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

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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/Blow) 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/Blow 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/Blow 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.

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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


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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 NOTCH1 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.1,5,7,17,18 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/B0 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 2005,24 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-2003 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 < 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.23 ERG and BAALC transcripts were quantified for 187 adult T-ALL (63 LALA-94, 124 GRAALL) by real-time RT-PCR as described.23 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 (mΔCt = 2ΔCt; ERG or Ct BAALC − Ct ABL/6) was then calculated. The expression levels of ERG and BAALC relative to ABL were expressed as 2mΔCt.

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 method29 and then compared by the log-rank test28 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/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
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 142 cases, alone (94 cases), or in association to 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, \(P = .007\)).

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 1. Characteristics of the patients’ cohort according to the LALA-94 and GRAALL trials.**

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

NA indicates not applicable.
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 \( P = .0002 \)) 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 high 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; \( P = .002 \)).

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).

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
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 or even a poor prognosis in adults. 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 (~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. 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. Recently, Moellering et al designed a
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. 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-ALL 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. 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. 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%). 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. 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
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 **TLX1**/*H11001* 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.

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

<table>
<thead>
<tr>
<th>Factor</th>
<th><strong>BAALC expression</strong></th>
<th><strong>ERG expression</strong></th>
<th>**ERG/**BAALC expression</th>
<th><strong>P</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>High, n (%)</td>
<td>Low, n (%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>187</td>
<td>47 (25)</td>
<td>140 (75)</td>
<td></td>
</tr>
<tr>
<td><strong>TCR</strong> subsets analyzed, no. (%)</td>
<td>157</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immediate</td>
<td>51 (33)</td>
<td>24 (47)</td>
<td>27 (53)</td>
<td></td>
</tr>
<tr>
<td>Pre-up</td>
<td>66 (42)</td>
<td>10 (15)</td>
<td>56 (85)</td>
<td></td>
</tr>
<tr>
<td>TCR</td>
<td>40 (25)</td>
<td>8 (20)</td>
<td>32 (80)</td>
<td></td>
</tr>
<tr>
<td><strong>EGIL, no. (%)</strong></td>
<td>184</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or 2</td>
<td>51 (30)</td>
<td>24 (47)</td>
<td>27 (53)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>104 (57)</td>
<td>15 (14)</td>
<td>89 (86)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>25 (13)</td>
<td>7 (28)</td>
<td>18 (72)</td>
<td></td>
</tr>
<tr>
<td><strong>Genotype subsets analyzed, no. (%)</strong></td>
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<td></td>
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<td></td>
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<tr>
<td><strong>CALM-AF10</strong></td>
<td>7 (4)</td>
<td>3 (43)</td>
<td>4 (57)</td>
<td></td>
</tr>
<tr>
<td><strong>SIL-TAL1</strong></td>
<td>16 (9)</td>
<td>2 (13)</td>
<td>14 (87)</td>
<td></td>
</tr>
<tr>
<td><strong>TLX1</strong></td>
<td>40 (21)</td>
<td>1 (2)</td>
<td>39 (98)</td>
<td></td>
</tr>
<tr>
<td><strong>TLX3</strong></td>
<td>17 (9)</td>
<td>3 (18)</td>
<td>14 (82)</td>
<td></td>
</tr>
<tr>
<td>None of the above</td>
<td>106 (57)</td>
<td>37 (35)</td>
<td>69 (65)</td>
<td></td>
</tr>
<tr>
<td><strong>N/F</strong> mutation</td>
<td>127 (68)</td>
<td>34 (27)</td>
<td>93 (73)</td>
<td></td>
</tr>
</tbody>
</table>

**Clinical subsets analyzed**

| Sex, male, no. (%)       | 151 (81) | 33 (70) | 118 (64) | 77 (82) | 74 (80) | 86 (81) | 65 (80) | NS       |
| Age > 35 y, no. (%)     | 64 (34)  | 16 (34) | 48 (34)  | 31 (33) | 33 (35) | 34 (32) | 30 (37) | NS       |
| WBC × 10^9/L, no. (%)   | 48 (43)  | 43 (55) | 55 (73)  | 37 (73) | 3 (37)  | 64 (40) | 40 (40) | 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       |
| Relapse, no. (%)        | 172 (92) | 42 (90) | 130 (93) | 85 (90) | 87 (94) | 96 (90) | 77 (95) | NS       |

**NS** indicates not significant.

*P* < .05.

---

**Table 4. Multivariate analyses for EFS and OS**

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

*By backward stepwise selection from the full 6-covariate logistic regression model (see “Methods”).
†GRAALLA-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.
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References

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Raouf Ben Abdelali, Vahid Asnafi, Thibaut Leguay, Nicolas Boissel, Agnès Buzyn, Patrice Chevallier, Xavier Thomas, Stephane Lepretre, Françoise Huguet, Norbert Vey, Martine Escoffre-Barbe, Emmanuelle Tavernier, Oumedaly Reman, Nathalie Fegueux, Pascal Turlure, Philippe Rousselot, Jean-Yves Cahn, Veronique Lheritier, Yves Chalandon, Marie-Christine Béné, Elizabeth Macintyre, Hervé Dombret, Norbert Ifrah and for the Group for Research on Adult Acute Lymphoblastic Leukemia