have highlighted that deregulation of specific molecular targets, such as *NOTCH1*[21] and *JAK/STAT*[23] pathways and *BCL2*,[24] *PIM1*,[25] and *IL7R*[26] oncogenes, predicted sensitivity to specific inhibitors.

The aim of this study was to design a comprehensive interphase fluorescence *in situ* hybridization (CI-FISH) test as a robust and comprehensive diagnostic molecular-cytogenetic

tool to investigate known type A and recurrent type B genetic changes. Validation in retrospective cohorts of pediatric and adult patients enabled accurate genetic classification in 80% of cases and identified targetable lesions in approximately 85%.

Materials and Methods

Study Cohort

A total of 338 patients with T-ALL, enrolled in the UK (Medical Research Council) and Italian (Gruppo Italiano per le Malattie Ematologiche dell'Adulto (GIMEMA)-Italian Pediatric Hematology and Oncology Group) clinical trials, were included in this study (Table 1). All patients or their parents or guardians gave informed consent for sample collection and molecular analyses, in agreement with the Declaration of Helsinki. The study was approved by the local bioethical committee (research project 3397/18). There were 225 children and 113 adults, with a male-female ratio of 2.8 (250 males and 88 females). According to the immunophenotype, 223 cases were classified as ETP-ALL/near−ETP-ALL ($n = 45$) or non−ETP-ALL ($n = 178$) (Supplemental Table S1). Previously identified *NOTCH1/FBXW7* mutations were present in 139 of 215 patients investigated. The panel of genes involved in T-ALL were inferred from available gene expression profile, molecular cytogenetics, and sequencing data.[4−6,9,10] CI-FISH studies were performed in a two-step diagnostic algorithm (Figure 1).[16,27]

FISH Probes

DNA clones were selected to study 21 oncogenes, whose genomic rearrangements were known to affect *TAL/LMO*, *HOXA*, *TLX1*, *TLX3*, *MEF2C*, or *NKX2-1* deregulation, for classification of cases into these specific subgroups (Supplemental Table S2). An additional 49 genomic clones were chosen for investigation of genomic imbalances and/or translocations that involved other T-ALL−related genes and loci (Supplemental Table S3). For each gene or locus, clones were selected according to the type(s) of cytogenetic abnormalities under investigation. Break-apart probe sets were designed to study structural rearrangements, such as translocations, tandem duplications, and inversions, that involved promiscuous genes. To investigate deletions that involved oncosuppressor genes, bacterial artificial chromosomes (BACs) and/or fosmids, spanning the entire or part of a gene, was used (Supplemental Tables S2−S4).

To characterize the fusion partners of promiscuous genes, for example, *MLLT10* with *PICALM*, *DDX3X*, *HNRNPH1*, or *NAP1L1*, *NUP214* with *SET*, *SQSTM1*, or *ABL1*, *NUP98* with *RAP1GDS1* or *PSIP1*, and *KMT2A* with *MLLT10*, *MLLT1*, *ELL*, *AFF1*, or *AFDN*, dual color dual fusion probe sets were designed (Supplemental Table S4). A specific dual color break-apart assay was designed (Figure 2, A−D) to characterize *TLX1* intrachromosomal rearrangements in six patients (PGTALL106, PGTALL160, PGTALL195, PGTALL209, PGTALL215, and PGTALL275). Commercial probes were used to study centromeric regions of selected chromosomes to rule out numerical chromosomal changes. In all cases that harbored a monoallelic *ETV6* deletion, *CDKN1B*, an oncosuppressor gene that mapped 800 Kb of centromeric *ETV6*, was also tested.

Directly labeled FISH probes were designed from BACs (RPCI-11 Human Male BAC Library and Caltech BAC CTB, CTC, and CTD Libraries), P1-derived artificial chromosomes (PACs; RPCI-1 and RPCI-5; Human Male PAC Library), and Fosmids (WIBR-2 Human Fosmid Library; National Center for Biotechnology Information, *https://www.ncbi.nlm.nih.gov/genome/gdv*; UCSC Genome Browser, University of California, Santa Cruz, *https://genome.ucsc.edu*, last accessed July 30, 2019).

Bacterial clones were cultured in Luria-Bertani medium that contained antibiotics; plasmid DNA was extracted using Qiagen Plasmid Midi Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. DNA was labeled with a Nick Translation Kit (Abbott Molecular, Des Plaines, IL) using SpectrumOrange dUTP, SpectrumGreen dUTP, and SpectrumAqua dUTP Vysis (Abbott Molecular). Dual or

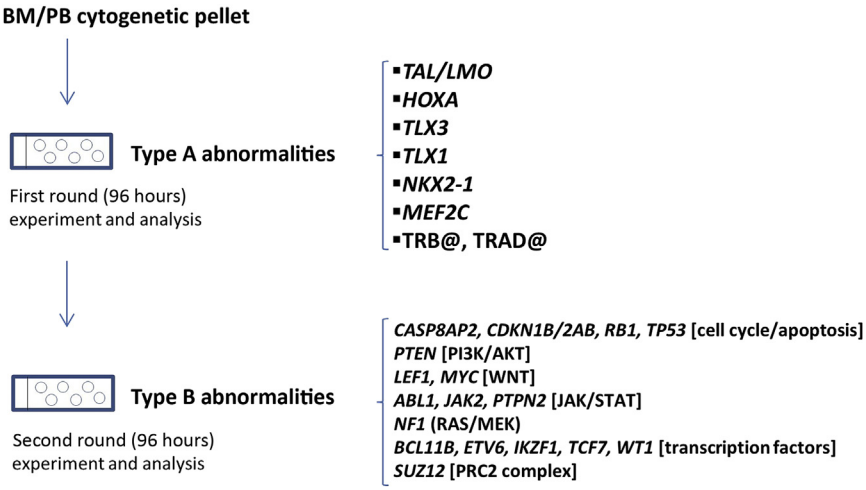
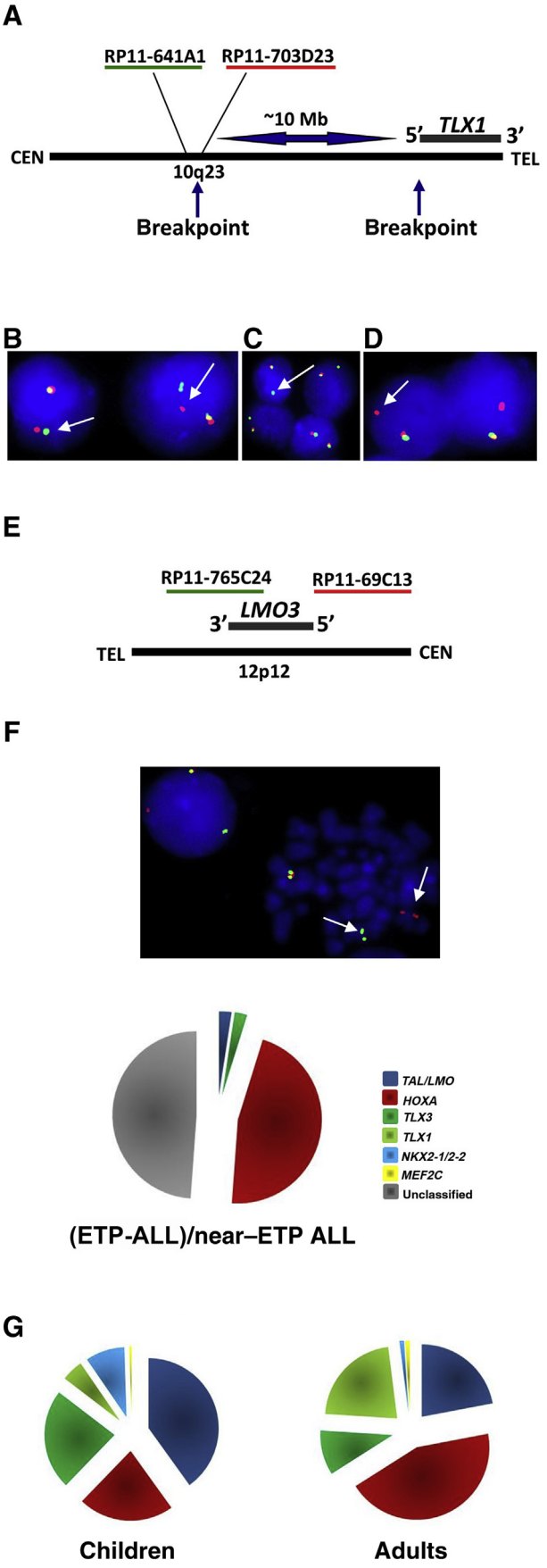


Figure 1 Schematic representation of the comprehensive fluorescence *in situ* hybridization flowchart applied to diagnostic samples of T-cell acute lymphoblastic leukemia. Cytogenetic pellets can be obtained after direct or 24-hour culturing. The first round provides information on type A abnormalities and TR@ involvement in approximately 4 days; the second round detects additional recurrent abnormalities (type B) in an additional 4 days. All tests are performed each time. BM, bone marrow; PB, peripheral blood; TF, transcription factors.



triple color assays were designed by combining differentially labeled probes into a ready-to-use working solution.

FISH Protocol

Briefly, after labeling, probes were resuspended in a 10% dextran sulfate, 50% formamide, and 4× saline citrate hybridization mixture with 20 μg of Human Cot-1 DNA Invitrogen (Fisher Scientific, Milano, Italy). Slides were prepared from diagnostic peripheral blood (PB) and/or bone marrow (BM) cytogenetic pellets, spotted in six to eight round areas on each slide. Slides were pretreated in a Coplin jar with protease solution [Dulbecco's phosphate-buffered saline (DPBS), 0.01 mol/L hydrochloride, and 0.01% pepsin] at 37°C for 30 minutes, rinsed with DPBS at room temperature for 5 minutes, and fixed in DPBS, 4% formaldehyde, and 0.05 mol/L magnesium chloride at room temperaturefor 8 minutes. After a postfixation wash with DPBS, slides were dehydrated through a cold ethanol series (70% to 85% to 100%) of 2 minutes each. Hybridization mix was applied to slides (3 μL to each area), and areas were covered by a round 10-mm coverslip and sealed with rubber cement. Slides and probes were co-denaturated on a hot plate at 76°C for 10 minutes, hybridized overnight in a moist chamber at 37°C, and washed with 0.4× saline citrate and 0.3% Nonidet P-40 (Sigma-Aldrich, St. Louis, MO) at 74°C for 2 minutes and at room temperature for 2 minutes. Slides were counterstained with DAPI (0.5 μL 0.1% DAPI in 1 mL of Vectashield mounting medium, Vector Laboratories, Burlingame, CA), 30 μL each slide. Between 120 and 200 nuclei were analyzed using a fluorescence microscope (Olympus BX61, Olympus, Milano, Italy). When available, abnormal metaphases were analyzed using a highly sensitive CV-M4+CL progressive scan camera (JAI, Copenhagen, Denmark) and the CytoVision image analysis software version 4.5.4 (Leica Microsystems, Wetzlar, Germany).

Abnormal hybridization patterns were i) split signal (one fusion signal and separate green and red signals), ii) tandem duplication/trisomy (three signals), iii) deletion/monosomy (one signal), and iv) partial deletion (one fusion signal and one orange or one green signal). The cut-offs for the

Figure 2 **A:** Schematic representation of the long arm of chromosome 10 with mapping of *TLX1* and DNA clones used to define the breakpoints (**blue arrows**) of paracentric inversions and cryptic deletions. **B:** Fluorescence *in situ* hybridization (FISH) in case PGTALL106 with paracentric inversion. **C:** FISH in case PGTALL209 with a cryptic deletion. **D:** FISH in case PGTALL275 with paracentric inversion and an accompayning deletion. **White arrows** in **B**–**D** indicate the abnormal hybridization patterns. **E:** Schematic representation of the short arm of chromosome 12 with mapping of *LMO3* and of the clones used in a break-apart assay. **F:** FISH in case PGTALL44 with *LMO3* translocation (**arrows** indicate split green/orange signals). **G:** Pie charts show the distribution of the six main genetic groups according to the age (percentages were referred to the 261 classified cases (ie, 178 children and 83 adults)) and to an immature phenotype [45 cases with early T-cell precursor acute lymphoblastic leukemia (ETP-ALL)/near–ETP-ALL]. Original magnification, ×100 (**B**–**D** and **F**). CEN, centromere; TEL, telomere.

false-positive detection of abnormal clones were set at the upper values of abnormal patterns seen in 10 PB samples from healthy donors, from scoring 200 nuclei for each sample. Split, duplication, and trisomy patterns were considered to be positive when found in ≥3% of interphase cells and monosomy, deletion, and partial deletion when found in ≥6%. Because the atypical hybridization pattern with multiple signals (>5) was never observed in healthy controls, the cut-off levels for amplification was set at ≥0.5%.

Statistical Analysis

The χ^2 and Fisher exact tests (SPSS version 20; IBM, Armonk, NY) were used to analyze the distribution of type A abnormalities according to age and phenotype and to assess the correlation between recurrent type B aberrations, the six main genetic subgroups, and phenotype.

Shallow Whole-Genome Sequencing

Shallow whole-genome sequencing (sWGS)[28] is a low-coverage next-generation sequencing technique that has been designed to identify copy number variations. It was performed to characterize cases PGTALL215 and PGTALL2759 (Supplemental Table S1), which showed atypical hybridization patterns with the break-apart assay for the *TLX1* gene, as previously described.[28] The amplified fragments were sequenced on the HiSeq 3000 (Illumina Inc., San Diego, CA), according to the manufacturer's instructions. The minimal number of reads per sample was set qat 10 million (mean coverage of 0.4×). Using a bin size of 100 Kb, the R-Bioconductor package QDNAseq.hg19[29] was applied to visualize the DNA copy-number profile and call genomic aberrations. Each genome profile (line view and chromosome view) was manually checked for genomic abnormalities. All profiles were visualized using the online tool ViVar (Center for Medical Genetics, *http://cmgg.be/vivar*; last accessed July 30, 2019).[30]

Results

Rearrangements and Clonality

In a two-step diagnostic workflow (Figure 1), CI-FISH detected 890 abnormalities in 325 of 338 cases (96%), with a median and a mean of 3 per case (range, 1 to 8). No chromosomal aberrations were identified in 13 cases that displayed a normal hybridization pattern with all the CI-FISH assays (Supplemental Table S1). Genomic imbalances were prevalent, including 455 deletions, 56 duplications, and nine amplifications. A total of 49 trisomies were detected, with the most frequent being gains of chromosomes 8 ($n = 17$), 6 and 7 ($n = 6$ cases each), 10 ($n = 5$), 19 ($n = 4$), and 4, 18, and 21 ($n = 3$ each); only one monosomy (chromosome 18) was identified (PGTALL202) (Supplemental Table S1). Balanced and unbalanced translocations accounted for 316 events and involved T-cell receptors in 117 cases. Numerical aberrations, consistent with triploidy and tetraploidy, were observed in four cases. Clonal primary changes were observed in 15% to 97% of cells, in agreement with the level of PB/BM leukemic infiltration by morphologic analysis (data not shown). Secondary changes were detected in the main clone or subclones at a varible percentage of 3% to 90%. In a single case (PGTALL4) (Supplemental Table S1), a subclonal *NUP214-ABL1* amplification was detected in 0.5% of cells.

Type A Abnormalities

CI-FISH successfully classified 261 of 326 T-ALL cases (80%) into one of the six established genetic categories, whereas 65 cases remained unclassified (Table 1). In 12 cases complete screening for abnormalities that involved primary oncogenes was not possible because of the lack or poor quality of samples, for example, low efficiency of CI-FISH experiment (>20% of cells lacking hybridization signals), insufficient material, and/or weak hybridization signals.

TAL/LMO was the most highly represented group, comprising 89 positive cases. Genomic abnormalities classifying cases within the *TAL/LMO* group involved *TAL1* ($n = 49$), *LMO2* ($n = 19$), *TAL2* ($n = 7$), *LMO1* ($n = 5$), *TAL1* and *LMO1/2* ($n = 6$), *TAL1* and *LMO3* ($n = 1$), *LMO3* ($n = 1$) (Figure 2, E and F), and *LYL1* with *LMO2* ($n = 1$) (Supplemental Table S1). The *HOXA* subgroup comprised 76 cases, harboring translocations of *MLLT10* ($n = 24$), *HOXA* ($n = 22$), *NUP214* ($n = 10$), *KMT2A* ($n = 10$), and *NUP98* ($n = 9$). In one case (PGTALL 30), both *PICALM-MLLT10* and *TRG-HOXA* rearrangements were present (Supplemental Figure S1). *TLX3* rearrangements were found in 50 cases and involved various partners: *BCL11B* ($n = 37$), *CDK6* ($n = 2$), or undetermined ($n = 11$).

TLX1 was involved in balanced ($n = 23$) or unbalanced ($n = 4$) rearrangements with *TR@* ($n = 19$) or non-*TR@* partners ($n = 8$). Six of the latter cases had intrachromosomal rearrangements: a cryptic deletion (PGTALL215), paracentric inversions (PGTALL106, PGTALL160, PGTALL195, and PGTALL209), or paracentric inversion with accompanying deletions (PGTALL275) (see below). Notably, all these intrachromosomal rearrangements resulted in the juxtaposition of *TLX1* close to regulatory sequences (chromosome 10: 92,668,000 to 92,770,000) located approximately 10 Mb upstream of its 5′ end (dbSuper, *http://asntech.org/dbsuper/index.php*; EnhancerAtlas, *http://www.enhanceratlas.org*; and VISTA Enhancer Browser, *https://enhancer.lbl.gov*; last accessed July 30, 2019).

The 17 cases assigned to the *NKX2-1* group harbored translocations of *NKX2-1* ($n = 15$), *NKX2-2* ($n = 1$), or *MYB* ($n = 1$). Lastly, *SPI.1* and *RUNX1* translocations, both underlying *MEF2C* overexpression,[6] were detected in one child and one adult, respectively.

Patients were allotted to the main categories based on age ($P < 0.001$) (Figure 2G). The higher prevalence of *TAL/LMO*

Table 2 Recurrent Secondary Changes in 338 Pediatric and Adult Cases of T-Cell Acute Lymphoblastic Leukemia

Gene/locus	Cases, no./total no. (%)							*P* value*
	Overall	*TAL/LMO*	*HOXA*	*TLX3*	*TLX1*	*NKX2-1/2-2*	*MEF2C*	
CDKN2AB	207/326 (63)	67 (77)	**31 (42)**	38 (81)	22 (85)	13 (76)	0	<0.001
CASP8AP2	41/324 (13)	**21 (24)**	8 (11)	1 (2)	2 (11)	3 (18)	0	0.06
TP53	9/165 (5)	0	**6 (10)**	0	0	0	0	NS
RB1	6/217 (3)	0	3 (5)	1 (5)	0	0	0	NS
PTEN	27/333 (8)	**17 (19)**	3 (4)	0	0	0	0	<0.001
PTPN2	22/331 (7)	0	3 (4)	**9 (18)**	**8 (30)**	2 (12)	0	<0.001
NUP214-ABL1	8/325 (2)	0	1 (1)	**6 (12)**	**1 (4)**	0	0	0.001
NF1	19/330 (6)	0	5 (6)	1 (2)	0	0	1	<0.001
SUZ12	19/330 (6)	0	5 (6)	1 (2)	0	0	1	<0.001
NOTCH1/FBXW7	139/215 (65)	21 (44)	38 (75)	22 (81)	12 (80)	15 (94)	2	<0.001
LEF1	24/313 (8)	9 (10)	1 (1)	2 (4)	0	**5 (29)**	0	0.003
MYC	15/306 (5)	9 (10)	2 (3)	1 (3)	1 (4)	0	0	NS
ETV6	15/264 (6)	0	5 (8)	1 (3)	**5 (25)**	0	0	0.004
WT1	14/259 (5)	0	7 (11)	5 (14)	1 (5)	0	0	0.005
BCL11B	9/271 (3)	1 (1)	2 (3)	0	2 (11)	**3 (18)**	0	0.012
IKZF1	6/214 (3)	0	1 (2)	2 (6)	0	1 (20)	0	NS
MYB	14/280 (5)	2 (3)	6 (10)	2 (6)	1 (4)	0	0	NS
TCF7	21/283 (7)	0	**13 (21)**	0	0	0	1	<0.001
DUP(9Q)	14/332 (4)	4 (5)	3 (4)	0	2 (7)	1 (6)	0	NS
Trisomy 8	17/306 (5)	3 (3)	2 (3)	2 (5)	4 (16)	0	0	NS
Trisomy 6	6/280 (2)	2 (3)	3 (4)	0	0	0	0	NS
Trisomy 7	6/318 (2)	4 (5)	1 (1)	1 (2)	0	0	0	NS

Although the table shows distribution of secondary changes in classified cases, the overall percentage was estimated on the total samples analyzed for each gene or abnormality, including unclassified and undetermined cases. The percentage was rounded up if >0.5 and down if ≤0.5; the deletion of *NF1* and *SUZ12* was considered as a unique event because they were lost together in cases with del(17)(q11.2); *NOTCH1/FBXW7* mutations are summed with the other *secondary changes*; privileged association and inverse correlations are in bold.

*χ^2 test.

NS, nonsignificant.

(40% versus 22%; $P = 0.0007$) and *TLX3* (23% versus 10%; $P = 0.0092$) in children and of *TLX1* in adults (5% versus 22%; $P = 0.0005$) confirmed previous reports.[1,5,11] Two novel findings that emerged from this study were the significant association of the *NKX2-1/2-2* group with childhood (9% versus 1%; $P = 0.0083$) and of *HOXA* with adult T-ALL (44% versus 22%; $P = 0.0061$). Correlating the genetic groups with phenotypes, *HOXA* was the most highly represented group in ETP-ALL/near−ETP-ALL (46.3% of cases; $P < 0.001$) (Figure 2G).

Characterization of Intrachromosomal Rearrangements of *TLX1*

The hybridization pattern of the *TLX1* probe set RP11-108L7 (centromeric to *TLX1*) and RP11-107I14 (telomeric to *TLX1*) (Supplemental Table S2) detected not only balanced *TLX1* translocations but also two new rearrangements. Namely, in cases PGTALL106 (Figure 2B), PGTALL160, PGTALL195, and PGTALL209 (Figure 2C), the hybridization pattern was consistent with a paracentric inversion, whereas in cases PGTALL215 and PGTALL275 (Figure 2D), a cryptic deletion was found at the centromeric side of the gene. In these two latter cases, sWGS confirmed two different genomic losses at 10q23 and precisely indicated their smaller extent (Supplemental Figure S2). The break-apart FISH probe set (RP11-641A1 and RP11-703D23), specifically designed to characterize the centromeric breakpoints of these intrachromosomal rearrangements (Figure 2A), showed that inversions and deletions shared the same breakpoint, approximately 10 Mb centromeric of *TLX1* that placed *TLX1* close to known enhancer sequences (Figure 2, A−D).

Recurrent Type B Abnormalities and Their Distribution

Type B aberrations were detected in 229 of 262 classified cases (87%), 53 of 65 unclassified cases (81%), and 11 of 12 cases (91%) for which screening for primary changes was incomplete (Supplemental Table S1). Among them, 17 genomic rearrangements, including partial chromosomal losses and gains or balanced translocations, and three chromosomal trisomies were considered to be recurrent because they were found in five or more cases (Table 2). An unequal distribution of cooperating genetic defects was also identified for the different genetic subtypes (Figure 3).

Chromosome Deletions

One or more deletions were found in 267 of 338 cases (79%). Overall, the most frequently deleted genes and loci

were *CDKN2AB* (63%), *CASP8AP2* (13%), *PTEN* (8%), *LEF1* (8%), *TCF7* (7%), *PTPN2* (7%), *NF1/SUZ12* (6%), and *ETV6* (6%) (Table 2). Deletions of *CDKN2AB*, including monoallelic and/or bi-allelic, partial or complete, were found in 76% to 85% of *TAL/LMO*-, *TLX1/3*-, and *NKX2-1/2*-2- but only in 42% of *HOXA*- positive cases ($P < 0.001$). Within the *HOXA* group, *CDKN2AB* deletions were detected in 50% of non−ETP-ALL and 31.5% of ETP-ALL cases. Deletions of 6q were the second most frequent losses (47 of 324 cases). Widely heterogeneous, they involved chromosomal bands 6q14 to 6q23 and encompassed the oncosuppressor *CASP8AP2*/6q14 in 41 cases (approximately 13%) (Supplemental Table S5). Loss of *CASP8AP2* was significantly associated with the *TAL/LMO* group ($P = 0.01$). *PTEN* deletions occurred in 8% of the entire study cohort but were enriched within the *TAL/LMO* subgroup in 19% of positive cases ($P = 0.05$). *ETV6* deletions co-occurred with *CDKN1B* deletions in all cases and were significantly associated with *TLX1*, whereas *PTPN2* deletions were enriched within the *TLX1* and *TLX3* groups. Losses of *LEF1* and *BCL11B* were highly recurrent in the *NKX2-1/2-2* subgroup. Lastly, genomic losses of *TCF7* and *TP53* were exclusively found in the *HOXA* subgroup.

Gains or Amplifications and Trisomies

Recurrent genomic gains involved *MYB* and a 9q34 region of variable size. Three copies of *MYB* were detected in 14 cases (5%), which resulted from tandem duplication ($n = 11$) or large 6q duplications ($n = 3$); dup(9)(q34) involved *NOTCH1*, *NUP214*, *ABL1*, and/or *TAL2* and showed no specific association with the main genetic subgroups. Unique recurrent amplifications involved the *NUP214-ABL1* fusion gene in 8 cases, seen as extrachromosomal and intrachromosomal in five and three cases, respectively. The *NUP214-ABL1* rearrangement was found in highly variable clone sizes, ranging from 0.5% to 100%, and was confined to the *TLX* (*TLX1* 4% and *TLX3* 12%) and *HOXA* (1%) categories.

Balanced Translocations

The most recurrent balanced translocations involved the *MYC* oncogene [15 of 306 cases (5%)], with *TR@* ($n = 7$) or non-*TR@* ($n = 8$) partners. Rare translocations and rearrangements, occurring in <1% of cases, involved *JAK2* (PGTALL50, PGTALL59, and PGTALL144), *CCND2* (PGTALL63 and PGTALL172), and *ETV6* (PGTALL69 and PGTALL234) (Supplemental Table S1).

Discussion

The CI-FISH assay was successfully applied in pediatric and adult T-ALL and identified an abnormal signal pattern in almost all cases (98%), allowing genetic classification in 80% of them. Compared with the single-nucleotide polymorphism (SNP) array, CI-FISH was more accurate in identification of small clonal and subclonal changes, thus providing a more favorable approach for genetic classification of T-lymphoblastic lymphomas, in which BM leukemic infiltration is ≤25%.[3] Moreover, SNP array detection is limited to unbalanced rearrangements, characterized by partial chromosomal gains and losses, whereas CI-FISH clearly identified balanced changes that are undetected by SNP array. However, an integrated approach that included SNP array, with next-generation techniques, for example, whole genome, exome, and RNA sequencing, provided accurate genetic classification in 91% of childhood T-ALL.[10] Although sequencing approaches will likely replace molecular cytogenetics in the future because it provides information on the entire genomic landscape of sequence variants, currently diagnostic laboratories are still better equipped to perform FISH.

Besides detecting all known type A abnormalities, CI-FISH unveiled new targets and new mechanisms of gene deregulation by identifying cryptic chromosomal aberrations, new *TR@* rearrangements, and gene promiscuity. As expected for early events, type A abnormalities were always present in the main clone in a percentage of positive cells consistent with the degree of BM and PB leukemic infiltration. Overall, 80% of cases were classified into one of the six major genetic groups (ie, *TAL/LMO*, *HOXA*, *TLX1*, *TLX3*, *NKX2-1*, and *MEF2C*).[4−8,10]

The *HOXA* subgroup first emerged as being significantly associated with adult T-ALL. However, here, its significant association with the ETP-ALL and near−ETP-ALL phenotype was also confirmed (Figure 2G).[5,31]

Two additional significant associations were the known association of *TLX1* in adults and the newly emerged link between the *NKX2-1* subgroup and childhood T-ALL. Interestingly, these two subgroups share a cortical thymocytic arrest and a similar and overlapping gene expression profiling,[6] suggesting that the two oncogenes mark closely related leukemogenic entities occurring in distinct patient age groups.

Of interest, because the testing of primary oncogenes was expanded to all four members of the rhombotin family of cysteine-rich LIM domain genes, not only *LMO1* and *LMO2* but also *LMO3* were found to be recurrently involved in *TRB* translocations in T-ALL (Figure 2, E and F). *LMO3* shares structural and functional homology with the other family members and has been found to act as an oncogene in neuroblastoma.[32] Normally silenced in T-lymphocytes, *LMO3* has been already reported as a new leukemogenic target in T-ALL, resulting from the t(7;12)(q34;p13) translocation that positions the *TRB* enhancer close to *LMO3*, leading to its transcriptional activation.[33] On the basis of the structural similarities among the three members of the *LMO* family,[34,35] as well as the association of both *LMO1/2* and *LMO3* with *SIL-TAL1*, cases with *TRB-LMO3* were interpreted as likely belonging to the *TAL/LMO* group.

Another discovery was that approximately 30% of *TLX1* rearrangements were independent of *TR@* translocations.

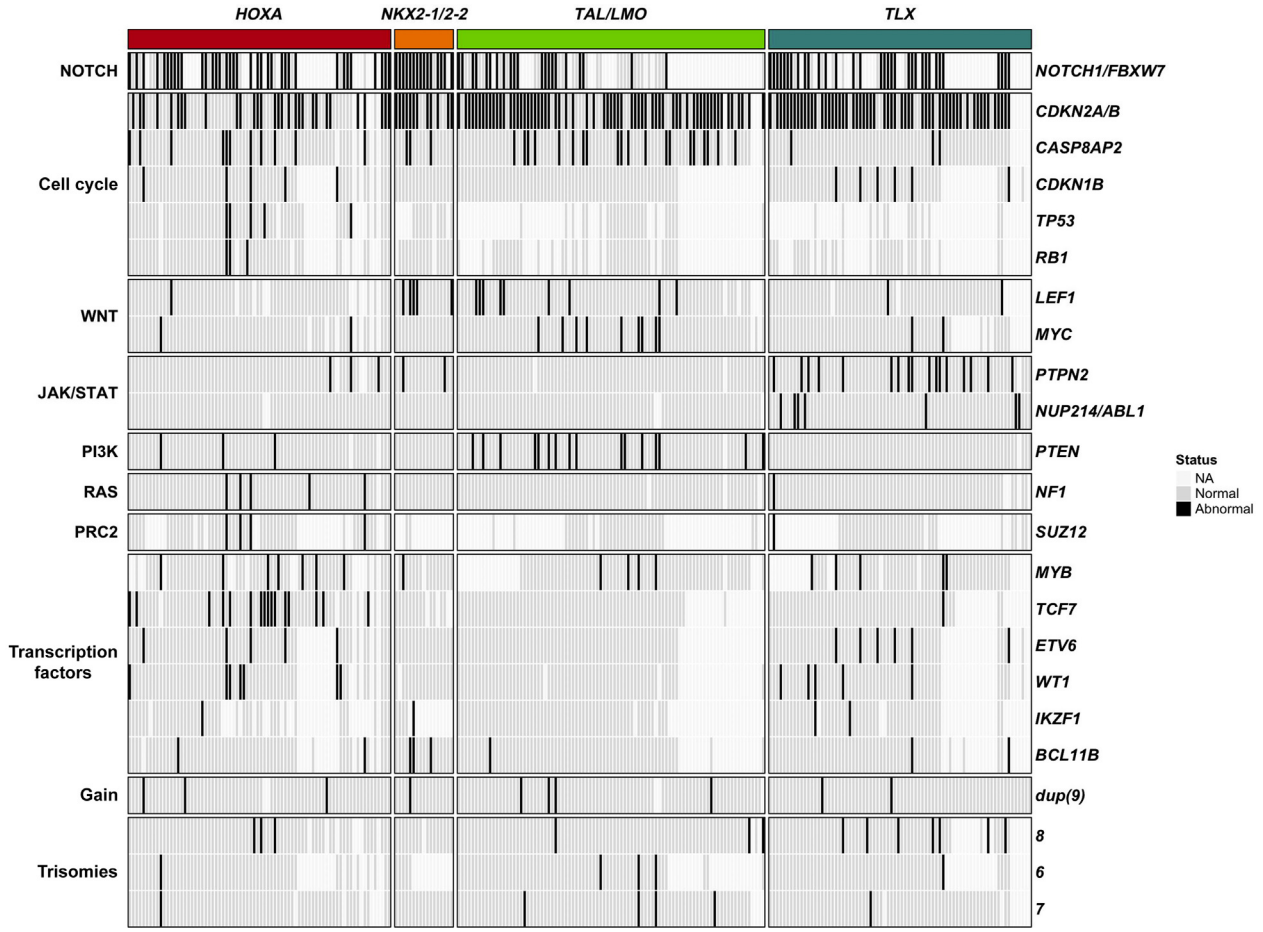


Figure 3 The heat-map shows the distribution of type B abnormalities according to the main genetic groups (*TLX1* and *TLX3* were grouped together; the two cases of *MEF2C*-positive T-cell precursor acute lymphoblastic leukemia were not included). NA, not applicable.

Most of these non-*TR@* abnormalities involved paracentric inversions and/or cryptic interstitial deletions that invariably juxtaposed *TLX1* to nearby regulatory sequences within 10q23. Of note, these cases shared the same 10q23 breakpoints reported by Liu et al[10] in approximately 29% of pediatric *TLX1*-positive T-ALL cases, thus indicating that intrachromosomal rearrangements are the second most frequent mechanism underlying *TLX1* transcriptional activation (Figure 2A).

Another noteworthy finding was the higher percentage of *HOXA*-positive cases than previously reported in both children[5] and adults,[36] probably because CI-FISH has the capacity to detect translocations that involve the full range of promiscuous genes leading to *HOXA* expression. Interestingly, one *HOXA*-positive leukemia case was found to carry two primary hits, *PICALM-MLLT10* and *TRG-HOXA*, within the same leukemic clone (Supplemental Figure S1).[37] The novelty of this case lies in the involvement of the *TRG* locus as a recurrent translocation partner of T-ALL–related oncogenes.[38] The involvement of TRG should be tested for future molecular-cytogenetic assays, especially to elucidate as yet unknown mechanisms of gene deregulation and prognostic biomarkers.

In keeping with the accepted model of a multistep leukemogenic process, CI-FISH detected multiple type B aberrations, confirming that accumulation of several abnormalities is necessary for disease progression and for informing on specific associations among different cooperative events. Overall, at least one actionable pathway was identified in approximately 85% of cases (Table 2 and Figure 3). The most frequent rearrangements included genes involved in cell cycle and apoptosis (*CDKN2A*, *CDKN1B*, *CASP8AP2*, *RB1*, and *TP53*; 65%), *WNT* (*LEF1*, *MYC* 13%), *PI3K/AKT* (*PTEN* 8%), *JAK/STAT* (*PTPN2*, *NUP214-ABL1* 8%), and *RAF/MEK/ERK* (*NF1* 6%) signaling pathways or of the epigenetic complex *PRC2* (*SUZ12* 6%). Preferential associations were corroborated between primary and secondary changes, such as that of *PTEN* and *MYC* with *TAL/LMO* and *NUP214-ABL1* and *PTPN2* with *TLX1/3*.[9–15] Of interest, previously unknown combinations, such as *TCF7* and *TP53* deletions in *HOXA*-positive cases or *LEF1* in the *NKX2-1* group, were demonstrated. Specific links were also observed between secondary changes and an immature phenotype. As expected, *NOTCH/FBXW7* mutations and *CDKN2AB* deletions were

underrepresented in the ETP-ALL and near−ETP-ALL group, which, on the other hand, had a high incidence of *TCF7*, *WT1*, *ETV6*, *RB1*, *NF1*, and *TP53* deletions (Supplemental Table S6). In addition to genomic rearrangements, mutations of members and modulators of the *NOTCH* pathway (*NOTCH1* and/or *FBXW7*) were detected in 65% of the cases. As previously reported by Zuurbier et al,[39] a significant lower frequency of *NOTCH1/FBXW7* mutations was found in cases belonging to the *TAL/LMO* subgroup (Table 2), which, on the other hand, showed a closer link with the *PI3K/AKT* pathway (19% of *PTEN* deletions versus 8% in the overall cohort, $P = 0.05$).[10,40]

Taken together, these findings confirmed that the synergistic effect of specific deregulated genes and pathways is necessary for leukemia onset, maintenance, and progression, suggesting that identification of multiple specific targets,[22−26,41] within the same leukemia sample, should be considered for combinatorial therapeutic intervention.[42−44]

Conclusion

In T-ALL, genetic markers have not yet been integrated into risk stratification and for treatment options as a complementary approach to the standard evaluation of minimal residual disease. The translation of molecular findings into clinical practice remains challenging because of the genetic complexity and heterogeneity of T-ALL and the observation that focusing on single markers can provide misleading information.[45]

One major issue has been the identification of the most convenient diagnostic approach for the detection of these multiple heterogenous concurrent events in individual patients and to select the most reliable markers and pathways to be exploited in treatment and monitoring. This study highlighted CI-FISH as a powerful assay in the diagnostic workup of pediatric and adult T-ALL and T-lymphoblastic lymphoma. Coupled with targeted sequencing of recurrently mutated genes, it provides comprehensive genetic diagnosis of T-ALL for detection of actionable biomarkers in the context of clinical trials.

Acknowledgments

We thank Donatella Beacci and Silvia Romoli for their help in the preparation of biological samples.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.jmoldx.2020.02.004*.

References

1. Girardi T, Vicente C, Cools J, De Keersmaecker K: The genetics and molecular biology of T-ALL. Blood 2017, 129:1113−1123
2. Marks DI, Rowntree C: Management of adults with T-cell lymphoblastic leukemia. Blood 2017, 129:1134−1142
3. Borowitz MJ, Chan JK, Dowing JR, Le Beau MM, Arber: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues: Precursor Lymphoid Neoplasm. Lyon, IARC, 2017. pp. 199−213
4. Ferrando AA, Neuberg DS, Staunton J, Loh ML, Huard C, Raimondi SC, Behm FG, Pui CH, Downing JR, Gilliland DG, Lander ES, Golub TR, Look AT: Gene expression signature define novel oncogenic pathways in T cell acute lymphoblastic leukemia. Cancer Cell 2002, 1:75−87
5. Belver L, Ferrando A: The genetics and mechanisms of T cell acute lymphoblastic leukaemia. Nat Rev Cancer 2016, 16:494−507
6. Homminga I, Pieters R, Langerak AW, de Rooi JJ, Stubbs A, Verstegen M, Vuerhard M, Buijs-Gladdines J, Kooi C, Klous P, van Vlierberghe P, Ferrando AA, Cayuela JM, Verhaaf B, Beverloo HB, Horstmann M, de Haas V, Wiekmeijer AS, Pike-Overzet K, Staal FJ, de Laat W, Soulier J, Sigaux F, Meijerink JP: Integrated transcript and genome analyses reveal NKX2-1 and MEF2C as potential oncogenes in T cell acute lymphoblastic leukemia. Cancer Cell 2011, 19:484−497
7. Van Vlierberghe P, Pieters R, Beverloo HB, Meijerink JP: Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia. Br J Haematol 2008, 143:153−168
8. Coustan-Smith E, Mullighan CG, Onciu MM, Behm FG, Raimondi SC, Pei D, Cheng C, Su X, Rubnitz JE, Basso G, Biondi A, Pui CH, Downing JR, Campana D: Early T-cell precursor leukemia: a subtype of very risk acute lymphoblastic leukemia identified in two independent cohorts. Lancet Oncol 2009, 10:147−156
9. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, et al: The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 2012, 481:157−163
10. Liu Y, Easton J, Shao Y, Maciaszek J, Wang Z, Wilkinson MR, et al: The genomic landscape of pediatric and young adult T-lineage acute lymphoblastic leukemia. Nat Genet 2017, 49:1211−1218
11. Durinck K, Goossens S, Peirs S, Wallaert A, Van Loocke W, Matthijssens F, Pieters T, Milani G, Lammens T, Rondou P, Van Roy N, De Moerloose B, Benoit Y, Haigh J, Speleman F, Poppe B, Van Vlierberghe P: Novel biological insights in T-cell acute lymphoblastic leukemia. Exp Hematol 2015, 43:625−639
12. La Starza R, Borga C, Barba G, Pierini V, Schwab C, Matteucci C, Lema Fernandez AG, Leszl A, Cazzaniga G, Chiaretti S, Basso G, Harrison CJ, Te Kronnie G, Mecucci C: Genetic profile of T-cell acute lymphoblastic leukemias with MYC translocations. Blood 2014, 124:3577−3582
13. La Starza R, Barba G, Demeyer S, Pierini V, Di Giacomo D, Gianfelici V, Schwab C, Matteucci C, Vicente C, Cools J, Messina M, Crescenzi B, Chiaretti S, Foà R, Basso G, Harrison CJ, Mecucci C: Deletions of the long arm of chromosome 5 define subgroups of T-cell acute lymphoblastic leukemia. Haematologica 2016, 101:951−958
14. La Starza R, Messina M, Gianfelici V, Pierini V, Matteucci C, Pierini T, Limongi MZ, Vitale A, Roti G, Chiaretti S, Foà R, Mecucci C: High PIM1 expression is a biomarker of T-cell acute lymphoblastic leukemia with JAK/STAT activation or t(6;7)(p21;q34)/TRB@-PIM1 rearrangement. Leukemia 2018, 32:1807−1810
15. Vicente C, Schwab C, Broux M, Geerdens E, Degryse S, Demeyer S, Lahortiga I, Elliott A, Chilton L, La Starza R, Mecucci C, Vandenberghe P, Goulden N, Vora A, Moorman AV, Soulier J, Harrison CJ, Clappier E, Cools J: Targeted sequencing identifies associations between IL7R-JAK mutations and epigenetic modulators in T-cell acute lymphoblastic leukemia. Haematologica 2015, 100:1301−1310

16. La Starza R, Lettieri A, Pierini V, Nofrini V, Gorello P, Songia S, Crescenzi B, Te Kronnie G, Giordan M, Leszl A, Valsecchi MG, Aversa F, Basso G, Biondi A, Conter V, Cazzaniga G, Mecucci C: Linking genomic lesions with minimal residual disease improves prognostic stratification in children with T-cell acute lymphoblastic leukaemia. Leuk Res 2013, 37:928−935
17. O'Connor D, Enshaei A, Bartram J, Hancock J, Harrison CJ, Hough R, Samarasinghe S, Schwab C, Vora A, Wade R, Moppett J, Moorman AV, Goulden N: Genotype-specific minimal residual disease interpretation improves stratification in pediatric acute lymphoblastic leukemia. J Clin Oncol 2018, 36:34−43
18. Richter-Pechańska P, Kunz JB, Hof J, Zimmermann M, Rausch T, Bandapalli OR, Orlova E, Scapinello G, Sagi JC, Stanulla M, Schrappe M, Cario G, Kirschner-Schwabe R, Eckert C, Benes V, Korbel JO, Muckenthaler MU, Kulozik AE: Identification of a genetically defined ultra-high-risk group in relapsed pediatric T-lymphoblastic leukemia. Blood Cancer J 2017, 7:e523
19. Ferrando A: NOTCH mutations as prognostic markers in T-ALL. Leukemia 2010, 24:2003−2004
20. Petit A, Trinquand A, Chevret S, Ballerini P, Cayuela JM, Grardel N, Touzart A, Brethon B, Lapillonne H, Schmitt C, Thouvenin S, Michel G, Preudhomme C, Soulier J, Landman-Parker J, Leverger G, Macintyre E, Baruchel A, Asnafi V; French Acute Lymphoblastic Leukemia Study Group (FRALLE): Oncogenetic mutations combined with MRD improve outcome prediction in pediatric T-cell acute lymphoblastic leukemia. Blood 2018, 131:289−300
21. Genescà E, Lazarenkov A, Morgades M, Berbis G, Ruíz-Xivillé N, Gómez-Marzo P, Ribera J, Juncà J, González-Pérez A, Mercadal S, Guardia R, Artola MT, Moreno MJ, Martínez-López J, Zamora L, Barba P, Gil C, Tormo M, Cladera A, Novo A, Pratcorona M, Nomdedeu J, González-Campos J, Almeida M, Cervera J, Montesinos P, Batlle M, Vives S, Esteve J, Feliu E, Solé F, Orfao A, Ribera JM: Frequency and clinical impact of CDKN2A/ARF/CDKN2B deletions as assessed by in-depth genetic analysis in adult T cell acute lymphoblastic leukemia. J Hematol Oncol 2018, 11:96
22. Roti G, Stegmaier K: New approaches to target T-ALL. Front Oncol 2014, 4:170
23. Maude SL, Dolai S, Delgado-Martin C, Vincent T, Robbins A, Selvanathan A, Ryan T, Hall J, Wood AC, Tasian SK, Hunger SP, Loh ML, Mullighan CG, Wood BL, Hermiston ML, Grupp SA, Lock RB, Teachey DT: Efficacy of JAK/STAT pathway inhibition in murine xenograft models of early T-cell precursor (ETP) acute lymphoblastic leukemia. Blood 2015, 125:1759−1767
24. Kawashima-Goto S, Imamura T, Tomoyasu C, Yano M, Yoshida H, Fujiki A, Tamura S, Osone S, Ishida H, Morimoto A, Kuroda H, Hosoi H: BCL2 inhibitor (ABT-737): a restorer of prednisolone sensitivity in early T-cell precursor-acute lymphoblastic leukemia with high MEF2C expression. PLoS One 2015, 10:e0132926
25. De Smedt R, Peirs S, Morscio J, Matthijssens F, Roels J, Reunes L, Lintermans B, Goossens S, Lammens T, Van Roy N, Touzart A, Jenni S, Tsai YC, Lovisa F, Mussolin L, Serafin V, Van Nieuwerburgh F, Deforce D, Uyttebroeck A, Tousseyn T, Burkhardt B, Klapper W, De Moerloose B, Benoit Y, Macintyre E, Bourquin JP, Basso G, Accordi B, Bornhauser B, Meijerink J, Vandenberghe P, Van Vlierberghe P: Pre-clinical evaluation of second generation PIM inhibitors for the treatment of T-cell acute lymphoblastic leukemia and lymphoma. Haematologica 2019, 104:e17−e20
26. Li Y, Buijs-Gladdines JG, Canté-Barrett K, Stubbs AP, Vroegindeweij EM, Smits WK, van Marion R, Dinjens WN, Horstmann M, Kuiper RP, Buijsman RC, Zaman GJ, van der Spek PJ, Pieters R, Meijerink JP: IL-7 receptor mutations and steroid resistance in pediatric T cell acute lymphoblastic leukemia: a genome sequencing study. PLoS Med 2016, 13:e1002200
27. Gorello P, La Starza R, Varasano E, Chiaretti S, Elia L, Pierini V, Barba G, Brandimarte L, Crescenzi B, Vitale A, Messina M, Grammatico S, Mancini M, Matteucci C, Bardi A, Guarini A, Martelli MF, Foà R, Mecucci C: Combined interphase fluorescence in situ hybridization elucidates the genetic heterogeneity of T-cell acute lymphoblastic leukemia in adults. Haematologica 2010, 95:79−86
28. Van Roy N, Van Der Linden M, Menten B, Dheedene A, Vandeputte C, Van Dorpe J, Laureys G, Renard M, Sante T, Lammens T, De Wilde B, Speleman F, De Preter K: Shallow whole genome sequencing on circulating cell-free DNA allows reliable noninvasive copy-number profiling in neuroblastoma patients. Clin Cancer Res 2017, 23:6305−6314
29. Scheinin I, Sie D, Bengtsson H, van de Wiel MA, Olshen AB, van Thuijl HF, van Essen HF, Eijk PP, Rustenburg F, Meijer GA, Reijneveld JC, Wesseling P, Pinkel D, Albertson DG, Ylstra B: DNA copy number analysis of fresh and formalin-fixed specimens by shallow whole-genome sequencing with identification and exclusion of problematic regions in the genome assembly. Genome Res 2014, 24:2022−2032
30. Sante T, Vergult S, Volders PJ, Kloosterman WP, Trooskens G, De Preter K, Dheedene A, Speleman F, De Meyer T, Menten B: ViVar: a comprehensive platform for the analysis and visualization of structural genomic variation. PLoS One 2014, 9:e113800
31. Bond J, Marchand T, Touzart A, Cieslak A, Trinquand A, Sutton L, Radford-Weiss I, Lhermitte L, Spicuglia S, Dombret H, Macintyre E, Ifrah N, Hamel JF, Asnafi V: An early thymic precursor phenotype predicts outcome exclusively in HOXA-overexpressing adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study. Haematologica 2016, 101:732−740
32. Aoyama M, Ozaki T, Inuzuka H, Tomotsune D, Hirato J, Okamoto Y, Tokita H, Ohira M, Nakagawara A: LMO3 interacts with neuronal transcription factor, HEN2, and acts as an oncogene in neuroblastoma. Cancer Res 2006, 65:4587−4597
33. Simonis M, Klous P, Homminga I, Galjaard RJ, Rijkers EJ, Grosveld F, Meijerink JP, de Laat W: High-resolution identification of balanced and complex chromosomal rearrangements by 4C technology. Nat Methods 2009, 6:837−842
34. Boehm T, Foroni L, Kaneko Y, Perutz MF, Rabbitts TH: The rhombotin family of cysteine-rich LIM-domain oncogenes: distinct members are involved in T-cell translocations to human chromosomes 11p15 and 11p13. Proc Natl Acad Sci U S A 1991, 88:4367−4371
35. Sang M, Ma L, Sang M, Zhou X, Gao W, Geng C: LIM-domain-only proteins: multifunctional nuclear transcription coregulators that interacts with diverse proteins. Mol Biol Rep 2014, 41:1067−1073
36. Soulier J, Clappier E, Cayuela JM, Regnault A, García-Peydró M, Dombret H, Baruchel A, Toribio ML, Sigaux F: HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood 2005, 106:274−286
37. Bergeron J, Clappier E, Cauwelier B, Dastugue N, Millien C, Delabesse E, Beldjord K, Speleman F, Soulier J, Macintyre E, Asnafi V: HOXA cluster deregulation in T-ALL associated with both a TCRD-HOXA and a CALM-AF10 chromosomal translocation. Leukemia 2006, 20:1184−1187
38. Gesk S, Martın-Subero JI, Harder L, Luhmann B, Schlegelberger B, Calasanz MJ, Grote W, Siebert R: Molecular cytogenetic detection of chromosomal breakpoints in T-cell receptor gene loci. Leukemia 2003, 17:738−745
39. Zuurbier L, Homminga I, Calvert V, te Winkel ML, Buijs-Gladdines JG, Kooi C, Smits WK, Sonneveld E, Veerman AJ, Kamps WA, Horstmann M, Petricoin EF 3rd, Pieters R, Meijerink JP: NOTCH1 and/or FBXW7 mutations predict for initial good prednisone response but not for improved outcome in pediatric T-cell acute lymphoblastic leukemia patients treated on DCOG or COALL protocols. Leukemia 2010, 24:2014−2022

40. Zuurbier L, EF3rd Petricoin, Vuerhard MJ, Calvert V, Kooi C, Buijs-Gladdines JG, Smits WK, Sonneveld E, Veerman AJ, Kamps WA, Horstmann M, Pieters R, Meijerink JP: The significance of PTEN and AKT aberrations in pediatric T-cell acute lymphoblastic leukemia. Haematologica 2012, 97: 1405−1413
41. Bertacchini J, Heidari N, Mediani L, Capitani S, Shahjahani M, Ahmadzadeh A, Saki N: Targeting PI3K/AKT/mTOR network for treatment of leukemia. Cell Mol Life Sci 2015, 72:2337−2347
42. Peirs S, Matthijssens F, Goossens S, Capitani S, Shahjahani M, Ahmadzadeh A, Saki N: ABT-199 mediated inhibition of BCL-2 as a novel therapeutic strategy in T-cell acute lymphoblastic leukemia. Blood 2014, 124:3738−3747
43. Daigle SR, Olhava EJ, Therkelsen CA, Basavapathruni A, Jin L, Boriack-Sjodin PA, Allain CJ, Klaus CR, Raimondi A, Scott MP, Waters NJ, Chesworth R, Moyer MP, Copeland RA, Richon VM, Pollock RM: Potent inhibition of DOT1L as treatment of MLL-fusion leukemia. Blood 2013, 122:1017−1025
44. Deveau AP, Forrester AM, Coombs AJ, Wagner GS, Grabher C, Chute IC, Léger D, Mingay M, Alexe G, Rajan V, Liwski R, Hirst M, Steigmaier K, Lewis SM, Look AT, Berman JN: Epigenetic therapy restores normal hematopoiesis in a zebrafish model of NUP98-HOXA9-induced myeloid disease. Leukemia 2015, 29:2086−2097
45. Ma J, Wu M: The indicative effect of Notch1 expression for the prognosis of T-cell acute lymphocytic leukemia: a systematic review. Mol Biol Rep 2012, 39:6095−6100