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ABSTRACT

Background
The prognostic value of the ectopic activation of *TLX3* gene expression, a major oncogenetic event associated with pediatric T-cell acute lymphoblastic leukemia, is controversial. Likewise, the frequency and the prognostic significance in pediatric T-cell acute lymphoblastic leukemia of the newly characterized *NUP214-ABL1* fusion transcript is not yet clear.

Design and Methods
Two hundred children with T-cell acute lymphoblastic leukemia were treated in the French FRALLE-93 study from 1993 to 1999. The expression of *TLX3*, *TLX1* and *SILTAL1* genes was analyzed in samples from 92 patients by real-time quantitative reverse transcriptase polymerase chain reaction. Most of these samples were further studied for *NUP214-ABL1* and *CALM-AF10* fusion transcripts.

Results
The median follow-up was 7.9 years. At 5 years the overall survival (± standard deviation, %) was 62 (±3%) and leukemia-free survival was 58 (±3%). Patients with T-cell acute lymphoblastic leukemia positive for *TLX3* had a poorer survival compared to those with T-ALL negative for *TLX3* (overall survival: 45±11% *vs.* 57±5%, p=0.049). In multivariate analysis, *TLX3* expression was an independent adverse risk factor predicting relapse with a hazard ratio of 2.44 (p=0.017) and an overall survival with a hazard ratio of 3.7 (p=0.001). *NUP214-ABL1* was expressed in 16.6% of patients with *TLX3*-positive T-ALL (3 of 18); all of the patients with this association died before completion of the treatment. *SILTAL* expression did not significantly affect the prognosis of patients with T-cell acute lymphoblastic leukemia. Only three of 92 patients expressed the *TLX1* gene and all three are alive.

Conclusions
TLX3 gene expression is an independent risk factor predicting poor survival in childhood T-cell acute lymphoblastic leukemia. When co-expressed with *TLX3*, *NUP214-ABL1* transcripts may increase the risk of poor survival.

Key words: childhood T-cell acute lymphoblastic leukemia, *TLX3/HOX11L2*, *NUP214-ABL1*, *SIL-TAL1*, outcome.

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Introduction

T-cell acute lymphoblastic leukemia (T-ALL) accounts for 10 to 15% of cases of childhood acute lymphocytic leukemia and is often associated with unfavorable features. The prognosis of this disease, although improved over the last years because of the use of more intensive treatments and risk-adapted therapy, remains unsatisfactory.[1-5] Thus, the identification of prognostically relevant molecular markers at diagnosis will be fundamental to guide risk-adapted strategies and contribute to the optimization of T-ALL treatment.

T-ALL are characterized by recurrent translocations affecting the T-cell receptor loci (*TCR*). Consequently, enhancer elements from the *TCRA/D* locus (14q11) and *TCRB* locus (7q34) come close to target genes and activate their ectopic expression. This mechanism implies a number of transcription factors such as the helix-loop-helix genes (*TAL1/SCL*, *TAL2*, *LYL1*),[6-10] the LIM-domain-only (*LMO1*, *LMO2*) genes, the homeobox genes of class-I (*HOXA10*, *HOXA11*)[11,12] or class-II (*HOX11/TLX1*, *HOX11L2/TLX3*)[13,14] and the *NOTCH1* gene.[15] The same genes are frequently targeted during the malignant transformation of T cells by others mechanisms, such as interstitial deletion on chromosome 1 for *TAL1/SCL* or point mutations for *NOTCH1*.[16,17]

Other translocations, not involving *TCR* loci, have also been described: the t(10;11)(p13;q14) translocation,[18,19] resulting in the *CALM-AF10* fusion gene; the cryptic t(5;14) (q34;q32) translocation,[14] resulting in the ectopic transcription of *TLX3/HOX11L2* and the episomal 9q34 recombination between *NUP214* and *ABL1* genes.[20] These two latter events are highly specific to T-ALL, occurring in, respectively, 20-24% and 5-6% of cases. For an unexplained reason, *NUP214-ABL1* fusion appears closely associated with *TLX1* and *TLX3* deregulation. Until recently there have been no reports of this episomal recombination in others types of leukemia and very little is known about its frequency in pediatric T-ALL. Furthemore, it is still unclear how these genetic lesions affect the prognosis of patients with T-ALL. Few cohorts of patients have been analyzed so far and for more recently characterized lesions such as the *NUP214-ABL1* fusion gene, no studies in childhood T-ALL are available. Preliminary analyses of the prognostic value of frequent lesions such as ectopic *TLX3* activation have led to contrasting conclusions.[21-14]

Here, we analyzed the prognostic value of *TLX3* expression on the clinical outcome of 92 patients with T-ALL enrolled in the FRALLE-93 study between 1993-1999. Moreover, to gain insights into the relationship between *TLX3* and other oncogenetic events we extended our analysis to *SILTAL* and *TLX1* gene expression, as well as to molecular lesions such as *NUP214-ABL1* and *CALM-AF10* gene fusions which have not yet been extensively evaluated in pediatric T-ALL.

Design and Methods

Patients and the FRALLE-93 trial design

Between 01.1993 and 31.12.1999, 1395 children (778 boys and 617 girls) with ALL were included in the FRALLE-93 protocol. The leukemia was classified as B-cell lineage in 1195 cases (86%) and T-cell lineage in 200 cases (14%). All the patients with T-ALL were included in this study and uniformly assigned to the high-risk group. Informed consent was obtained from the patients' parents in all cases. The treatment design is summarized in *Online Supplementary Figure S1*. High-risk patients were assigned to three different treatment subgroups according to their response to prednisone at day 8 and the blast count in bone marrow evaluated at day 21. Prednisone response was defined as *good* when the number of blasts circulating in the blood was < 1000/μL at day 8 or *poor* when the circulating blast count was >1000/μL at day 8. The persistence of blasts in the bone marrow at day 21 was categorized as M1 when the blast count was <5%, M2 when the count was between 5% and 25% and M3 when it was >25%. Group C1, patients, who had a good response to prednisone and M1 or M2 bone marrow involvement, received two delayed intensification blocks; group C2, patients considered at a very high risk because of poor response to prednisone and/or M3 bone marrow involvement, received six intensification blocks, followed by autologous stem cell transplantation or, group C3, by allogeneic stem cell transplantation if an HLA-matched sibling was available. Central nervous system prophylaxis consisted in triple intrathecal injection with methylprednisolone, methotrexate, cytarabine and 18 Gy radiotherapy. The details of the treatment for group C2 have been published elsewhere[25] The main characteristics of the patients studied are presented in Table 1. T-cell lineage was assessed on the basis of expression of CD3, CD2, CD5 and CD7 antigens and exclusion of B-cell-associated antigens according to the European EGIL recommendations.[26] Karyotypes and fluorescence *in situ* hybridization (FISH) analysis of t(5;14) translocation of cases analyzed for *TLX3* expression have already been reported.[27] Molecular studies for *SILTAL*, *TLX3* and *TLX1* were possible in 92 out 200 samples (46%). This was mainly related to the fact that systematic cryopreservation of T-ALL blasts was not performed in all the participating centers. Additional analysis of *CALM-AF10* and *NUP214-ABL1* was carried out in 84 and 52 patients, respectively.

Molecular studies

RNA was extracted and reverse transcribed according to previously described procedures.[21] Expression of *SILTAL*, *TLX3* and *TLX1* was analyzed by real time quantitative polymerase chain reaction (RQ-PCR) on a Taqman 7700 or 7500 real time PCR device (Applied Biosystems, Les Ulis, France) and their levels of expression were quantified relative to the expression of the endogenous control gene, *ABL*. Primers and probes for *ABL1* and *SILTAL* as well as the RQ-PCR procedures are described in the Europe-Against-Cancer Program.[28,29]

TLX1 and *TLX3* gene expression was detected as reported elsewhere.[21] *CALM-AF10* fusion transcripts, both 5' and 3' fusions, and the more frequent *NUP214-ABL1* fusion transcripts were screened for as reported previously.[20, 30]

Statistical analysis

Two different descriptive analyses were performed, the first on the overall population of patients, the second on the sub-population of patients for whom molecular data were available. Variables considered were patients', age and gender, and disease and treatment characteristics. Leukemia-free survival was defined as survival without evidence of relapse or progression. All patients still alive in first continuous remission were censored at their last follow-up. Probabilities of overall survival and leukemia free survival were calculated using the Kaplan-Meier estimate. Further analyses were performed only on patients with available molecular data to evaluate the clinical significance of the oncogenetic events. Patient-, disease-, and treatment-related variables of the groups divided according to oncogenetic events were compared using the χ^2 statistic for categorical variables and the Mann-Whitney test for continuous variables. The log-rank test and Cox proportional hazards model were used to determine the univariate and independent prognostic importance of several variables. All variables with a p value less than 0.2 in the univariate analysis or differing in distribution were entered into the model. The type I error rate was fixed at 0.05 for the determination of factors associated with time to event outcomes. Statistical analyses were performed with SPSS (Inc., Chicago, USA) software packages.

Table 1. Clinical characteristic of the overall pediatric T-ALL cohort and of the study population.

Total	*FRALLE-93 T-ALL cohort n=200*	*Study population n=92*
Median age, years, at diagnosis (range)	9.15 (1.1-19.5)	9.7 (1.7-18.5)
Median year of diagnosis	1996 (93-99)	1997 (93-99)
Patients' gender: male/female	139/61 69% - 31%	63/29 68% - 32%
Median WBC at diagnosis, $\times10^9$/L (range)	98,85 (0,6-1247)	149.5 (0.6-736)
WBC >50 $\times10^9$/L	126 (63%)	68 (74%)
Mediastinal involvement	141 (70%)	66 (71%)
Response to pre- phase treatment		
PPR	109 (57%)	45 (52%)
GPR	82 (43%)	41 (39%)
M status		
M1	135 (69%)	68 (73%)
M2	21 (11%)	11 (12%)
M3	40 (20%)	11 (12%)
NE	4	2
Risk group of the protocol		
C1	100 (59%)	46 (55%)
C2	44 (26%)	23 (28%)
C3	24 (14%)	14 (17%)
Stem cell transplant (SCT)		
allogeneic	32 (16%)	16 (17%)
autologous	39 (20%)	18 (20%)
no SCT	129 (65%)	58 (63%)
Follow-up, years (range)	7.9 (4.3-11.8)	7.4 (4.2-11.8)
5-year leukemia-free survival	58±3%	64±5%
5-year overall survival	62 ± 3%	56 ± 5%

WBC, white blood cell count; GPR: good prednisone response; PPR: poor prednisone response; M1-2-3 status: percentage of blasts in the bone marrow at day 21.

Results

Patients and FRALLE-93 trial design

The biological and clinical characteristics of the entire cohort of FRALLE-93 T-ALL patients are summarized in Table 1. One hundred and sixty-eight patients out of 200 reached complete remission. The follow-up period ranged from 4.3 to 11.8 years (median, 7.9 years). The overall and leukemia-free survival rates of the FRALLE-93 T-ALL population were 62% (±3%) and 58% (±3%), respectively, at 5 years and 60% (±4%) and 56% (±4%) at 10 years. As shown in Table 1 the subgroup of 92 patients who underwent genetic analysis is representative of the total population, as there were no differences in clinical presentation, initial response to chemotherapy, or subsequent treatments between the subgroup and the total population.

Frequency and distribution of the genetic lesions among the study population

High and specific over-expression of *TLX3* and *TLX1* was found in 21.7% (20/92) and 3.2% (3/92) of the cases, respectively. *SILTAL* and *CALM-AF10* 5'- or 3'-fusion transcripts were detected in an additional 21.7% (20/92) and 4.7% (4/84) of samples tested. *NUP214-ABL1* fusion transcripts were found in three (5.8%) of 52 T-ALL samples: two had a fusion between *NUP214* exon 29 and *ABL1* exon 2 and one between *NUP214* exon 30 and *ABL1* exon 2. All the three cases of T-ALL positive for a *NUP214-ABL1* fusion were also positive for *TLX3* activation.

Clinical and biological characteristics at diagnosis according to the oncogenetic lesions

Five distinct molecular subgroups could be identified within the study population *SILTAL, HOX11L2/TLX3, HOX11/TLX1, CALM-AF10* and *NUP214-ABL1*. Except for the *NUP214-ABL1* gene fusion, none of the other lesions studied was found to be co-expressed in the same leukemic sample. As shown in Table 2 there were no differences between these molecular subgroups in sex ratio, age or mediastinal involvement, although initial leukocytosis did differ. The white blood cell count was significantly higher in the *SILTAL* subgroup than in the *TLX3, TLX1* and *NUP214-ABL1* subgroups. Marked hyperleukocytosis and mediastinal involvement were also noted in the group of patients with *CALM-AF10* T-ALL. Conventional cytogenetic analysis was conducted in 169 patients and was normal in half of these cases

(87/169). Within the *TLX3* subgroup, only four cases displayed abnormal karyotypes involving chromosome 5 (addition of 5q, monosomy 5, and deletion of 5q). FISH analysis confirmed the presence of translocation of the *TLX3* locus on 5q35 in all the cases analyzed (8/16). In the *SILTAL* subgroup, six cases displayed karyotype abnormalities, mostly involving chromosome 6 (deletion of 6p, n=1, or 6q, n=3). No 10q24 chromosome rearrangement was detected in the *TLX1* subgroup. In the *CALM-AF10* subgroup, the karyotypes were normal in three of four cases. Episomal distribution of *NUP214-ABL1* was confirmed by FISH analysis in the only case studied in this subgroup. Overall, the frequency of cytogenetic alterations was similar in the different oncogenetic subgroups.

Analysis of the prognostic impact of oncogenetic events

TLX3 and *SILTAL*. The clinical response to treatment and the survival of patients in each oncogenetic subgroup are detailed in Table 3. Kaplan-Meier estimates of overall survival for patients with *TLX3* and *SILTAL* lesions are represented in Figure 1. T-ALL positive for *TLX3* gene expression did not differ significantly from *TLX3*-negative ones with respect to prednisone-poor response (p=0.11), bone marrow blast count (M status) at day 21 (p=0.81), complete remission rate (p=0.54) and treatment subgroups: C1 *vs.* C2 *vs.* C3 (p=0.44). Patients with *SILTAL*-positive T-ALL had hyperleukocytosis and many had a poor response to prednisone at day 8 (58%), but they had an excellent response to induction chemotherapy (95% M1 for *SILTAL*-positive patients *vs.*

Table 2. Clinical and biological characteristics at diagnosis of the patients divided according to the presence of oncogenetic lesions.

	FRALLE-93 T-ALL cohort	*SILTAL positive*	*TLX3 positive*	*TLX1 positive*	*NUP214-ABL1 positive*	*CALM-AF10 positive*
Total number, n	200	20/92	20/92	3/92	3/52	4/84
(%)		21.7%	21.7%	3.2%	5.8%	4.7%
Gender, male/female	139/61	13/7	13/7	2 / 1	3/0	3 /1
Age, median, years	9.15	6.5	9.25	9.8	11.6	9.8
(range)	(1.1-19.5)	(2.3-17.8)	(3.2-18.5)	(4.8-11.8)	(5-13.5)	(4.3-16.4)
WBC, median	98.85	238.5	116.7	140	66	426
(range, $\times10^9$/L)	(0.6-1247)	(3.5-636)	(1.9 - 736)	(5.4-256)	(12.4-736)	(260-606)
<50 $\times10^9$/L (n)	75	1	7	1	1	0
50-99 $\times10^9$/L (n)	25	2	3	0	1	0
≥100 $\times10^9$/L (n)	100	17	10	2	2	4
Mediastinal involvement, (n)	141	13	14	0	1	4
Karyotype						
Abnormal, n	82	6	4	1	0	0
Normal, n	87	11	12	1	2	4
Not evaluable, n	31	3	4	1	1	0

Table 3. Clinical response to treatment, distribution and type of events, and survival status in subgroups divided according to the presence of oncogenetic lesions.

Total number, (n)	*FRALLE-93 T-ALL (200)*	*all TLX3 neg. (72)*	*TLX3 pos (20)*	*SILTAL pos (20)*	*TLX1 pos (3)*	*NUP214-ABL1 pos (3)*	*CALM-AF10 pos (4)*
Response to prephase, n							
died before evaluation	4	2	0	0	0	0	0
GPR <1000 blasts/μL	109	32	13	8	3	0	4
PPR >1000 blasts/μL	82	35	6	11	0	3	0
not evaluated	5	3	1	1	0	0	0
M status at day 21, n							
died before evaluation	4	2	0	0	0	0	0
M1	135	54	14	19	2	1	3
M2	21	8	3	1	1	0	1
M3	40	8	3	0	0	2	0
Type of event, n							
No event[a]	110	42	9	12	3	0	3
Failure	20	5	1	0	0	0	0
early deaths	4	1	0	0	0	0	0
relapses	66[b]	24	10[a]	8[d]	0	3	1
toxic deaths	3	0	0	0	0	0	0
Leukemia-free survival % ± SE	58±3	60±5	45±11	70±10	not evaluable	not evaluable	not evaluable
Overall survival % ± SE	62±3	57±5	45±11	80±9	not evaluable	not evaluable	not evaluable
Alive, n	122	64	9	15	3	0	1

[a]Continuous complete remission; [b]Relapses occurred in bone marrow (BM) (n=42), central nervous system (CNS) (n=6), BM+CNS (n=5), testis (n=3) and mediastinum (n= 10); [C]Relapses occurred in BM(n= 9) and testis (n=1) ; [d]Relapses occurred in BM (n=3), CNS (n=3), BM+CNS (n=1) and mediastinum (n=1).

65% MI for *SILTAL*-negative patients, p=0.008) (Table 3). In univariate analysis only two factors were significantly correlated with a lower 5-year overall survival rate: a poor response to prednisone (p=0.001) and *TLX3* status (p=0.049) (Table 4). A shorter leukemia-free survival was significantly correlated only to a poor response to prednisone (p=0.002). It is noteworthy that M3 status did not significantly predict patients' outcome (leukemia-free survival p=0.11; overall survival p=0.09). Patients with *SILTAL*-positive T-ALL appeared to have a better long-term outcome than those without this genetic lesion, although the difference was not statistically significant (p=0.1) (Figure 1). In a multivariable analysis, a poor response to prednisone at day 8 and over-expression of the *TLX3* gene independently predicted adverse outcome. The hazard ratios for 5-year leukemia-free survival and overall survival for patients with a poor response to prednisone were 3.6 (p=0.01) and 4.8 (p<0.0001), respectively. The corresponding hazard ratios for patients with *TLX3*-positive T-ALL were 2.44 (p 0.017) and 3.7 (p=0.001), respectively (Table 5).

Outcome of T-ALL in other oncogenetic groups

A detailed description of the other subgroups is given in Table 3. The low number of positive samples in each

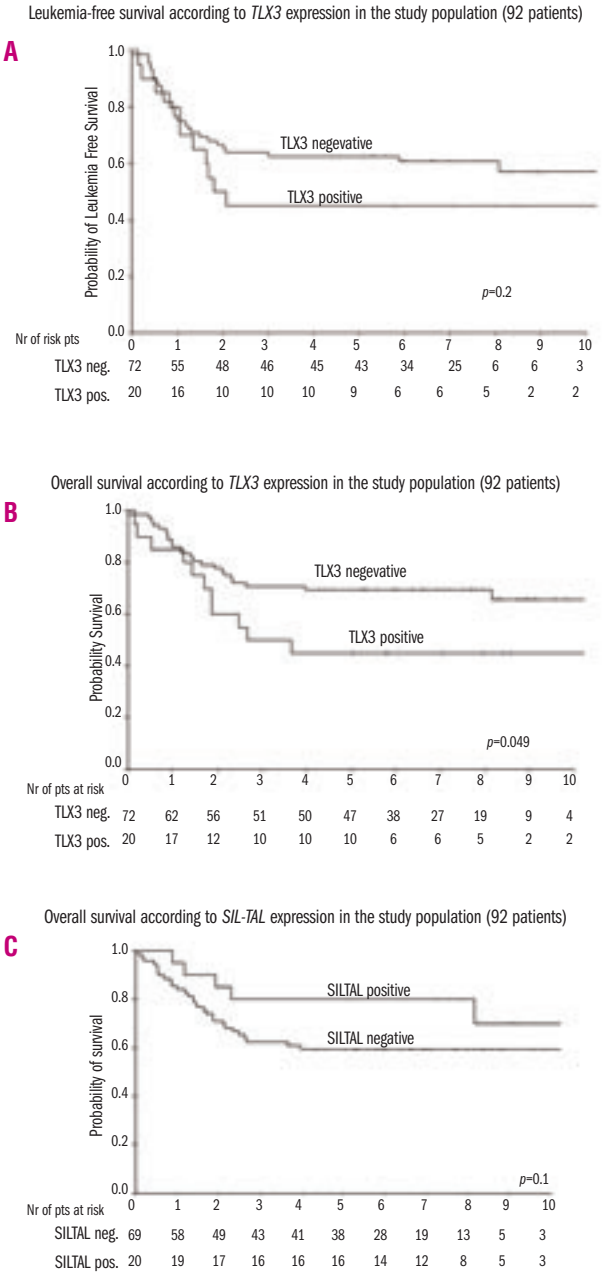

Figure 1. Kaplan Meier analysis of (A) leukemia-free survival according to *TLX3* expression and (B) overall survival according to *TLX3* expression and (C) overall survival according to SILTAL expression.

Table 4. Univariate analysis of factors associated with 5-year overall survival (OS) and leukemia-free survival (LFS) in the study population.

	OS	*p*	*LFS*	*p*
Age				
Below median	67±7%		63±7%	
Median or above	61±7%		54±7%	
		0.47		0.4
Year of diagnosis				
Before median	61±7%		54±7%	
Median or after	67±7%		63±7%	
		0.56		0.44
White blood cell count				
< median (149.5 ×10^9/L)	65±7%		59±7%	
≥ median	63±7%		59±7%	
		0.93		0.89
Response to steroids at day 8				
Good	80±6%		73±6%	
Poor	46±8%		41±8%	
		0.001		0.002
M status				
M1+ M2	68±5%		63±5%	
M3	45±15%		36±14%	
		0.09		0.11
TLX3				
negative	69±5%		63±6%	
positive	45±11%		45±11%	
		0.049		0.2
SILTAL				
negative	59±6%		55±6%	
positive	80±9%		70±10%	
		0.1		0.26

M1-2-3 status: percentage of blasts in the bone marrow at day 21.

Table 5. Multivariate analysis of factors associated with leukemia-free survival and overall survival at 5 years in the FRALLE-93 T-ALL study population.

Leukemia-free survival[a]	*Hazard ratio*	*95% confidence interval*	*p value*
Poor response to prednisone	3.57	7.14-1.72	0.001
TLX3 positive *vs.* negative	2.43	5.26-1.17	0.017
Overall survival[a,b]			
Poor response to prednisone	4.76	11.11-2.08	<0.0001
TLX3 positive *vs.* negative	3.7	8.33-1.69	0.001

[a]Cox regression model with all covariates associated with a p value less than 0.2 by univariate analysis: [b]same as for leukemia-free survival but without SILTAL status which had a p value of 0.26.

group precluded a reliable statistical evaluation of their clinical impact, nevertheless all three patients with *TLX1* expression had an excellent outcome whereas all three patients with the association of *NUP214-ABL1* and *TLX3* expression died before completion of their treatment.

Discussion

In the present study, we analyzed the prognostic value of five different genetic abnormalities, *SILTAL*, *TLX3*, *TLX1*, *CALM-AF10* and *NUP214-ABL1* in a large panel of cases of pediatric T-ALL treated in the FRALLE-93 trial. We were able to classify at the molecular level up to 50% (47/92) of the T-ALL cases studied and demonstrate that *TLX3* overexpression is an independent risk factor in pediatric T-ALL, predicting an inferior outcome. That risk may increase when the *NUP214-ABL1* fusion is co-expressed.

Very little is known about the role of the *TLX3* gene in leukemic hematopoiesis. Recently, unsupervised hierarchical analysis of T-ALL gene expression profiles demonstrated a major cluster sharing a common gene expression signature, which included *TLX1*, *TLX3*-expressing cases and *HOXA*-translocated cases.[12] Nervertheless, the contributions of *TLX3* and *TLX1* to the leukemic process could be quite different since actual data on clinical outcome seem to be opposing for these two lesions in adult T-ALL[22,31,32] as well in pediatric T-ALL.[24] Moreover, the high frequency of ectopic *TLX3* activation in pediatric T-ALL suggests that this gene plays a crucial role. Conflicting results have been reported[21-24] on the prognostic value of *TLX3* activation in pediatric T-ALL. Confounding elements, such as heterogeneity of patient cohorts and clinical trials and differences in therapeutic strategies, might explain such discrepancies.

The survival of patients with T-ALL has improved over the past 10 years, especially in paediatric co-operative studies, because of more intensive post-induction chemotherapy.[2,3] Nevertheless, a subgroup usually identified as being at very high risk, generally including prednisone-poor responders and/or *slow* responders to early chemotherapy (M3), still have a high mortality rate. Our analysis confirmed the pejorative impact of a poor response to prednisone on both leukemia-free and overall survival in the FRALLE-93 T-ALL cohort and showed that *TLX3* activation is of significant value in predicting worse outcome in terms of overall and leukemia-free survival independently of the prednisone response at day 8. Interestingly, we observed that *TLX3* expression was more significantly associated with inferior survival than was M3 status (*TLX3* positivity, $p \geq 0.049$ *vs.* M3 status, $p=0.09$). Our data confirm the findings of Ferrando[22] in a cohort of 59 children and young adults treated at St. Jude hospital,[33] and the more recent results of van Grotel *et al.* in two independent cohorts of 72 and 53 children treated according to the DCOG ALL7/8 and COALL-97 protocols, respectively.[24] In the latter study, with a median follow up of 3 years, *TLX3* expression was significantly predictive of a poor outcome in both univariate (log-rank $p=0.014$) and multivariate analysis (log-rank $p=0.039$). As in our study, the patients had a high median white blood cell count (135×10^9/L) and similarly, the most marked hyperleukocytosis was observed in the *SILTAL* and *CALM-AF10* subgroups.

Our data reinforce results from adult T-ALL studies.[31,34] An analysis of potential risk factors among the 92 T-ALL patients treated in the LALA94 trial[31] clearly indicated an association between *TLX3* expression and inferior overall survival (13% *vs.* 47%; $p=0.009$). Similar data emerged from the analysis of 100 patients with T-ALL treated in the GMALL 05/93 and 06/99 trials between 1993 and 2000, reported by Baldus *et al.*[34]

Our results do, however, differ from those reported by Cavé *et al.*[23] These authors did not observe any pejorative effect associated with *TLX3* expression in a cohort of 138 T-ALL cases treated with EORTC 58881 (n=75) and 58951(n=78) protocols between 1989 and 2002. As in the case of the FRALLE-93 protocol, the general design of the 58881 trial had many similarities with that of the ALL-BFM90 trial and treatment results of T-ALL patients included in the very high risk and standard risk groups were also similar, with global leukemia-free survival rates of 61.7%±3.6 and 63.8%±6.7, respectively, at 8 years. The overall survival of T-ALL patients reported by Cavé *et al.* was 78%, thus higher than in the 58881 study. This difference could result from a bias in sample recruitment over the period, reflecting the better outcome of patients treated in the more recent 58951 study. In any case, the low number of events in that cohort of patients does not allow the detection of significant differences among subgroups.

Some features of the patient population at diagnosis in the Cavé's study differed strikingly from our and from other studies. Firstly, the median white blood cell count in the EORTC cohort was lower than in the FRALLE cohort (49×10^9/L *vs.* 98.85×10^9/L); this was also the case for the subgroups expressing *TLX3* (34×10^9/L *vs.* 120×10^9/L), *SILTAL* (124×10^9/L *vs.* 238.5×10^9/L) and *TLX1* (29×10^9/L *vs.* 140×10^9/L). Secondly, the median age of patients positive for *TLX3*, and *TLX1* was lower than in the FRALLE cohort (6 and 5 *vs.* 9.2 and 9.8 years, respectively) whereas the median age of SILTAL- positive patients was higher (11 *vs.* 6 years). These differences are difficult to explain but they could be revelant to the interpretation of the results.

Besides the differences in patient populations and treatment efficacy, another explanation of the difference in the prognostic relevance of *TLX3* may be the presence of associated oncogenetic events. The recent finding of *NUP214-ABL1* episomal fusions within T-ALL patients positive for *TLX3* or *TLX1* activation could support this hypothesis. We observed a global frequency of *NUP214-ABL1* of 5.8%, which is in agreement with a previous report,[20] with the frequency increasing to 16% (3 of 18) among the *TLX3*-positive subgroup. Our data show that this association may effectively worsen clinical outcome, as suggested by Graux *et al.*, and a search for *NUP214-ABL1* should be systematically included in the investigation of *TLX3*-positive cases of T-ALL.

In keeping with the studies mentioned above, patients

with *SILTAL*-positive T-ALL showed very high white blood cell counts at diagnosis and were more frequently poor responders to prednisone at day 8 of treatment. Nevertheless, *SILTAL* expression did not affect significantly either leukemia-free survival or overall survival. One possible explanation is that *TAL1* expression and/or reactivation is a frequent oncogenetic event associated with T-ALL, even in the absence of structural alterations of its locus.[12,21] The levels of *TAL1* expression are highly variable and not directly linked to the underlying mechanism of activation. Thus, it would be interesting, as in the case of *TLX1* expression,[35] to identify what level of expression has a direct impact on the prognosis.

We observed a favorable outcome in patients with *TLX1*-positive T-ALL, which appears consistent with observations in adults suggesting a positive association between *TLX1* expression and *good responder* patient.[32]

Finally, we were unable to confirm previous data suggesting a pejorative role of *CALM-AF10* fusion transcripts.[30,36] but with only four positive cases and one event in this subgroup, statistical analysis of the clinical impact of this lesion would not be reliable.

In conclusion, our study highlights the importance of *TLX3* activation as a new and powerful risk predictor in pediatric patients with T-ALL. *TLX3* expression and the presence of *NUP214-ABL1* should be taken into account when evaluating patients with high-risk disease in order to improve outcome in pediatric T-ALL.

Authorship and Disclosures

PB, JL-P, GL, AB: conception and design of the study; GL, J-LP, LD, FS, AB: administrative support; PB, J-LP, VA, JMC, SF, VG, YP, MB, TL, CS, LD, AH, AB: provision of study materials or patients; PB, JL-P, VA, JMC, MFA: collection and assembly of the data; PB, ML, JL-P, AB: data analysis and interpretation; PB, JL-P: manuscript writing. The authors reported no potential conflicts of interest.

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