Pediatric-inspired intensified therapy of adult T-ALL reveals the favorable outcome of *NOTCH1/FBXW7* mutations, but not of low *ERG/BAALC* expression: a GRAALL study

*Raouf Ben Abdelali,[1] *Vahid Asnafi,[1] Thibaut Leguay,[2] Nicolas Boissel,[3] Agnès Buzyn,[4] Patrice Chevallier,[5] Xavier Thomas,[6] Stephane Lepretre,[7] Françoise Huguet,[8] Norbert Vey,[9] Martine Escoffre-Barbe,[10] Emmanuelle Tavernier,[11] Oumedaly Reman,[12] Nathalie Fegueux,[13] Pascal Turlure,[14] Philippe Rousselot,[15] Jean-Yves Cahn,[16] Veronique Lheritier,[6] Yves Chalandon,[17] Marie-Christine Béné,[18] Elizabeth Macintyre,[1] Hervé Dombret,[3] and Norbert Ifrah,[19] for the Group for Research on Adult Acute Lymphoblastic Leukemia

[1]Université Paris 5 Descartes, Hôpital Necker-Enfants-Malades, Assistance Publique-Hopitaux de Paris (AP-HP), Laboratoire d'hématologie and Centre National de la Recherche Scientifique Unité Mixte de Recherche 8147, Paris, France; [2]Department of Hematology, Centre Hospitalier du Haut Lévêque, Pessac, France; [3]University Paris 7, Hôpital Saint-Louis, AP-HP, Department of Hematology and Institut Universitaire d'Hématologie, Paris, France; [4]Department of Hematology, Hôpital Necker-Enfants-Malades, AP-HP, Paris, France; [5]Department of Hematology, Hôpital Hôtel-Dieu; Nantes, France; [6]Department of Hematology, Hôpital Edouard Herriot, Lyon, France; [7]Department of Hematology, Centre Henri Becquerel, Rouen, France; [8]Department of Hematology, Hôpital Purpan, Toulouse, France; [9]Department of Hematology, Institut Paoli Calmettes, Marseille, France; [10]Department of Hematology, CHU Rennes, Rennes, France; [11]Department of Hematology, Institut de Cancérologie de la Loire, Saint Priest en Jarez, France; [12]Department of Hematology, Centre Hospitalier, Caen, France; [13]Department of Hematology, University Hospital Montpellier, Montpellier, France; [14]Department of Hematology, Centre Hospitalier Dupuytren, Limoges, France; [15]Department of Hematology, Université Versailles Saint-Quentin-en-Yvelines Versailles, France; [16]Department of Hematology and Unité Mixte de Recherche 5525, Centre National de la Recherche Scientifique-UJF, Grenoble, France; [17]Division of Hematology, University Hospital of Geneva, Geneva, Switzerland; [18]Department of Immunology, Faculté de Médecine, Université Henri Poincaré Nancy I, Nancy, France; and [19]Pôle de recherche et d'enseignement supérieur de l'Université de Nantes-Angers-Le Mans, Centre Hospitalier Universitaire Angers service des Maladies du Sang et Inserm U 892, Angers, France

Despite recent progress in the understanding of acute lymphoblastic leukemia (T-ALL) oncogenesis, few markers are sufficiently frequent in large subgroups to allow their use in therapeutic stratification. Low *ERG* and *BAALC* expression (*E/B*^{low}) and *NOTCH1/FBXW7 (N/F)* mutations have been proposed as powerful prognostic markers in large cohorts of adult T-ALL. We therefore compared the predictive prognostic value of *N/F* mutations versus *E/B*^{low} in 232 adult T-ALLs enrolled in the LALA-94 and Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) protocols. The outcome of T-ALLs treated in the pediatric-inspired GRAALL trials was significantly superior to the LALA-94 trial. Overall, 43% and 69% of adult T-ALL patients were classified as *E/B*^{low} and *N/F* mutated, respectively. Strikingly, the good prognosis of *N/F* mutated patients was stronger in more intensively treated, pediatric-inspired GRAALL patients. The *E/B* expression level did not influence the prognosis in any subgroup. *N/F* mutation status and the GRAALL trial were the only 2 independent factors that correlated with longer overall survival by multivariate analysis. This study demonstrates that the *N/F* mutational status and treatment protocol are major outcome determinants for adults with T-ALL, the benefit of pediatric inspired protocols being essentially restricted to the *N/F* mutated subgroup. (*Blood*. 2011;118(19):5099-5107)

Introduction

The outcome of acute lymphoblastic leukemia (ALL) has been considerably improved in pediatric cases, with 5-year overall survival (OS) rates now reaching > 80%.[1] In adults with Philadelphia chromosome (Ph)-negative ALLs, even if the complete remission (CR) rate reaches 90%, long-term therapeutic results remain less satisfactory, with a 5-year OS rate of 45%.[1] The Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study group recently reported a significant improvement in the outcome of Ph-negative adult ALL using a pediatric-inspired, intensified treatment protocol.[2] T-ALL corresponds to a heterogeneous group of acute leukemias, which account for 35% of Ph-negative adult ALLs. The classic initial prognostic factors used for ALL therapeutic stratification are predominantly clinical: age, WBC count, and CNS involvement.[3] These undoubtedly identify prognostically distinct subgroups among patients with B lineage ALL, but they are less appropriate for the stratification of T-ALL.

Although recent advances have led to spectacular progress in the understanding of T-ALL oncogenesis,[4] the large number of molecular markers identified in this process and the fact that most are only present in minor subgroups limit their use for therapeutic stratification. Recognized oncogenic pathways in T-ALL include transcriptional activation of several proto-oncogenes, submicroscopic deletions of tumor suppressor genes, and *NOTCH1* and/or *FBXW7* mutations.[5,6] The NOTCH1 signaling pathway has been shown to be an essential factor in normal and pathologic T lymphoid development.[7,8] In T-ALL, NOTCH1 abnormalities

Submitted February 1, 2011; accepted July 14, 2011. Prepublished online as *Blood* First Edition paper, August 11, 2011; DOI 10.1182/blood-2011-02-334219.

The online version of this article contains a data supplement.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 USC section 1734.

*R.B.A. and V.A. contributed equally to this study.

were first identified in the t(7;9)(q34;q34.3), which juxtaposes the C-terminal region of human *NOTCH1* to the TCRβ enhancer, leading to aberrant expression of a truncated dominant active and ligand-independent form of NOTCH1 (called TAN-1) in rare patients (< 1%).[9] In mouse models, constitutive activation of NOTCH1 signaling induced T-ALL, as did transplantation with TAN1-expressing hematopoietic progenitor cells.[10-13] In 2004, activating *NOTCH1* mutations were reported in ~ 50% of childhood T-ALLs.[6] These mutations involve either the heterodimerization (HD) domain, when they probably facilitate cleavage of the NOTCH receptor, and/or the negative regulatory PEST domain, when they probably increase the half-life of intracellular NOTCH (ICN). An alternative mechanism of constitutive NOTCH1 activation by loss-of-function mutations of *FBXW7*, leading to inhibition of ubiquitin-mediated degradation of activated NOTCH1, has also been reported.[14-16]

NOTCH1 and/or *FBXW7* (*N/F*) mutations lead to activation of the NOTCH1 pathway in > 70% of both pediatric and adult T-ALL and have been reported to be of favorable outcome in pediatric T-ALL. Whether this is also the case in adult T-ALL remains controversial.[5,17,18] Low *ERG* and *BAALC* (*E/B*) expression, initially described in acute myeloid leukemia,[19,20] was reported to predict a highly favorable outcome in 41% of adult T-ALLs by the German Multicenter Study Group for Adult Acute Lymphoblastic Leukemia.[21,22] This group also showed that *N/F* mutations are associated with better event-free survival (EFS) for *E/B*^{low} patients but are not predictive of a better outcome in the overall cohort of adult T-ALL patients.[17]

We therefore compared the predictive prognostic value of *N/F* mutations versus *E/B* expression levels in 232 adult T-ALLs enrolled in the French LALA-94 and GRAALL trials.

Methods

Trials and patients

The LALA-94 multicenter prospective randomized trial has been previously reported.[23] The present study was restricted to 87 patients with available DNA and/or cDNA among the 236 T-ALL adult patients included. The CR rate and outcome of these 87 patients did not differ significantly from the overall cohort; their 3-year OS was estimated at 45% (95% CI, 34%-55%) versus 41% (95% CI, 34%-47%). The LALA-94 protocol was replaced by pediatric-inspired GRAALL03-05 protocols, which were more intensive, especially in terms of nonmyelotoxic drug doses (steroids, vincristine, and L-asparaginase) and included a steroid prephase with evaluation of peripheral blood corticosensitivity before the initiation of induction chemotherapy. The GRAALL-2003 protocol was a multicenter phase 2 trial that enrolled 76 adults with T-ALL between November 2003 and November 2005,[2] 56 of whom had available DNA and/or cDNA and are reported here. These 56 patients were representative of the overall GRAALL-2003 T-ALL population, with a 3-year OS of 68% (95% CI, 54%-79%) versus 67% (95% CI, 55%-77%). The multicenter randomized GRAALL-2005 trial is an ongoing phase 3 trial that is very similar to the GRAALL-2003 phase 2 but included 2 prospective randomized comparisons: (1) to receive or not an intensified sequence of hyperfractionated cyclophosphamide during induction and late intensification (this was given to all patients in the GRAALL-2003); and (2) in B-lineage CD20-positive ALL patients only, to receive or not rituximab during induction, consolidation, late intensification, and maintenance. At the time of the first planned interim analysis, 119 adults with T-ALL had been randomized in the GRAALL-2005 between May 2006 and March 2009. We report here on 89 patients of these patients, for whom DNA and/or cDNA was available. As before, these 89 patients were representative of the whole GRAALL-2005 T-ALL population, with a 3-year OS of 60% (95% CI, 46%-72%) versus 74% (95% CI, 47%-89%; $P = .73$). Consent was obtained from all patients at trial entry according to the Declaration of Helsinki. The study was in accordance with local and multicenter research ethical committee approval. The present study is composed of a total of 232 T-ALL patients, including 87 LALA-94 and 145 GRAALL (GRAALL-2003 and GRAALL-2005) cases. Median follow-up was 7.7 and 3.0 years for LALA-94 and GRAALL patients, respectively.

T-ALL diagnosis and molecular analysis

Diagnosis of T-ALL was based on the World Health Organization 2008 criteria, defined by expression of cytoplasmic and/or surface CD3, and negativity of CD19 and MPO.[24] TCR-based classification of T-ALL was performed as described.[25] DNA and RNA were extracted from fresh and/or cryopreserved bone marrow or blood samples as described.[26] Patients were selected for this study according to the availability of DNA and/or cDNA of acceptable quality (C_t ABL < 30) for molecular analysis. Direct sequencing of *NOTCH1* and *FBXW7* was performed centrally at the Necker Hospital for the 232 adult T-ALL patients as described.[5]

ERG and BAALC transcripts were quantified for 187 adult T-ALL (63 LALA-94, 124 GRAALL) by real-time RT-PCR as described.[22] ERG, BAALC, and ABL transcripts were quantified in duplicate in the same experiment, and 3 independent experiments were performed for each sample. The mean cycle number difference ($m\Delta C_t = \Sigma[C_t$ ERG or C_t BAALC $- C_t$ ABL]/6) was then calculated. The expression levels of *ERG* and *BAALC* relative to *ABL* were expressed as $2^{m\Delta Ct}$.

To classify T-ALL patients in the same way as the GMALL group, cases were dichotomized at the *ERG* median expression level into low and high expressers, and defined as *BAALC* low with expression levels between the first and third quartiles and as *BAALC* high with expression levels in the upper quartile as reported.[22] *N/F* mutational status and *E/B* expression levels were compared with immunophenotype, TCR rearrangement status, fusion transcript detection (SIL-TAL and CALM-AF10), and oncogenic transcript quantification (TLX1 and TLX3).[26]

Statistical analysis

Binary variables were compared with the Fisher exact test. The Mann-Whitney test was used for median comparisons. OS and EFS were calculated from the date of prephase initiation. Events accounting for EFS were failure of remission induction, relapse, and death in first CR. Failure time data were estimated by the Kaplan-Meier method[27] and then compared by the log-rank test[28] Cumulative incidence estimations took into account competing risks and were compared by the Gray test.[29] For multivariate analysis, we used a backward stepwise selection Cox model,[30] with the following covariates: trial (GRAALL vs LALA or GRAALL-2005 vs GRAALL-2003), NF status, WBC with a 100×10^9/L cut-off, age with a 35-year cut-off, cortical immunophenotype, *TLX1* overexpression, and prophase sensitivity for GRAALL patients. P value < .05 was considered to indicate statistical significance. All calculations were performed using the STATA/SE Version 10.0 software (Stata Corporation) and the R Version 1.5.1 software (R Development Core Team).

Results

T-ALL outcome in LALA-94 versus GRAALL-2003/GRAALL-2005 trial

The overall CR rate was 92% (n = 214 of 232 patients). At 3 years, cumulative incidence of relapse, EFS, and OS were estimated at 47% (95% CI, 40%-54%), 45% (95% CI, 38%-51%), and 58% (95% CI, 51%-64%), respectively. As previously reported in overall GRAALL-2003 Ph-negative ALL patients,[2] the outcome of patients with T-ALL was significantly better when treated according to the GRAALL trials compared with the LALA-94 trial. CR rates were comparable (92% in both LALA-94 and GRAALL subgroups). However, the 3-year cumulative incidence of relapse

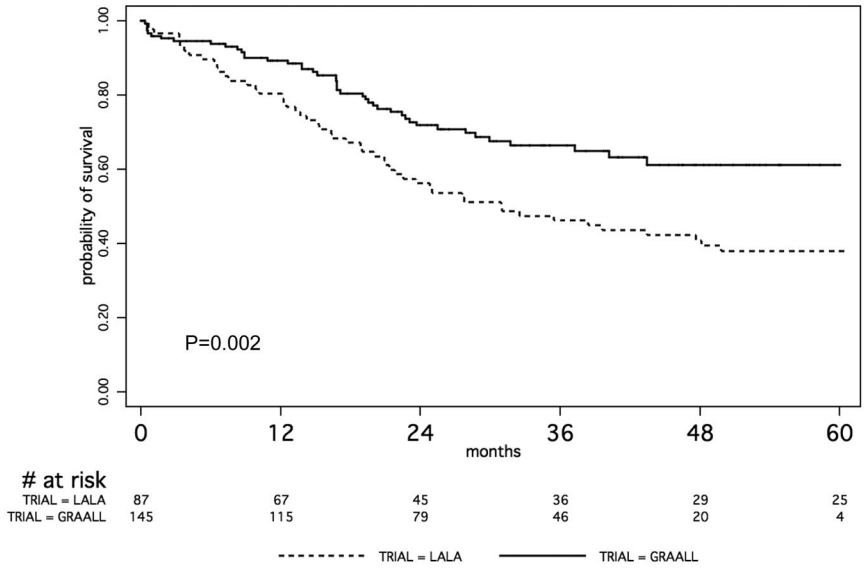

Figure 1. Kaplan-Meier estimates of OS of adult T-ALL according to the LALA-94 versus GRAALL trials. Patients treated in GRAALL (black line) have a significantly better outcome than those enrolled in LALA-94 (dashed line).

was 63% (95% CI, 52%-74%) compared with 36% (95% CI, 28%-46%; $P = .0001$), 3-year EFS was 34% (95% CI, 24%-44%) and 54% (95% CI, 45%-62%; $P = .001$), and 3-year OS was 45% (95% CI, 38%-51%) and 58% (95% CI, 51%-64%; $P = .002$; Figure 1), in the LALA-94 and GRAALL subgroups, respectively. These results remained essentially unchanged after censoring patients who received allogeneic stem cell transplantation at the time of transplantation (not shown). As shown in Table 1, the only significant difference between the GRAALL and LALA-94 subgroups was the higher proportion of patients with marked leukocytosis in the LALA-94 trial.

According to the United Kingdom/Eastern Cooperative Oncology Group (UK/ECOG) risk classification, which defines high-risk T-ALL on the basis of age (35 years or older) and initial WBC $> 100 \times 10^9$/L only, 141 of the 232 patients had high-risk ALL (61%) and 91 had standard-risk ALL (39%). This 2-risk classification was significantly predictive of shorter EFS (40% vs 53% at 3 years; $P = .04$) and OS (50% vs 70% at 3 years; $P = .002$), but the cumulative incidence of relapse was not statistically higher in the high-risk group (50% vs 43.5% at 3 years; $P = .51$).

Clinical, immunophenotypic, and oncogenic features correlated to N/F mutational status

NOTCH1 mutations were identified in 142 (61%) of the 232 T-ALL patients. HD mutations were present in 119 cases, alone (94 cases), in association with mutations of the proline glutamate serine threonine domain (PEST; 24 cases), or as internal duplication (1 case). PEST mutations were detected in 18 cases as a unique *NOTCH1* mutation. The other types of mutations were rare: only 6 cases harbored *NOTCH1* internal duplication, alone in 4 cases and in association to HD or PEST, in one case each. *FBXW7* mutations were identified in 46 patients (20%), of whom 29 cases were also *NOTCH1* mutated. The most frequent *FBXW7* mutations were arginine substitution at R465 (19 cases), R505 (11 cases), R479 (7 cases), and R689 (3 cases). The 6 remaining *FBXW7* mutations were G423 (2 cases), G477 (1 case), S516 (1 case), V504 (1 case), and a stop insertion (1 case). Five of the latter cases were also *NOTCH1* HD mutated (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). Overall, 159 of 232 adult T-ALL patients (69%) were *NOTCH1* and/or *FBXW7* mutated. The mutation rate of *NOTCH1* and/or *FBXW7* (*N/F*) was similar in the LALA-94 (61 of 87; 70%) and GRAALL (98 of 145; 68%) cohorts.

N/F mutated patients did not significantly differ from unmutated patients with respect to age, sex, or CNS involvement at diagnosis (Table 2), but WBC counts $> 100 \times 10^9$/L were found in 27% of *N/F* mutated cases versus 43% of unmutated cases ($P = .02$). As a consequence, the proportion of UK/ECOG high-risk patients was higher in the unmutated group (71% vs 56%; $P = .03$). An *N/F* mutated status was also more frequently observed in T-ALL with a cortical/pre-αβ immunophenotype (62% vs 43%; $P = .008$) or with *TLX1* overexpression (37 of 44, $P = .007$).

Table 1. Characteristics of the patients' cohort according to the LALA-94 and GRAALL trials.

	GRAALL	LALA-94	All	*P*
Patients, N	145	87	232	
Median age, y (range)	30 (16-59)	28 (15-55)	29 (15-59)	.2
Age ≥ 35 y	36%	31%	36%	.48
WBC, 10^9/L, median (range)	32 (0.9-645)	71 (1.4-620)	48 (0.9-645)	.01
WBC ≥ 100 × 109/L, %	26%	41%	32%	.03
UK-ECOG high-risk, %	61%	64%	61%	.68
CNS involvement, %	10%	7%	9%	.49
Prephase sensitivity, %	54%	NA	NA	NA

NA indicates not applicable.

Outcome of adult T-ALL patients based on N/F mutational status

There was no significant difference in CR rate according to *N/F* status (94% vs 88% for *N/F* mutated and GL cases, respectively; $P = .11$). In CR patients, the 3-year cumulative incidence of relapse was significantly lower in the *N/F* mutated subgroup (41% [95% CI, 33%-50%] vs 61% [95% CI, 48%-74%]; $P = .003$). This resulted in a longer 3-year EFS (52% [95% CI, 43%-60%] vs 31% [95% CI, 20%-42%]; $P = .0001$) and OS (62% [95% CI, 53%-70%] vs 48% [95% CI, 35%-60%]; $P = .0002$) in the *N/F* mutated subgroup (Figure 2).

As patient outcome was highly dependent on the treatment they received (LALA-94 vs GRAALL, as shown in Figure 1), we then analyzed the prognostic value of *NOTCH1* and/or *FBXW7* mutations in

Table 2. Clinical, immunophenotypic (EGIL[31] and TCR subsets[32]), and genotypic characteristics of adult T-ALL as a function of *NOTCH1/FBXW7* status

		NOTCH1		*FBXW7*		*NOTCH1 and/or FBXW7*		
	Total	Mut n (%)	WT n (%)	Mut (%)	WT n (%)	Mut n (%)	WT n (%)	*P*
Total, no. (%)	232	142 (61)	90 (39)	46 (20)	186 (80)	159 (69)	73 (31)	
TCR subsets analyzed, no. (%)	189							
Immature	72 (38)	42 (58)	30 (42)	12 (17)	60 (83)	46 (64)	26 (36)	NS
Pre-αβ	72 (38)	50 (69)	22 (31)	16 (22)	56 (78)	57 (79)	15 (21)	.008*
TCR^+	45 (24)	20 (44)	25 (56)	9 (20)	36 (80)	25 (56)	20 (44)	NS
EGIL, no. (%)	224							
1 or 2	73 (32)	43 (59)	30 (41)	8 (11)	65 (89)	46 (63)	27 (37)	NS
3	125 (56)	86 (69)	39 (31)	33 (26)	92 (74)	96 (77)	29 (23)	.008*
4	26 (12)	10 (38)	16 (62)	4 (15)	22 (85)	14 (54)	12 (46)	NS
Genotype subsets analyzed, no. (%)	214							
CALM-AF10	13 (6)	4 (31)	9 (69)	4 (31)	9 (69)	6 (46)	7 (54)	NS
SIL-TAL1	16 (7)	7 (44)	9 (56)	2 (12)	14 (88)	8 (50)	8 (50)	NS
TLX1	44 (21)	34 (77)	10 (23)	7 (16)	37 (84)	37 (84)	7 (16)	.007*
TLX3	23 (11)	15 (65)	8 (35)	8 (35)	15 (65)	19 (83)	4 (17)	NS
None of the above	118 (55)	68 (58)	50 (42)	22 (19)	96 (81)	74 (63)	44 (37)	NS
Clinical subsets analyzed								
Sex, male, no. (%)	183 (79)	114 (80)	69 (77)	35 (76)	148 (80)	127 (80)	56 (77)	NS
Median age, y	30	29	30	28	30	29	30	NS
Age > 35 y, no. (%)	84 (36)	52 (37)	32 (36)	13 (28)	71 (38)	56 (35)	28 (38)	NS
WBC, 10^9/L, median	48	44	52	37	52	42	76	NS
WBC > 100×10^9/L, no. (%)	74 (32)	40 (28)	34 (38)	11 (24)	63 (34)	43 (27)	31 (43)	.02*
Mediastinal involvement, no. (%)	105 (48)	70 (51)	35 (42)	26 (56)	79 (45)	78 (51)	27 (40)	NS
CNS involvement, no. (%)	20 (9)	10 (7)	10 (11)	4 (9)	16 (9)	13 (8)	7 (10)	NS
CR, no. (%)	214 (92)	136 (96)	78 (87)	41 (89)	173 (93)	150 (94)	64 (88)	NS
Relapse, no. (%)	94 (44)	48 (35)	46 (59)	18 (44)	76 (44)	55 (37)	39 (61)	.001*

NS indicates not significant.
*$P < .05$

LALA-94 and GRAALL patients separately. Strikingly, the good prognosis of *N/F* mutated patients was only found in more intensively treated, pediatric-inspired GRAALL patients, as opposed to less intensively treated LALA-94 patients. As shown in Figure 3A for EFS, the better outcome associated with *N/F* mutations was highly significant in GRAALL patients (65% [95% CI, 54%-74%] vs 30% [95% CI, 17%-45%] at 3 years, $P = .0001$), whereas there was no significant difference among LALA-94 patients (35% [95% CI, 23%-47%] vs 31% [95% CI, 15%-49%] at 3 years, $P = .10$). A similar yet even larger difference was observed for OS, when comparing GRAALL patients (74% [95% CI, 62%-82%] vs 51% [95% CI, 33%-66%] at 3 years $P = .002$) and LALA-94 patients (48% [95% CI, 34%-60%] vs 26% [95% CI, 11%-44%] at 3 years; $P = .03$; Figure 3B). Furthermore, as shown in Figure 4A and Figure 4B for EFS and OS, respectively, the favorable impact of N/F mutation was also observed when GRAALL-2003 and GRAALL-2005 patients were analyzed separately. Of note, we also observed that *N/F* mutated T-ALL GRAALL patients were more frequently sensitive to the steroid prephase than unmutated cases (61% vs 40%; $P = .02$).

This clearly suggests that treatment intensity and modalities may modulate the prognostic impact of *N/F* mutations in adult T-ALL. It is even reasonable to question the benefit of pediatric-inspired treatment intensification in patients with *N/F* unmutated T-ALL.

Clinical, immunophenotypic, and oncogenic features correlated to E/B expression level

ERG and BAALC transcripts were expressed as continuous variables by quantitative PCR, but T-ALLs were classified as *ERG* and/or *BAALC* low or high expressers using the techniques and cut-off criteria of Baldus et al.[22] The majority (35 of 47; 74%) of *BAALC* high expressers were also classified as *ERG* high ($P = .0001$). Patients with high *BAALC* expression were less frequently males (70% vs 84%; $P = .03$). More than half of the *BAALC* high patients had blasts of an immature immunophenotype ($P < .0001$), and all but one of the *TLX1* expressing cases were *BAALC* low (Table 3). High expression of *BAALC* and/or *ERG* was more frequent in T-ALL patients without recurrent recognized molecular genetic markers (67% vs 44%; $P = .002$). *ERG* high expressers, but not *BAALC* high expressers, had higher WBC at diagnosis (median, 73×10^9 vs 37×10^9; $P = .02$).

T-ALLs were then classified as *ERG* low/*BAALC* low (*E/B*^{low}) versus other *E/B* expression patterns, which were combined, as previously reported.[22] *E/B*^{low} T-ALLs corresponded to 43% (81 of 187) of cases, had lower WBC at diagnosis (median, 40 vs 64; $P = .04$), and were more frequently associated with *TLX1* overexpression (32% vs 13%; $P = .002$).

In the *E/B*^{low} T-ALL group, the proportion of UK/ECOG high-risk patients (49 of 81; 60%) was similar to that found in the remaining combined group (64 of 106; 60%), and included 29 of 53 (55%) *N/F* mutated and 20 of 28 (71%) *N/F* unmutated patients. No other significant clinical and biologic features were associated with the *E/B*^{low} group.

Outcome of adult T-ALL patients based on E/B expression levels

CR rate was not different in the group of *E/B*^{low} T-ALL compared with others (95% vs 90%; $P = .28$). In CR patients, 3-year cumulative incidence of relapse was similar in both *E/B*^{low} and the combined group (41% [95% CI, 30%-54%] vs 46% [95% CI, 36%-58%]; $P = .53$). Similarly, 3-year EFS (52% [95% CI, 40%-63%] vs 45% [95% CI, 35%-55%]; $P = .22$) and OS (65%

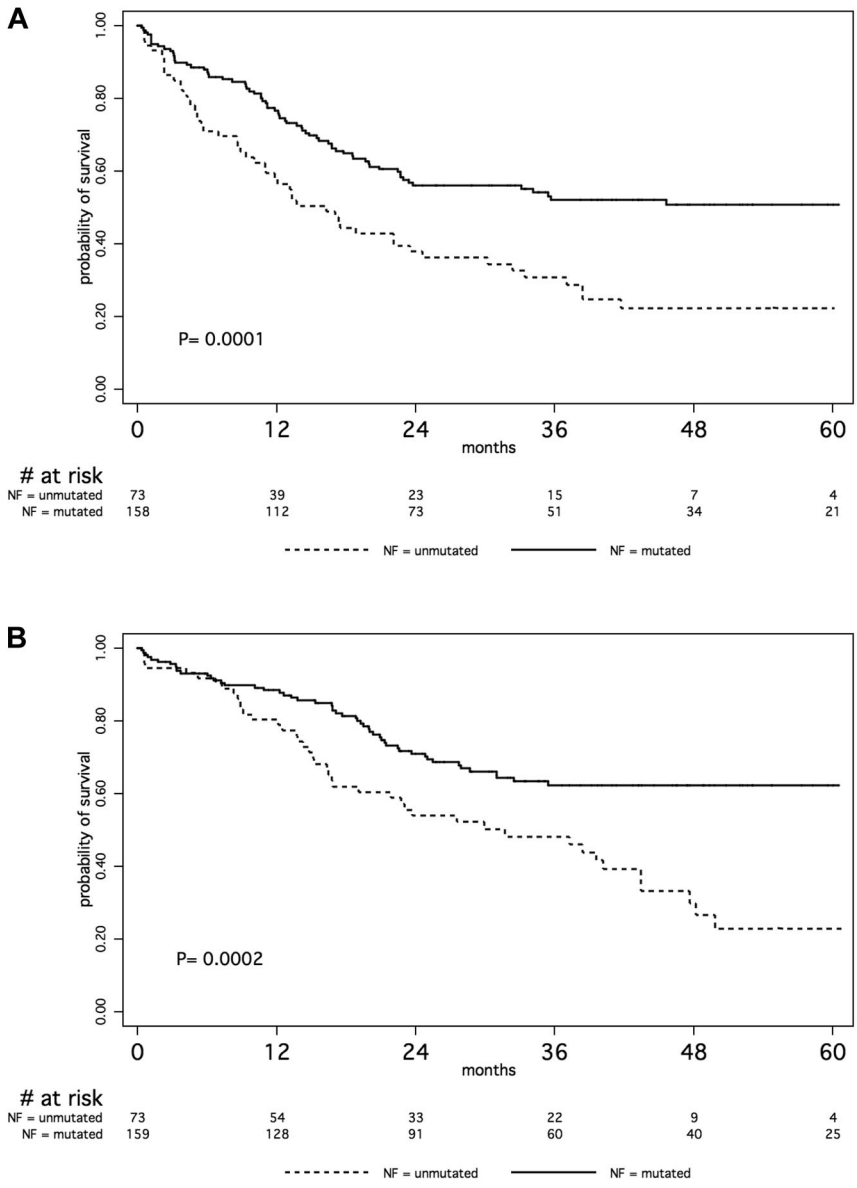

Figure 2. Impact of *NOTCH1/FBXW7* mutational status on outcome of adult T-ALL patients treated on the LALA-94 or GRAALL trials. (A) EFS and (B) OS. Kaplan-Meier analyses showing a significantly lower risk of event or death for patients with mutated *NOTCH1* and/or *FBXW7* (black line) compared with *NOTCH1/FBXW7* unmutated patients (dashed line).

[95% CI, 52%-75%] vs 60% [95% CI, 49%-69%]; $P = .15$) were not different between subgroups, although, in keeping with the GMALL results, there was a trend toward a better outcome in the *E/B*^{low} group.

When LALA-94 and GRAALL patients were analyzed separately, the *E/B* expression level did not influence the prognosis in any subgroup, even if a trend toward a better outcome was observed in *E/B*^{low} patients treated in the former LALA-94 trial (not shown). There were too few patients to allow analysis of the prognostic impact of *E/B* status in the *N/F* unmutated group. Finally, no interaction was found between the prognostic value of *NOTCH1* and/or *FBXW7* and the lack of prognostic value of high expression of *BAALC* and/or *ERG* (not shown).

NOTCH1/FBXW7 mutation is an independent prognosis factor

The results of multivariate analyses for EFS and OS are summarized in Table 4. As shown, *N/F* mutation status was an independent prognostic factor for longer EFS and OS, together with GRAALL protocols and *TLX1* overexpression. A similar analysis was performed for the subset of GRAALL-treated patients, after introducing resistance to the steroid prephase and GRAALL-2005 versus GRAALL-2003 trial as potential prognostic factors. As shown, *N/F* mutation status remained the strongest prognostic factor for longer EFS and OS in these patients. *TLX1* overexpression also positively influenced EFS, whereas age of 35 years or older had a negative impact on EFS, but not OS, in these patients.

Overall, these analyses show that *N/F* mutational status and the treatment strategy are major determinants for outcome in adults with T-ALL, with the benefit of pediatric inspired protocols being essentially restricted to the *N/F* mutated subgroup.

Discussion

The GRAALL group has previously reported that a pediatric-inspired intensification therapy improves the outcome of Ph1-negative ALL adult patients in an age-dependent fashion.[2] ALL relapses are, however, still frequently fatal, and identification of the

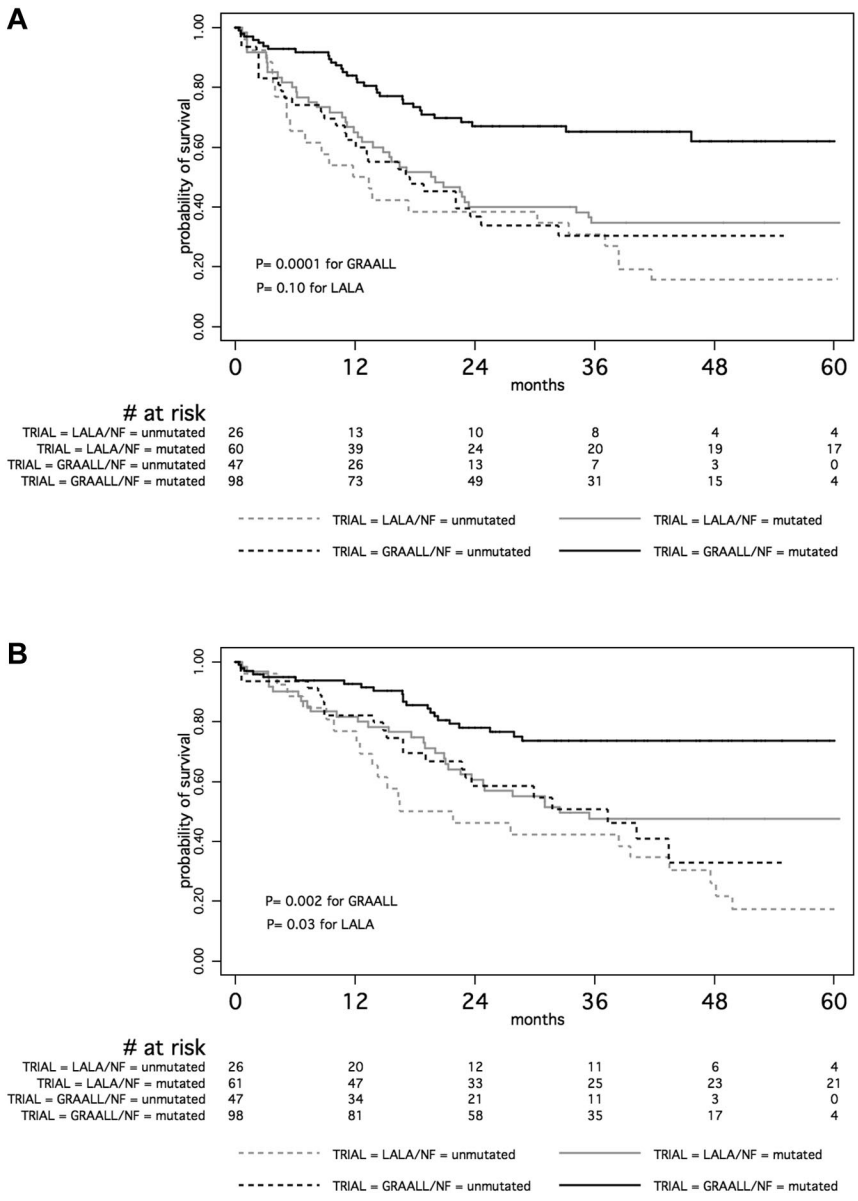

Figure 3. Impact of *NOTCH1/FBXW7* mutational status on adult T-ALL patients outcome according to the LALA-94 versus GRAALL trials. (A) EFS and (B) OS. Kaplan-Meier analyses, shown within the GRAALL trial a highly significantly better EFS and OS of patients with mutated *NOTCH1* and/or *FBXW7* (black line) compared with unmutated *NOTCH1/FBXW7* patients (dashed line).

most pertinent biologic risk factors for early and effective therapeutic stratification is needed. We here show that the *N/F* mutational status is probably such a candidate for T-lineage ALL.

We had previously reported a favorable prognostic impact of *N/F* mutations in adult T-ALL, but other studies in adult or pediatric T-ALL have reported variable results[17,18,33] or even a poor prognosis in adults.[34] Although some of these discrepancies may result from the analysis of small series, the data presented here would suggest that, for adult T-ALL, the therapeutic regimen probably differentially impacts patient outcome across various patient subsets. *N/F* mutated T-ALL respond very well to the pediatric-inspired GRAALL regimen compared with the preceding LALA-94 trial, whereas little benefit was seen for *N/F* unmutated cases, suggesting that alternative schedules should be considered for this subgroup ($\sim$ 30% of T-ALLs). One important limitation to our study is its retrospective character and the fact that the GRAALL patients were included in 2 successive and similar protocols. However, we demonstrated (Figure 4; Table 4) that the conclusions remain statistically valid after considering the GRAALL03 and GRAALL05 trials as independent variables. It will be important to confirm these findings in a prospective study.

The incidence of *NOTCH1* mutations (61%) and *FBXW7* mutations (20%) is similar here to that reported in adult and pediatric cohorts.[5,6,17,33] We observed an association between isolated heteroduplex domain (HD) mutations and *FBXW7* mutations, highlighting their synergistic functional consequences. HD mutations facilitate cleavage of the NOTCH1 receptor, whereas *FBXW7* mutations lead to the inhibition of ubiquitin-mediated degradation of intracellular cleaved form of Notch1 (ICN1), the active form of NOTCH1. By contrast, because *FBXW7* mutations and PEST mutations both increase the half-life of ICN1, they were, as expected, mutually exclusive.

Efforts to antagonize the NOTCH pathway have relied on blocking the generation of ICN using inhibitors of the γ-secretase complex (GSI). Preliminary data in humans have shown that treatment with GSI had a modest apoptosis activity, and a dose-limiting intestinal toxicity, which was reduced by the addition of glucocorticoid to GSI.[35] Recently, Moellering et al designed a

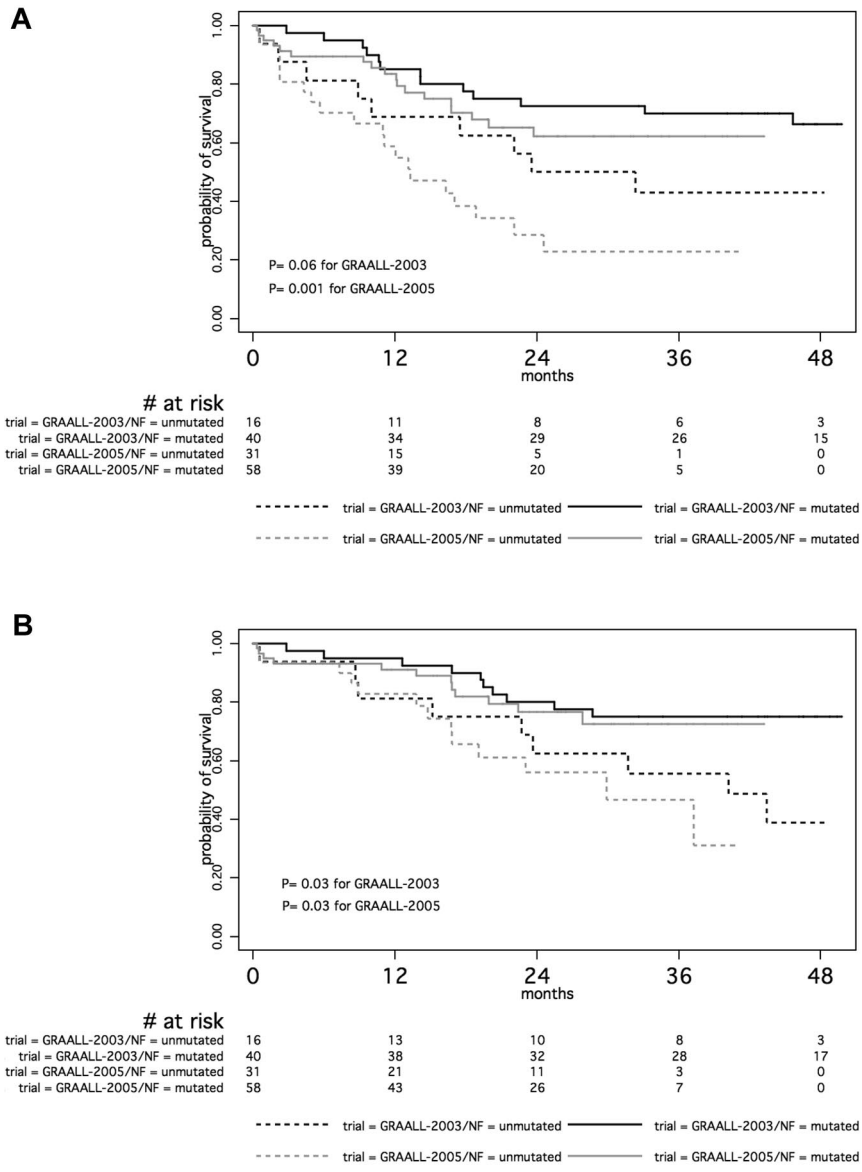

Figure 4. Impact of *NOTCH1/FBXW7* mutational status on adult T-ALL patients outcome according to the GRAALL-2003 versus GRAALL-2005 trials. (A) EFS and (B) OS. Kaplan-Meier analyses show that in both trials GRAALL-2003 and GRAALL-2005 patients with mutated *NOTCH1* and/or *FBXW7* (solid lines) have a significantly lower risk of death or relapse than those with unmutated *NOTCH1/FBXW7* (dashed lines).

small synthetic α-helical peptide, SAHM1, that acts by directly targeting a critical protein-protein interface in the NOTCH1 transactivation complex, leading to transcriptional inhibition downstream of ICN1 production.[36] Compared with GSI, this drug has the advantage of avoiding intestinal toxicity and probably also being effective in the case of isolated *FBXW7* mutations. Optimal evaluation of such therapeutic options should be undertaken in T-ALLs cases with a known mutated *N/F* status and should arguably be restricted to *N/F* mutated T-ALLs. This implies that information regarding *N/F* mutational status should be available for individual patients within a time frame compatible with such therapeutic stratification.

Because alternative prognostic markers in adult T-ALL have been described, we undertook a comparison of their relative pertinence to *N/F* status. *ERG* and *BAALC* are expressed at high levels in hematopoietic progenitors.[37,38] Their relevance in hematologic malignancies was first established in acute myeloid leukemia, where high expression of *BAALC* or *ERG* predicts a worse outcome in patients with normal cytogenetics.[19,20] The GMALL group has shown that high *ERG* and/or *BAALC* expression identifies a subgroup (59%) of adult T-ALL patients with a higher risk of relapse (4-year relapse-free survival, 33%) and decreased survival (4-year OS 30%).[21,22] In addition, recent data demonstrate that high expression of such genes as *ERG* is a feature of early T-cell precursor leukemia, which defines a minor subtype of very high-risk pediatric T-ALL.[39] In the current study, using the same methods and cut-off criteria as the GMALL group, we found a similar proportion of *ERG/BAALC* low patients (43%) and confirmed the association of *ERG* and, especially, *BAALC* expression with immunophenotypically immature T-ALLs, absence of *TLX1*$^+$, and cortical cases. However, we did not observe any significant prognostic impact of *E/B* expression levels on the relapse rate or OS in either LALA-94 or GRAALL trials, although a slight trend for higher CR rates and longer EFS in *E/B* low cases was in keeping with GMALL data. This may reflect the difference in therapeutic regimens.

In practice, E/B transcript expression is a continuous spectrum, and the classification into low or high group is based on arbitrary cut-offs, which are probably difficult to apply in multicenter and/or interprotocol settings. In acute myeloid leukemia, *BAALC* expression higher than the median expression of the overall cohort is classified as high, whereas in T-ALL, *BAALC* expression in the

Table 3. Clinical, immunophenotypic, and genotypic characteristics of adult T-ALL as a function of *ERG/BAALC* expression level

		BAALC expression		*ERG* expression		*ERG/BAALC* expression		
	Total	High, n (%)	Low, n (%)	High, n (%)	Low, n (%)	Combined, n (%)	Low, n (%)	*P*
Total, no. (%)	187	47 (25)	140 (75)	94 (50)	93 (50)	106 (57)	81 (43)	
TCR subsets analyzed, no. (%)	157							
Immature	51 (33)	24 (47)	27 (53)	27 (53)	24 (47)	34 (67)	17 (33)	NS
Pre-αβ	66 (42)	10 (15)	56 (85)	28 (42)	38 (58)	32 (48)	34 (52)	NS
TCR^+	40 (25)	8 (20)	32 (80)	25 (63)	15 (37)	25 (63)	15 (37)	NS
EGIL, no. (%)	184							
1 or 2	55 (30)	25 (45)	30 (55)	34 (62)	21 (38)	41 (75)	14 (25)	.001*
3	104 (57)	15 (14)	89 (86)	41 (39)	63 (61)	46 (44)	58 (56)	NS
4	25 (13)	7 (28)	18 (72)	17 (68)	8 (32)	17 (68)	8 (32)	NS
Genotype subsets analyzed, no. (%)	186							
CALM-AF10	7 (4)	3 (43)	4 (57)	4 (57)	3 (43)	4 (57)	3 (43)	NS
SIL-TAL1	16 (9)	2 (13)	14 (87)	9 (56)	7 (44)	9 (56)	7 (44)	NS
TLX1	40 (21)	1 (2)	39 (98)	13 (32)	27 (68)	14 (35)	26 (65)	.002*
TLX3	17 (9)	3 (18)	14 (82)	8 (47)	9 (53)	8 (47)	9 (53)	NS
None of the above	106 (57)	37 (35)	69 (65)	62 (58)	44 (42)	70 (66)	36 (34)	.002*
N/F mutation	127 (68)	34 (27)	93 (73)	66 (52)	61 (48)	74 (58)	53 (42)	NS
Clinical subsets analyzed								
Sex, male, no. (%)	151 (81)	33 (70)	118 (84)	77 (82)	74 (80)	86 (81)	65 (80)	NS
Median age, y	30	30	30	30	30	29	30	NS
Age > 35 y, no. (%)	64 (34)	16 (34)	48 (34)	31 (33)	33 (35)	34 (32)	30 (37)	NS
Median, WBC $\times 10^9$/L	48	43	55	73	37	64	40	.04*
WBC > 100 $\times 10^9$/L, no. (%)	63 (34)	14 (30)	49 (35)	39 (42)	24 (26)	41 (39)	22 (27)	NS
Mediastinal involvement, no. (%)	90 (49)	20 (43)	70 (51)	42 (45)	48 (53)	46 (44)	44 (56)	NS
CNS involvement, no. (%)	15 (8)	3 (7)	12 (9)	5 (5)	10 (11)	7 (7)	8 (10)	NS
CR, no. (%)	172 (92)	42 (90)	130 (93)	85 (90)	87 (94)	95 (90)	77 (95)	NS
Relapse, no. (%)	70 (41)	13 (31)	57 (44)	36 (42)	34 (39)	41 (43)	29 (38)	NS

NS indicates not significant.
*$P < .05$.

upper quartile is considered as high. Technically, *N/F* mutation analysis is advantageous because it relies on robust, well-established DNA or cDNA sequencing, which is easier to standardize between centers and trials.

N/F mutations were associated with a cortical immunophenotype and *TLX*1^+ T-ALL, both of which have a favorable effect on outcome in the GRAALL study by univariate analysis. However, the prognostic value of *N/F* mutations was the only parameter to emerge from multivariate analysis. It is noteworthy that more classic parameters, such as high WBC, did not appear to be prognostically relevant, although this should be confirmed in larger patient numbers, along with the evaluation of corticosensitivity and other in vivo parameters of response to treatment, such as minimal residual disease.

In conclusion, our data show that *N/F* mutations identify a major subgroup (69%) of adult T-ALL with a highly favorable outcome, particularly within the pediatric inspired GRAALL03/05 trials. In an era when the number of potential prognostic markers in T-ALL has increased exponentially, *N/F* mutation analysis is emerging as a clinically pertinent diagnostic parameter for individual therapeutic stratification of T-ALL patients.

Acknowledgments

The authors thank A. Delannoy and S. Maury for their constructive comments on the manuscript as well as all French, Swiss, and Belgian participants, clinicians, biologists, and clinical research assistants, in the LALA-94 and the GRAALL 2003-2005 trials for collecting and providing data and samples.

Table 4. Multivariate analyses for EFS and OS

	EFS			OS		
Factor	**HR of event**	**95% CI**	***P***	**HR of death**	**95% CI**	***P***
All patients (209 patients)*	0.52	0.35-0.77	.001	0.47	0.30-0.72	.001
GRAALL protocols†						
N/F mutation	0.54	0.37-0.81	.002	0.53	0.34-0.83	.006
TLX1 overexpression	0.52	0.29-0.97	.038	0.42	0.19-0.93	.032
GRAALL patients (135 patients)‡	0.42	0.24-0.73	.002	0.36	0.19-0.70	.002
N/F mutation						
TLX1 overexpression	0.41	0.18-0.99	.05			§
Age > 35 years	1.78	1.01-3.12	.045			§

*By backward stepwise selection from the full 6-covariate logistic regression model (see "Methods").
†GRAALA-2003 or GRAALL-2005 versus LALA-94.
‡By backward stepwise selection from the full 7-covariate logistic regression model (see "Methods").
§These 2 covariates were removed from the model with a *P* value of .11 and .15 for TLX1 overexpression and age ≥ 35, respectively.

This work was supported by the Association Laurette Fugain and the Comité département de la Ligue Contre le Cancer (E.M. laboratory). LALA-94was supported in part by Ministère de l'Emploi et de la Solidarité, France, Promoteur Hospices Civils de Lyon (PHRC 94-95-97.02). GRAALL was supported by Le Programme Hospitalier de Recherche Clinique, Ministère de l'Emploi et de la Solidarité, France (grants P0200701 and P030425/AOM03081) and the Swiss Federal Government.

The Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) includes the former France-Belgium Group for Lymphoblastic Acute Leukemia in Adults (LALA), the French Western-Eastern Group for Lymphoblastic Acute Leukemia, and the Swiss Group for Clinical Cancer Research.

Authorship

Contribution: R.B.A., V.A., H.D., E.M., and N.I. wrote the manuscript and oversaw conceptual development of the project; R.B.A. and V.A. performed and analyzed molecular data; and all authors contributed to supervision of clinical research and data collection.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

A complete list of the members of the GRAALL is available in the online supplemental Appendix.

Correspondence: Norbert Ifrah, Service des maladies du sang, Centre Hospitalier, 4 rue Larry, 49000 Angers, France; e-mail: NoIfrah@chu-angers.fr.

References

1. Pui CH, Evans WE. Treatment of acute lymphoblastic leukemia. *N Engl J Med.* 2006;354(2):166-178.
2. Huguet F, Leguay T, Raffoux E, et al. Pediatric-inspired therapy in adults with Philadelphia chromosome-negative acute lymphoblastic leukemia: the GRAALL-2003 study. *J Clin Oncol.* 2009; 27(6):911-918.
3. Rowe JM, Buck G, Burnett AK, et al. Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial. MRC UKALL XII/ECOG E2993. *Blood.* 2005;106(12):3760-3767.
4. Aifantis I, Raetz E, Buonamici S. Molecular pathogenesis of T-cell leukaemia and lymphoma. *Nat Rev Immunol.* 2008;8(5):380-390.
5. Asnafi V, Buzyn A, Le Noir S, et al. NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. *Blood.* 2009;113(17):3918-3924.
6. Weng AP, Ferrando AA, Lee W, et al. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 2004;306(5694): 269-271.
7. Maillard I, Fang T, Pear WS. Regulation of lymphoid development, differentiation, and function by the Notch pathway. *Annu Rev Immunol.* 2005; 23:945-974.
8. Radtke F, Wilson A, Mancini SJ, MacDonald HR. Notch regulation of lymphocyte development and function. *Nat Immunol.* 2004;5(3):247-253.
9. Ellisen LW, Bird J, West DC, et al. TAN-1, the human homolog of the Drosophila notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell.* 1991;66(4):649-661.
10. Allman D, Karnell FG, Punt JA, et al. Separation of Notch1 promoted lineage commitment and expansion/transformation in developing T cells. *J Exp Med.* 2001;194(1):99-106.
11. Aster JC, Xu L, Karnell FG, Patriub V, Pui JC, Pear WS. Essential roles for ankyrin repeat and transactivation domains in induction of T-cell leukemia by notch1. *Mol Cell Biol.* 2000;20(20): 7505-7515.
12. O'Neil J, Calvo J, McKenna K, et al. Activating Notch1 mutations in mouse models of T-ALL. *Blood.* 2006;107(2):781-785.
13. Pear WS, Aster JC, Scott ML, et al. Exclusive development of T cell neoplasms in mice transplanted with bone marrow expressing activated Notch alleles. *J Exp Med.* 1996;183(5):2283-2291.
14. Maser RS, Choudhury B, Campbell PJ, et al. Chromosomally unstable mouse tumours have genomic alterations similar to diverse human cancers. *Nature.* 2007;447(7147):966-971.
15. O'Neil J, Grim J, Strack P, et al. FBW7 mutations in leukemic cells mediate NOTCH pathway activation and resistance to gamma-secretase inhibitors. *J Exp Med.* 2007;204(8):1813-1824.
16. Thompson BJ, Buonamici S, Sulis ML, et al. The SCFFBW7 ubiquitin ligase complex as a tumor suppressor in T cell leukemia. *J Exp Med.* 2007; 204(8):1825-1835.
17. Baldus CD, Thibaut J, Goekbuget N, et al. Prognostic implications of NOTCH1 and FBXW7 mutations in adult acute T-lymphoblastic leukemia. *Haematologica.* 2009;94(10):1383-1390.
18. Mansour MR, Sulis ML, Duke V, et al. Prognostic implications of NOTCH1 and FBXW7 mutations in adults with T-cell acute lymphoblastic leukemia treated on the MRC UKALLXII/ECOG E2993 protocol. *J Clin Oncol.* 2009;27(26):4352-4356.
19. Baldus CD, Tanner SM, Ruppert AS, et al. BAALC expression predicts clinical outcome of de novo acute myeloid leukemia patients with normal cytogenetics: a Cancer and Leukemia Group B Study. *Blood.* 2003;102(5):1613-1618.
20. Marcucci G, Baldus CD, Ruppert AS, et al. Overexpression of the ETS-related gene, ERG, predicts a worse outcome in acute myeloid leukemia with normal karyotype: a Cancer and Leukemia Group B study. *J Clin Oncol.* 2005;23(36):9234-9242.
21. Baldus CD, Burmeister T, Martus P, et al. High expression of the ETS transcription factor ERG predicts adverse outcome in acute T-lymphoblastic leukemia in adults. *J Clin Oncol.* 2006;24(29): 4714-4720.
22. Baldus CD, Martus P, Burmeister T, et al. Low ERG and BAALC expression identifies a new subgroup of adult acute T-lymphoblastic leukemia with a highly favorable outcome. *J Clin Oncol.* 2007;25(24):3739-3745.
23. Thomas X, Boiron JM, Huguet F, et al. Outcome of treatment in adults with acute lymphoblastic leukemia: analysis of the LALA-94 trial. *J Clin Oncol.* 2004;22(20):4075-4086.
24. Swerdlow SH, Harris NL, Jaffe ES, et al, eds. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues* (4th ed). Lyon, France: IARC Press; 2008:150-154.
25. Asnafi V, Beldjord K, Boulanger E, et al. Analysis of TCR, pT alpha, and RAG-1 in T-acute lymphoblastic leukemias improves understanding of early human T-lymphoid lineage commitment. *Blood.* 2003;101(7):2693-2703.
26. Bergeron J, Clappier E, Radford I, et al. Prognostic and oncogenic relevance of TLX1/HOX11 expression level in T-ALLs. *Blood.* 2007;110(7): 2324-2330.
27. Kaplan E, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481.
28. Peto R, Peto J. Asymptotically efficient rank invariant test procedures. *J R Stat Soc.* 1972; 135(2):185-206.
29. Gray RJ. A class of k-sample tests for comparing the cumulative incidence of a competing risk. *Ann Stat.* 1998;16:1141-1154.
30. Cox D. Regression models and life tables. *J R Stat Soc.* 1972;34:187-220.
31. Bene MC, Castoldi G, Knapp W, et al. Proposals for the immunological classification of acute leukemias: European Group for the Immunological Characterization of Leukemias (EGIL). *Leukemia.* 1995;9(10):1783-1786.
32. Asnafi V, Buzyn A, Thomas X, et al. Impact of TCR status and genotype on outcome in adult T acute lymphoblastic leukemia: a LALA-94 study. *Blood.* 2005;105(8):3072-3078.
33. Breit S, Stanulla M, Flohr T, et al. Activating NOTCH1 mutations predict favorable early treatment response and long-term outcome in childhood precursor T-cell lymphoblastic leukemia. *Blood.* 2006;108(4):1151-1157.
34. Zhu YM, Zhao WL, Fu JF, et al. NOTCH1 mutations in T-cell acute lymphoblastic leukemia: prognostic significance and implication in multifactorial leukemogenesis. *Clin Cancer Res.* 2006; 12(10):3043-3049.
35. Real PJ, Tosello V, Palomero T, et al. Gamma-secretase inhibitors reverse glucocorticoid resistance in T cell acute lymphoblastic leukemia. *Nat Med.* 2009;15(1):50-58.
36. Moellering RE, Cornejo M, Davis TN, et al. Direct inhibition of the NOTCH transcription factor complex. *Nature.* 2009;462(7279):182-188.
37. Baldus CD, Tanner SM, Kusewitt DF, et al. BAALC, a novel marker of human hematopoietic progenitor cells. *Exp Hematol.* 2003;31(11):1051-1056.
38. Tanner SM, Austin JL, Leone G, et al. BAALC, the human member of a novel mammalian neuroectoderm gene lineage, is implicated in hematopoiesis and acute leukemia. *Proc Natl Acad Sci U S A.* 2001;98(24):13901-13906.
39. Coustan-Smith E, Mullighan CG, Onciu M, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. *Lancet Oncol.* 2009;10(2):147-156.