Clinical features and outcome of *SIL/TAL1*-positive T-cell acute lymphoblastic leukemia in children and adolescents: a 10-year experience of the AIEOP group

T-cell acute lymphoblastic leukemia (T-ALL) accounts for approximately 15% and 25% of childhood and adult cases of ALL, respectively,[1,2] and is associated with a less favorable outcome.[1] In recent years, however, more intensive and risk-adapted treatment has significantly improved the outcome of patients with T-ALL, leading to cure rates approaching 70% in children and adolescents, and 30%-40% in adults.[2-4]

Whether classical risk factors, such as age and white blood cell (WBC) count at diagnosis, are relevant in T-ALL patients is still a matter of debate. At the moment, treatment response, measured at different time points, is considered the most important factor for risk stratification. The identification of new prognostically relevant diagnostic markers, such as genetic abnormalities, could, however, be instrumental in refining risk-adapted treatment stratification in T-ALL.[5]

Mutation of *TAL1* (1p32) is a non-random genetic defect often present in childhood T-ALL. This gene encodes for a

Table 1. Clinical characteristics of patients according to genetic group.

	SIL/TAL1 positive		*SIL/TAL1* negative		Total		
	N	%	N	%	N	%	*P*
Total	56	15.6	303	84.4	359		
Gender							
Male	50	89.3	231	76.2	281	78.3	0.03
Female	6	10.7	72	23.8	78	21.7	
Age (years)							
1-5	16	28.6	94	31.0	110	30.6	0.31
6-9	13	23.2	94	31.0	107	29.8	
10-17	27	48.2	115	38.0	142	39.6	
Median value		9.5 years		8.1 years			
WBC count (x 10^9/L)							
<20	7	12.5	67	22.5	74	20.9	<0.001
≥20-<100	11	19.6	115	38.6	126	35.6	
≥100	38	67.9	116	38.9	154	43.5	
(5 not known)							
Median value		174 x10^9/L		67x10^9/L			
CNS involvement							
Positive	5	9.4	25	8.5	30	8.6	0.76
Negative	48	90.6	270	91.5	318	91.4	
(11 not known)							
Immunophenotype							
Early T	24	49.0	113	40.5	137	41.8	0.28
Cortical T	17	34.7	131	47.0	148	45.1	
Mature T	8	16.3	35	12.5	43	13.1	
Biclonal T	4		9		13		
T (not specified)	3		15		18		
Early response							
Response to PDN							
PGR	25	45.5	206	69.1	231	65.4	<0.001
PPR	30	54.5	92	30.9	122	34.6	
(6 not known)							
Response to IA							
CR	53	96.4	274	92.6	327	93.2	0.31
No CR	2	3.6	22	7.4	24	6.8	
(1 death, 7 not known)							
PCR-MRD TP1/TP2							
MRD-SR	5	11.6	34	16.0	39	15.2	0.77
MRD-IR	28	65.1	131	61.5	159	62.1	
MRD-HR	10	23.3	48	22.5	58	22.7	
(103 not evaluable)							
Final risk group							
Standard	4	7.1	33	10.9	37	10.3	0.05
Intermediate	20	35.7	150	49.5	170	47.4	
High	32	57.2	120	39.6	152	42.3	

WBC: white blood cells; CNS: central nervous system; PDN: prednisone; PGR: prednisone good responder; PPR: prednisone poor responder; CR: complete remission; PCR-MRD: polymerase chain reaction minimal residual disease; TP1: time point 1; TP2: time point 2; SR: standard risk; IR: intermediate risk; HR: high risk.

Table 2. Events according to genetic group.

	SIL/TAL1 positive		*SIL/TAL1* negative		Total	
	N	%	N	%	N	%
Total	56		303		359	
Death in induction	1	1.8	1	0.3	2	0.5
Resistant to protocol	1	1.8	4	1.3	5	1.4
Relapses (deaths)	14(9)	25.0	72(56)	23.8	86(65)	24.0
Site						
BM	5		44		49	
CNS	5		10		15	
Testis	1		2		3	
BM+others	1		9		10	
Other extramedullary	2*		7**		9	
Second malignant neoplasm	2	3.6	3	1.0	5	1.4
Death in CCR	1	1.8	8	2.6	9	2.5
After chemotherapy	0		5	1.6	5	1.4
After HSCT	1	1.8	3	1.0	4	1.1
Alive in CCR	37	66.1	215	71.0	252	70.2
5-year EFS		64.1%(6.8)		71.2%(2.6)		70.2%(2.4)
5-year survival		78.6%(5.5)		76.3%(2.5)		76.6%(2.2)

*BM: bone marrow; CNS: central nervous system; CCR: continuous complete remission; HSCT: hematopoietic stem cell transplantation; EFS: event-free survival; SE, standard error; *Sites: lymph nodes (1); lymph nodes and mediastinum (1); **Sites: lymph nodes and/or mediastinum (6); CNS and testis (1).*

protein with a basic helix-loop-helix motif, a DNA binding domain common to several transcriptional regulatory factors. Disruption of *TAL1* has been reported in up to 30% of T-ALL cases,[1] and is frequently associated with a submicroscopic interstitial deletion (90 Kb) between the 5' untranslated region (UTR) of the *TAL1* and the *SIL* genes (9%-26% of cases, depending on the different studies). The *SIL/TAL1* fusion product gives rise to the inappropriate expression of TAL1, which, in turn, may promote T-cell leukemogenesis.[1,6-8] The clinical relevance and the prognostic value of this rearrangement remain unclear.[1,9-12] Different studies have reported either a favorable or unfavorable outcome trend for *SIL/TAL1* positive patients.[1,8,12]

We evaluated the frequency and prognostic value of *SIL/TAL1* in a large cohort of children (age 1-17 years) with newly diagnosed T-ALL and treated at AIEOP centers according to the AIEOP-BFM ALL 2000 (September 2000-July 2006) and the subsequent AIEOP ALL R2006 (August 2006-December 2009) protocols. Treatment outlines and differences between the two protocols are summarized in the *Online Supplementary Table S1*. The study is registered at the US National Institutes of Health website (*http://clinicaltrials.gov; clinicaltrials.gov identifier: 00613457*).

Diagnosis of ALL was performed using cytomorphology and cytochemistry on bone marrow cells. T-cell origin of ALL was defined by a positive CD3 (either on cell surface or cytoplasmic) and a negative CD19 immunophenotype. Further subclassification (early, cortical or mature) was performed according to definition of the European Group for the Immunological Characterization of Leukemias (EGIL).[13] Early T-cell precursor (ETP) subtype was not routinely screened. All diagnoses were centrally reviewed and confirmed by a reference laboratory.

Patients were stratified as standard risk (SR), intermediate risk (IR) or high risk (HR) mainly on the basis of PCR-minimal residual disease (MRD) at day 33 [time point 1 (TP1)] and day 78 [time point 2 (TP2)], as previously published.[14] In addition, and regardless of the MRD results, patients with either prednisone (PDN) poor-response (PPR; ≥1000 circulating blasts/μL on day 8) or failure to achieve complete remission (CR) after induction phase IA were allocated to the HR arm.

Genomic DNA samples at diagnosis were retrospectively screened for the type 1 and type 2 *TAL1* deletions by PCR amplification using the BIOMED-1 primer sets and PCR conditions.[15]

The cycling protocol for *TAL1* deletion type 1 amplification consisted of 90 s at 94°C of initial denaturation, then 60 s at 60°C and 90 s at 72°C for the first cycle, followed by 35 cycles of 30 s at 94°C, 30 s at 60°C, 90 s at 72°C, and a final extension phase of 10 min at 72°C. The cycling protocol for TAL1 deletion type 2 amplification consisted of 3 min of initial denaturation at 92°C, followed by 40 cycles of 45 s at 92°C, 90 s at 60°C, 2 min at 72°C, and a final extension phase of 10 min at 72°C. PCR products were examined by 1% agarose gel electrophoresis and then sequenced by the Sanger method.

Overall, 359 of 382 children with T-ALL (92%) were screened for *SIL/TAL1* deletion type 1 and 52 of them (14.5%) were positive; 290 of 359 cases with biological material available were subsequently tested for deletion type 2 and only 4 of them (1.4%) were positive, as expected from the literature.[7] Since *SIL/TAL1* type 2 deletion is quite rare, the 69 *SIL/TAL1* type 1 negative patients who were not screened for type 2 deletion were included in the negative cohort. Thus a total of 56 cases (16%) were positive for *SIL/TAL1*. The characteristics of the *SIL/TAL1* positive and negative patients and data on their early response to treatment are reported in Table 1. Male gender was highly prevalent in the *SIL/TAL1* patients (P=0.03). There was

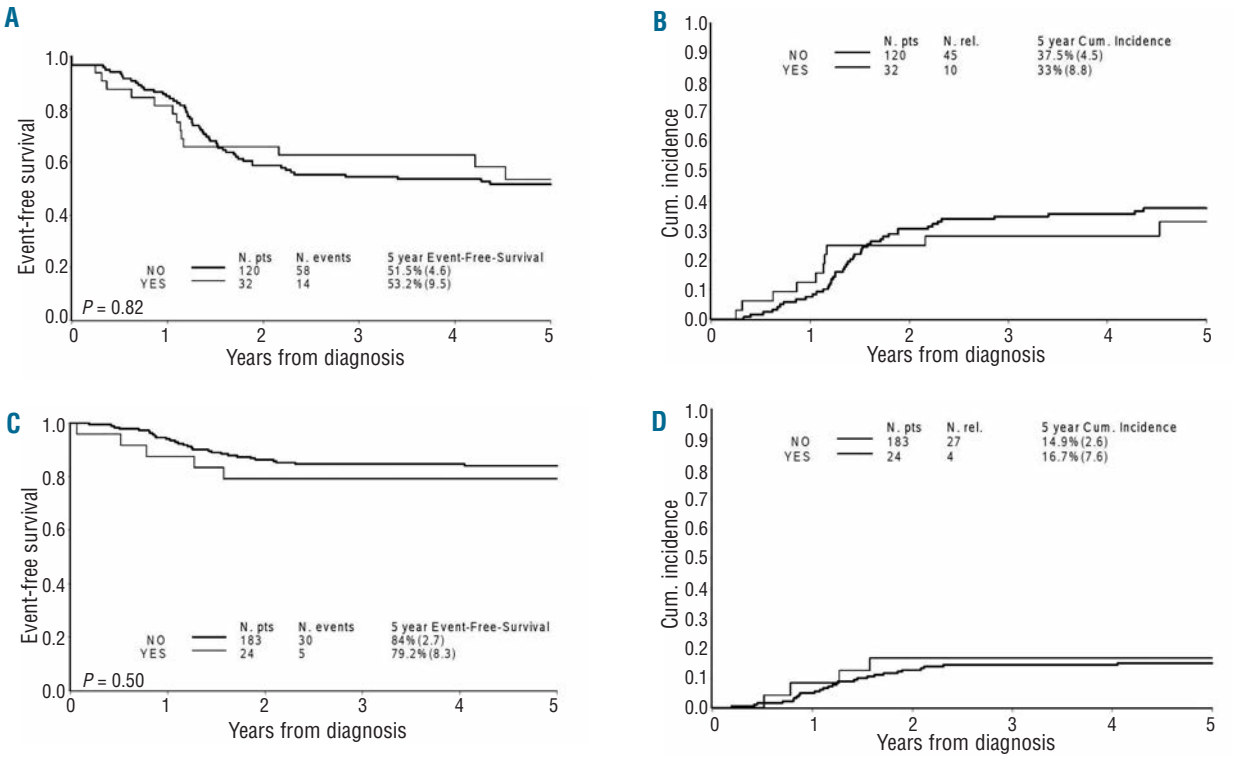

Figure 1. (A). Event-free survival in patients *SIL/TAL1* positive *versus SIL/TAL1* negative in the HR group. (B). Cumulative incidence of relapse in patients *SIL/TAL1* positive *versus SIL/TAL1* negative in the HR group. (C). Event-free survival in patients SIL/TAL1 positive *versus SIL/TAL1* negative in no HR group. (D). Cumulative incidence of relapse in patients *SIL/TAL1* positive *versus SIL/TAL1* negative in the no HR group.

no significant difference in age distribution between the two groups (P=0.31), with a tendency for more adolescents in the *SIL/TAL1* positive group. There was a statistical difference in WBC count at presentation between the two subgroups (P<0.001) with *SIL/TAL1* positive and negative patients having respectively a median WBC count of 174x10^9/L *versus* 67x10^9/L and a WBC count of 100x10^9/L or more in 68% *versus* 39% of patients. Detailed immunophenotype was available in 341 patients: 53 *SIL/TAL1* positive and 288 negative. The immunophenotype distribution was similar in the two subgroups (P=0.28). There was a significant difference in response to the PDN pre-phase between the two groups: 54.5% of *SIL/TAL1* positive children were PPR *versus* 31% of those who are negative (P<0.001). Data on morphological response at the end of phase IA were available for 351 patients; the rate of patients not in CR was 3.6% and 7.4% in *SIL/TAL1* positive *versus* negative cases (P=0.31). There was no significant difference in MRD distribution between the two subgroups (P=0.77). The percentage of HR patients was higher in the *SIL/TAL1* positive group compared to the group of negative patients mainly due to a higher frequency of PPR. The final risk stratification was as follows: 7% SR, 36% IR and 57% HR in *SIL/TAL1* positive *versus* 11%, 49% and 39% in *SIL/TAL1* negative patients (P=0.05).

The 5-year event-free survival (EFS) was 64.1% (SE 6.8) *versus* 71.2% (2.6), and the overall survival (OS) 78.6% (5.5) *versus* 76.3% (2.5) (P=0.34; P=0.82) for patients *SIL/TAL1* positive or negative, respectively. At a median follow up of 6.9 years, a total of 86 relapses were registered: 14 (25.0%) in the *SIL/TAL1* positive and 72 (23.8%) in the negative groups, respectively (Table 2). There was no significant difference in cumulative incidence of relapse (CIR) between the two genetic groups [5-year CIR of 26.1% (6.1) *versus* 23.8% (2.5) in *SIL/TAL1* positive or negative patients; P=0.72]. *SIL/TAL1* positive patients did, however, have a higher frequency of extramedullary relapses (8 of 14, 57% *versus* 19 of 72, 26%; P=0.01). There was no significant difference in EFS and CIR curves within HR and no HR patients (Figure 1A-D). In analysis according to age (1-9 years *versus* 10-17 years), the EFS for *SIL/TAL1* positive and negative patients, was 67.9% (9.9) *versus* 71.1% (3.4) (P=0.96) and 59.3% (9.5) *versus* 71.3 (4.2) (P=0.17), respectively. *SIL/TAL1* positivity did not have a significant prognostic value also when analyzed in a Cox regression model, after adjusting for factors known to potentially influence outcome (risk group, age and WBC count at diagnosis).

In this relatively large study, 16% of the children screened showed the presence of *SIL/TAL1* with a preponderance of breakpoint db1. As already reported,[11] no difference was observed in the two cohorts of children with regard to immunophenotypic features of the leukemic cells. The increase in *SIL/TAL1* frequency with age observed here, although not statistically significant, is in keeping with the results of Cavè *et al.* which suggest a modal distribution, with a higher incidence of this alteration in adolescents and young adults.[1,8] *SIL/TAL1* deletion was also significantly more frequent in males, strongly associated with higher initial WBC count and PPR, but, interestingly, no difference according to MRD response or response to phase IA was seen. Although in our study the cumulative incidence of relapses was similar for *SIL/TAL1* positive and

negative patients (both overall and also separately in non-HR or HR subgroups), a higher frequency of extramedullary relapses was observed in the group of positive patients. This original finding, not reported previously, suggests that this gene alteration might play a role in the migration process of leukemia T cells. The median time of extramedullary relapses in *SIL/TAL1* positive patients was very similar to that of relapses involving the bone marrow (0.9 *versus* 1.1 years from diagnosis).

In conclusion, our study shows an association of *SIL/TAL1* deletion with adolescence, higher WBC count at diagnosis, and PPR. No major differences in overall outcome were seen. EFS was slightly inferior in the *SIL/TAL1* positive subgroup, but there was no difference in survival. This finding may be due to the relapse sites. In fact, the excess of relapses in the *SIL/TAL1* positive subgroup is largely represented by patients suffering extramedullary relapse who have a higher probability of being subsequently rescued. This study, like many others aimed at assessing the role of single genetic lesions, was not able to detect a prognostic value in childhood T-ALL, suggesting that further investigations should take into account multiple genetic alterations rather than focus on single ones.

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