Toward a NOTCH1/FBXW7/RAS/PTEN-based oncogenetic risk classification of adult T-cell acute lymphoblastic leukemia: a Group for Research in Adult Acute Lymphoblastic Leukemia study

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Abstract

The Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) recently reported a significantly better outcome in T-cell acute lymphoblastic leukemia (T-ALL) harboring NOTCH1 and/or FBXW7 (N/F) mutations compared with unmutated T-ALL. Despite this, one third of patients with N/F-mutated T-ALL experienced relapse.

Reference


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Toward a NOTCH1/FBXW7/RAS/PTEN–Based Oncogenetic Risk Classification of Adult T-Cell Acute Lymphoblastic Leukemia: A Group for Research in Adult Acute Lymphoblastic Leukemia Study


Purpose
The Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) recently reported a significantly better outcome in T-cell acute lymphoblastic leukemia (T-ALL) harboring NOTCH1 and/or FBXW7 (N/F) mutations compared with unmutated T-ALL. Despite this, one third of patients with N/F-mutated T-ALL experienced relapse.

Patients and Methods
In a series of 212 adult T-ALLs included in the multicenter randomized GRAALL-2003 and -2005 trials, we searched for additional N/K-RAS mutations and PTEN defects (mutations and gene deletion).

Results
N/F mutations were identified in 143 (67%) of 212 patients, and lack of N/F mutation was confirmed to be associated with a poor prognosis. K-RAS, N-RAS, and PTEN mutations/deletions were identified in three (1.6%) of 191, 17 (8.9%) of 191, and 21 (12%) of 175 patients, respectively. The favorable prognostic significance of N/F mutations was restricted to patients without RAS/PTEN abnormalities. These observations led us to propose a new T-ALL oncogenetic classifier defining low-risk patients as those with N/F mutation but no RAS/PTEN mutation (97 of 189 patients; 51%) and all other patients (49%; including 13% with N/F and RAS/PTEN mutations) as high-risk patients. In multivariable analysis, this oncogenetic classifier remained the only significant prognostic covariate (event-free survival: hazard ratio [HR], 3.2; 95% CI, 1.9 to 5.15; \(P < .001\); and overall survival: HR, 3.2; 95% CI, 1.9 to 5.6; \(P < .001\)).

Conclusion
These data demonstrate that the presence of N/F mutations in the absence of RAS or PTEN abnormalities predicts good outcome in almost 50% of adult T-ALL. Conversely, the absence of N/F or presence of RAS/PTEN alterations identifies the remaining cohort of patients with poor prognosis.


INTRODUCTION
T-cell acute lymphoblastic leukemia (T-ALL) corresponds to a heterogeneous group that accounts for 30% of adult BCR-ABL–negative acute lymphoblastic leukemias (ALLs).1 Recognized T-ALL oncogenic pathways include proto-oncogene activation, tumor suppressor gene deletion, and activation of the Notch1 pathway by NOTCH1/FBXW7 (N/F) mutations,2,3 leading to various combinations of gene alterations and complex oncogenic networks.4-8 N/F mutations involve either the heterodimerization domain, probably facilitating cleavage of the NOTCH receptor, and/or the negative regulatory PEST domain,9 increasing the half-life of intracellular NOTCH. An alternative mechanism of constitutive Notch1 pathway activation involves loss-of-function mutations of FBXW7, leading to the inhibition of ubiquitin-mediated degradation of activated NOTCH1.10

Even if the complete remission (CR) rate in adults with BCR-ABL–negative ALLs reaches 90%,
long-term outcome remains unsatisfactory, with a 5-year overall survival (OS) rate of approximately 45%. Historical prognostic factors used for therapeutic stratification are predominantly initial clinical features, including age, WBC count, immunophenotype, and CNS involvement. Minimal residual disease (MRD) quantification is a strong prognostic factor but requires stringent standardization and is obviously not available at baseline. The Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) reported a significant improvement in the outcome of adults with BCR-ABL–negative ALL using a pediatric-inspired intensified treatment protocol, which in T-ALL was particularly beneficial for patients harboring N/F mutations, compared with unmutated patients. Despite this, approximately one third of patients with N/F-mutated T-ALL experience relapse, suggesting that other factors may dampen the positive effect of N/F and making the identification of a subgroup with a favorable outcome a desirable goal.

The two pro-proliferative Ras/Raf/MEK/ERK and PI3K/PTEN/Akt/mTOR pathways have also been reported to be deregulated in limited series of pediatric T-ALL, but corresponding data for adult T-ALL are scanty. More specifically, RAS, a regulator of the Ras/Raf/MEK/ERK pathways, and PTEN, the main negative regulator of the PI3K/PTEN/Akt/mTOR pathways, both play roles in cell proliferation and resistance to chemotherapy.

Here, we identified PTEN loss-of-function deletions/mutations or K-RAS/N-RAS activating mutations as two virtually mutually exclusive genetic abnormalities found in 23% of adult T-ALLs treated on GRAALL trials. Importantly, the absence of N/F or presence of RAS/PTEN alterations identifies the 50% of patients who are most likely to benefit from alternative therapies that target either the PI3K/PTEN/Akt/mTOR or the Ras/Raf/MEK/ERK pathways.

### PATIENTS AND METHODS

The GRAALL-2003 protocol was a multicenter phase II trial that enrolled 76 adults with T-ALL between November 2003 and November 2005, of whom 57 had material available for the present study and have been previously reported. The multicenter randomized GRAALL-2005 trial was the following phase III trial and was similar to the GRAALL-2003 trial, with the addition of the randomized evaluation of an intensified sequence of hyperfractionated cyclophosphamide during induction and late intensification. Between May 2006 and May 2010, 189 adults with T-ALL were randomly assigned in the GRAALL-2005 study. The present study concerns 155 of these patients, for whom diagnostic DNA and/or cDNA was available. As for the GRAALL-2003 trial, these 155 patients were representative of the total GRAALL-2005 T-ALL population, with a 3-year OS of 67% (95% CI, 60% to 74%). The GRAALL-2003 and GRAALL-2005 protocols are briefly described in the Data Supplement. Informed consent was obtained from all patients at trial entry. Both trials were conducted in accordance with the Declaration of Helsinki and approved by local and multicenter research ethical committees. In these trials, allogeneic (allo) stem-cell transplantation (SCT) was offered in first CR in patients who had a matched sibling or 10/10 fully matched unrelated donor and at least one of the following criteria: CNS involvement at diagnosis; early resistance to chemotherapy; or presence of activating mutations as two virtually mutually exclusive genetic abnormalities found in 23% of adult T-ALLs treated on GRAALL trials.

### Table 1: Patient flow diagram

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<tr>
<th>Category</th>
<th>GRAALL-2003</th>
<th>GRAALL-2005</th>
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<tr>
<td>Enrolled patients</td>
<td>N = 265</td>
<td>N = 189</td>
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<td>N/F analyzed</td>
<td>n = 212; 100%</td>
<td>n = 191; 90%</td>
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<tr>
<td>N/K-RAS analyzed</td>
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<tr>
<td>PTEN analyzed</td>
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<td>Total classified</td>
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<td>Fully analyzed (N/F, K/N-RAS, and PTEN)</td>
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<td>High risk with N/F unmutated</td>
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<tr>
<td>(K/N-RAS and/or PTEN not analyzed)</td>
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<tr>
<td>Low-risk patients</td>
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<tr>
<td>(N/F mutated/N/K-RAS unmutated/PTEN no defects)</td>
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<td>GRAALL-2005</td>
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<td>5-year EFS</td>
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<td>N/K-RAS mutated</td>
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<td>GRAALL-2003</td>
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<td>GRAALL-2005</td>
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<td>5-year OS</td>
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<tr>
<td>5-year EFS</td>
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<td>31%</td>
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<tr>
<td>PTEN defects</td>
<td>n = 21/92; 23%</td>
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<td>GRAALL-2003</td>
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<td>GRAALL-2005</td>
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<td>5-year OS</td>
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<tr>
<td>5-year EFS</td>
<td>29%</td>
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</table>

Fig 1. Patient flow diagram. EFS, event-free survival; GRAALL, Group for Research in Adult Acute Lymphoblastic Leukemia; N/F, NOTCH1/PBXW7; OS, overall survival.
corticosteroid after the first 1-week prephase; early resistance to chemotherapy after 1 additional week of treatment; and CR not achieved after first induction.

Among the 212 consecutive adults with T-ALL included in the present study (57 GRAALL-2003 and 155 GRAALL-2005 patients), 133 were eligible for allo-SCT and 67 actually received transplantation in first CR (16 GRAALL-2003 and 51 GRAALL-2005 patients). With a point date on December 31, 2011, the median follow-up time was 4.2 years (6.0 and 3.3 years for GRAALL-2003 and GRAALL-2005 patients, respectively). Complete methods are available in the Data Supplement.

Patient characteristics and CR rates were compared using either the Fisher’s exact test or the Mann-Whitney U test. Median comparisons were performed using the Mann-Whitney U test. OS and event-free survival (EFS) were calculated from the date of prephase initiation. Events accounting for EFS were induction failure, first hematologic relapse, and death from any cause in first CR. Cumulative incidence of relapse (CIR) and relapse-free survival (RFS) were calculated from the date of CR achievement. For the analysis of survival outcomes, SCT was not considered to be a censoring event in patients who received allo-SCT in first CR. OS and EFS were estimated using the Kaplan-Meier method and then compared using the log-rank test. Multivariable regressions were performed with the Cox model. CIR was estimated taking into account death in first CR for competing risk and then compared using cause-specific hazard Cox models. Specific hazards of relapse (SHRs) and hazard ratios (HRs) were given with 95% CIs. Interactions were assessed by introducing an interaction term in the Cox model. Prognostic discriminatory powers were evaluated by concordance probability estimates

![Graph A](image1.png)  ![Graph B](image2.png)  ![Graph C](image3.png)  ![Graph D](image4.png)

**Fig 2.** Event-free survival (EFS) and overall survival (OS) by NOTCH1/FBXW7 (N/F) status and trial. (A) EFS by N/F status. At 5 years, EFS was estimated at 32% (95% CI, 19% to 45%) in patients with unmutated N/F, compared with 69% (95% CI, 60% to 76%) in those with N/F mutation. The hazard ratio (HR) for shorter EFS in the former group was 2.65 (95% CI, 1.6 to 3.9; *P* < .001). (B) OS by N/F status. At 5 years, OS was estimated at 42% (95% CI, 29% to 55%) in patients with unmutated N/F, compared with 77.5% (95% CI, 61% to 88%) in those with N/F mutation. The HR for shorter OS in the former group was 2.45 (95% CI, 1.5 to 3.9; *P* < .001). (C) EFS by N/F status in the Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL)-2003 and GRAALL-2005 trials. For GRAALL-2003 patients, 5-year EFS was estimated at 27% (95% CI, 14% to 41%) in patients with unmutated N/F, compared with 67.5% (95% CI, 51% to 80%) in those with N/F mutation. The HR for shorter EFS in the former group was 2.3 (95% CI, 1.01 to 5.2; *P* = .04). For GRAALL-2005 patients, 5-year EFS was estimated at 25% (95% CI, 15% to 39%) in patients with unmutated N/F, compared with 77.5% (95% CI, 61% to 88%) in those with N/F mutation. The HR for shorter EFS in the former group was 2.65 (95% CI, 1.6 to 4.3; *P* < .001). (D) OS by N/F status in the GRAALL-2003 and GRAALL-2005 trials. For GRAALL-2003 patients, 5-year OS was estimated at 45% (95% CI, 21% to 67%) in patients with unmutated N/F, compared with 74% (95% CI, 63% to 81%) in those with N/F mutation. The HR for shorter OS in the former group was 2.4 (95% CI, 1.4 to 4.2; *P* = .0012), GL, germline.
Lack of N/F Mutation Identifies a Poor Prognostic Subset of Adult T-ALL

N/F mutations were identified in 143 (67%; 95% CI, 61% to 74%) of the 212 analyzed patients with T-ALL (Fig 1). The mutation rate of N/F was similar in the GRAALL-2003 (70%; 95% CI, 57% to 82%) and GRAALL-2005 (67%; 95% CI, 58% to 74%) cohorts. In keeping with our previous report,3,19 EFS and OS were significantly (P < .001 and P < .001, respectively) better in T-ALLs harboring N/F mutations, compared with unmutated T-ALL (Figs 2A and 2B, respectively). Furthermore, as shown in Figures 2C and 2D, the favorable impact of N/F mutation was also observed when GRAALL-2003 and GRAALL-2005 patients were analyzed separately.

Despite this, one third of patients with N/F mutations experienced an EFS event, mostly within the first 2 years of follow-up (Fig 2A). To identify a genetic surrogate for relapsing T-ALLs, we studied Ras/Raf/MEK/ERK and PI3K/PTEN/akt/mTOR pathway activation by N/K-RAS and PTEN alteration, respectively.

N/K-RAS Mutations Are Frequent Events in Adult T-ALL

Among the 212 patients with T-ALL tested for N/F mutations, 191 were explored for activating RAS mutations. K-RAS and N-RAS mutations were identified in three (2%; 95% CI, 0.3% to 5%) of 191 and 17 (9%; 95% CI, 5% to 14%) of 191 patients, respectively. Overall, 20 (11%; 95% CI, 7% to 16%) of 191 GRAALL T-ALLs harbored activating RAS mutations. Clinical, immunophenotypic and oncogenic features of the patients were analyzed according to the absence or presence of RAS mutations (Table 1), and full details of individual patients with RAS abnormalities are reported in the Data Supplement.

Patients with RAS mutations did not differ significantly from patients without mutations with respect to age, sex, or WBC counts greater than 100 × 10⁹/L at diagnosis (Table 1). CNS involvement was found in 25% of patients with RAS mutations versus 6% of patients without mutations (P = .02). RAS mutations were also more frequently observed in T-ALL with no classical oncogenic markers compared with T-ALLs harboring Tlx1/3, Sil-Tal1, or CALM-AF10 abnormalities (78% vs 50%, respectively; P = .03). No significant correlation was found with European Group for the Immunological Classification of Leukemias (N/K-RAS, NOTCH1, and/or FBF1/WT1, T-ALL, T-cell acute lymphoblastic leukemia; TCR, T-cell receptor).

To validate the CGH array findings, N/F (introns 2 and 8) genomic allele quantification by quantitative polymerase chain reaction system was then used to identify PTEN locus by high-resolution comparative genomic hybridization (CGH) array for 100 patients already screened for PTEN exon 7 mutations. Overall, PTEN deletions were detected in five (5%; 95% CI, 2% to 11%) of 100 patients. The deletions were mainly large, ranging from 60 to 7,464 kb, but were focal and intragenic in two patients (Fig 3A) and biallelic in one patient. Because the breakpoints were relatively heterogeneous, a common deleted region, including exon 2, was identified (Fig 3A, right panel).

To validate the CGH array findings, PTEN (introns 2 and 8) genomic allele quantification by quantitative polymerase chain reaction was performed. As shown in Figure 3A, all patients with PTEN deletions identified by CGH array demonstrated a low PTEN/ALBUMIN gene dosage ratio (range, 0.05 to 0.59) compared with 39 patients without deletions (range, 0.72 to 1.4). This genomic quantitative polymerase chain reaction system was then used to identify
PTEN deletion in the remaining 75 patients tested for PTEN mutations but not by CGH array. This allowed identification of one additional patient with PTEN deletion (PTEN/ALBUMIN ratio, 0.53). Overall, PTEN genomic deletions occurred in six (3%; 95% CI, 0.9% to 6%) of 175 patients. Two patients with heterozygous PTEN deletions also harbored PTEN mutations (Data Supplement). Altogether, PTEN genomic abnormalities by deletion and/or mutation were identified in 21 (12%; 95% CI, 78% to 18%) of 175 patients.

To determine whether the observed PTEN genomic abnormalities led to inactivation of PTEN expression and function, we then analyzed protein expression by immunophenotyping and Western blot in 82 and 57 T-ALLs, respectively, with available material. All tested patients harboring PTEN genomic alteration (four deletions and seven mutations) demonstrated loss of or low-level PTEN protein expression as measured by Western blot or flow analysis (Figs 3B and 3C).

**PTEN Genomic Abnormalities Occur Frequently in Unmutated N/F- and SIL-TAL1–Positive Adult T-ALLs but Are Mutually Exclusive With N/K-RAS Mutations**

Clinical, immunophenotypic, and oncogenic features of patients were analyzed as a function of PTEN status (Table 2). Full clinical, immunophenotypic, oncogenic, and karyotypic data of individual patients with PTEN abnormalities are reported in the Data Supplement. PTEN abnormalities were more frequent in unmutated N/F-ALLs; only eight (38%; 95% CI, 18% to 62%) of 21 T-ALLs with PTEN mutations/deletions harbored N/F mutations compared with 112 (73%; 95% CI, 65% to 80%) of 154 germline PTEN T-ALLs (P < .001). With respect to recurrent oncogenic subtypes, SIL-TAL1–positive patients demonstrated the highest rate of PTEN abnormalities; seven (33%; 95% CI, 15% to 57%) of 21 T-ALLs with PTEN mutations/deletions harbored SIL-TAL1 fusion compared with only nine (6%; 95% CI, 2.8% to 11.2%) of 149 PTEN wild-type T-ALLs (P = .001).
PTEN-altered patients did not significantly differ from wild-type patients with respect to sex, CNS involvement, or early sensitivity to corticosteroids or chemotherapy (Table 2), but WBC counts greater than 100 × 10^9/L at diagnosis were found in 62% of PTEN-altered patients compared with 23% of unmutated patients (P < .001). PTEN-altered status was also more frequently observed in patients younger than 35 years of age (P = .02) and in mature T-ALLs expressing surface TCR (47% vs 18% not expressing surface TCR; P = .006). Overall, PTEN alteration was more frequent in younger, mature, TCR-positive, SIL-TAL1–positive, N/F-unmutated patients with high leukemic bulk tumors. Interestingly, only one patient with RAS mutation was also mutated for PTEN but only within a subpopulation of leukemic cells (Data Supplement), suggesting that these two oncogenic alterations affecting two different interlinked pro-proliferative pathways may be virtually mutually exclusive in adult T-ALL.

N/K-RAS Mutations and PTEN Genomic Abnormalities Predict Similar Poor Outcome

Figures 4A, 4B, and 4C show that both N/K-RAS mutations and PTEN genomic abnormalities were associated with marked trends to shorter CIR, RFS, and OS (see the Data Supplement for PTEN abnormalities alone and within N/F subgroups). Because of their biologic pro-proliferative function, mutual exclusion, and similar poor prognostic significance, we regrouped all patients with N/K-RAS mutations or PTEN genomic abnormalities in one unique RAS/PTEN alteration subgroup. Figures 4D, 4E, and 4F illustrate the significant prognostic impact of these oncogenic alterations on CIR, RFS, and OS, respectively.

RAS, PTEN, and N/F Mutational Status Identifies a Strong Classifier in Adult T-ALL

We then analyzed how the presence of these virtually exclusive N/K-RAS mutations and PTEN genomic abnormalities may modulate the good prognosis associated with N/F mutations and whether prognostic interactions may exist between these two genomic pathways. For this purpose, we performed a multivariable Cox model for CIR, RFS, and OS, entering the two N/F and RAS/PTEN covariates, as well as their interaction term. As illustrated in Figures 5A, 5B, and 5C, this analysis indicated that the prognostic significance of N/F mutation was still observed but with significant interactions between N/F and RAS/PTEN mutations, indicating that the favorable impact of N/F mutation was only observed in patients without RAS/PTEN mutation (Figs 5A to 5C). Importantly, sensitivity analyses of patients treated as part of the GRAALL-2003 trial or during the GRAALL-2005 trial demonstrated that statistical significance of the classifier was consistent in both groups (Data Supplement).

These observations led us to propose a new T-ALL oncogenic classifier defining low-risk patients as those with N/F mutation but no RAS/PTEN mutation (here, 97 of 189 studied patients; 51%) and all other patients (49%) as high-risk patients. Figures 5E, 5F, and 5G show CIR, RFS, and OS according to this new strong oncogenic classifier. As a whole, 23 patients who would have been classified as low risk based on their N/F status joined the high-risk subgroup based on their RAS/PTEN status. Importantly, these patients did not differ from their N/F-mutated, RAS/PTEN-unaltered counterparts (Data Supplement). Comparing the oncogenic risk classification based only on the N/F mutational status to this refined oncogenic classifier, HRs for high-risk patients increased from 2.6 (95% CI, 1.7 to 4.0) to 3.25 (95% CI, 2.0 to 5.3) for EFS and from 2.5 (95% CI, 1.5 to 4.0) to 3.3 (95% CI, 1.9 to 5.8) for OS. Concordance probability estimates of the old N/F versus the new N/F/RAS-PTEN classifier were 0.603 (95% CI, 0.561 to 0.645) versus 0.633 (95% CI, 0.589 to 0.677) for EFS and 0.600 (95% CI, 0.552 to 0.647) versus 0.636 (95% CI, 0.587 to 0.684) for OS, respectively.

When adjusting the effect of the N/F/RAS-PTEN classifier to age (using the 35-year cutoff) and WBC count (using the 100 × 10^9/L cutoff), the oncogenic classifier remained the only significant prognostic covariate (EFS: HR, 3.2; 95% CI, 1.9 to 5.8; P < .001; and OS: HR, 3.2; 95% CI, 1.9 to 5.8; P < .001).

A limited subset of 89 patients (46 new low-risk and 43 new high-risk patients, according to this N/F-RAS-PTEN classifier) were evaluated for genomic immunoglobulin/TCR MRD level at time of CR achievement after the first induction course. Using the 10−4 MRD cutoff, there was only a nonstatistically significant trend toward a

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**Table 2. Characteristics of Patients With T-ALL According to Their PTEN Status (PTEN CGH array, PTEN/ALB allelic ratios, and PTEN exon 7 mutation)**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients</th>
<th>PTEN Altered</th>
<th>PTEN Not Altered</th>
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</tbody>
</table>

Abbreviations: CGH, comparative genomic hybridization; CHs, chemosen- sitivity; CR, complete remission; Cs, corticosteroid sensitivity; EGIL, European Group for the Immunological Classification of Leukemias; N/F, NOTCH1 and/or FBXW7; T-ALL, T-cell acute lymphoblastic leukemia; TCR, T-cell receptor.

*Significant using t test or Fisher’s exact test when appropriate.
T-ALL Oncogenetic Classifier

Fig 4. Cumulative incidence of relapse (CIR), relapse-free survival (RFS), and overall survival (OS) by N/K-RAS mutation or PTEN genomic abnormality. (A) CIR according to the presence of N/K-RAS mutation alone, PTEN genomic abnormality alone, or both (one single patient). At 5 years, CIR was estimated at 24% (95% CI, 17% to 33%) in patients with no N/K-RAS mutation or PTEN genomic abnormality, compared with 57% (95% CI, 38% to 80%) in those with N/K-RAS mutation and 64% (95% CI, 32% to 79%) in those with PTEN genomic abnormality. In the latter subgroups, the specific hazards of relapse (SHRs) were 2.8 (95% CI, 1.5 to 4.9) in the latter group (P = .003) and 2.1 (95% CI, 1.1 to 4.3; P = .028), respectively. (B) RFS according to the presence of N/K-RAS mutation alone, PTEN genomic abnormality alone, or both (one single patient). At 5 years, RFS was estimated at 75% (95% CI, 66% to 82%) in patients with no N/K-RAS mutation or PTEN genomic abnormality, compared with 42% (95% CI, 26% to 58%) in those with PTEN genomic abnormality. In the latter groups, SHRs for shorter OS were 2.0 (95% CI, 1.04 to 3.8; P = .033) and 2.0 (95% CI, 1.06 to 3.8; P = .028), respectively. (C) OS according to the presence of N/K-RAS mutation alone, PTEN genomic abnormality alone, or both (one single patient). At 5 years, OS was estimated at 69% (95% CI, 60% to 77%) in patients with no N/K-RAS mutation or PTEN genomic abnormality, compared with 45% (95% CI, 18% to 69%) in those with N/K-RAS mutation and 43% (95% CI, 20% to 64%) in those with PTEN genomic abnormality. In the latter groups, hazard ratios (HRs) for shorter RFS were 2.6 (95% CI, 1.3 to 5.0; P = .004) and 2.2 (95% CI, 1.1 to 4.3; P = .027), respectively. (D) OS according to the presence of N/K-RAS mutation and/or PTEN genomic abnormality. The HR for shorter OS in the latter group was 2.1 (95% CI, 1.3 to 3.6; P = .003). GL, germline.
Fig 5. Cumulative incidence of relapse (CIR), relapse-free survival (RFS), and overall survival (OS) by NOTCH1/FBXW7 (NF) and RAS/PTEN mutational status. (A) CIR according to the presence of NF or/and RAS/PTEN mutations. In patients with no N/K-RAS mutation or PTEN genomic abnormality, 5-year CIR was estimated at 15% (95% CI, 9% to 24%) in patients with NF mutation, compared with 50% (95% CI, 34% to 61%) in those without NF mutation. The specific hazard of relapse (SHR) was 3.3 (95% CI, 2.0 to 10.0) in the latter group (P < .001). Conversely, in those with N/K-RAS mutation or/and PTEN genomic abnormality, 5-year CIR was similarly poor in patients with NF mutation and in those without NF mutation (58%; 95% CI, 37% to 80% v 57%; 95% CI, 33% to 83%, respectively). The SHR was 1.25 (95% CI, 0.5 to 3.3) in the latter group (P = .78). (B) RFS according to the presence of NF or/and RAS/PTEN mutations. In patients with no N/K-RAS mutation or PTEN genomic abnormality, 5-year RFS was estimated at 85% (95% CI, 76% to 91%) in patients with NF mutation, compared with 45% (95% CI, 25% to 63%) in those without NF mutation. The hazard ratio (HR) for shorter RFS in the latter group was 4.0 (95% CI, 2.0 to 10.0) (P < .001). Conversely, in those with N/K-RAS mutation or/and PTEN genomic abnormality, 5-year RFS was similarly poor in patients with NF mutation and in those without NF mutation (36%; 95% CI, 13% to 69% v 43%; 95% CI, 17% to 67%, respectively). The HR for shorter RFS in the latter group was 1.1 (95% CI, 0.45 to 2.5; P = .78). (C) OS according to the presence of NF or/and RAS/PTEN mutations. In patients with no N/K-RAS mutation or PTEN genomic abnormality, 5-year OS was estimated at 82% (95% CI, 72% to 88%) in patients with NF mutation, compared with 37% (95% CI, 19% to 55%) in those without NF mutation. The HR for shorter OS in the former group was 0.7 (95% CI, 0.3 to 1.7; P = .34). (D) CIR according to the new NF, N/K-RAS, and PTEN oncogenic classifier. At 5 years, OS was estimated at 82% (95% CI, 72% to 88%) in low-risk patients, compared with 44% (95% CI, 33% to 55%) in high-risk patients. The HR for shorter OS in the latter group was 3.3 (95% CI, 1.9 to 5.8; P < .001). GL, germline.
higher MRD response rate in low-risk compared with high-risk patients (74% vs 60%, respectively; \(P = .18\)). When adjusting the effect of the N/F-RAS-PTEN classifier to age (using the 35-year cutoff), WBC count (using the \(100 \times 10^9/L\) cutoff), and MRD response (using the \(10^{-4}\) cutoff) in these 89 patients, the oncogenetic classifier remained the only significant prognostic factor for OS (HR, 4.8; 95% CI, 1.6 to 14.8; \(P = .006\)).

Taken together, these data demonstrate that the detection of RAS and PTEN mutations adds significant prognostic value to assessment of the N/F status in isolation and allows identification of a significant proportion (48%) of good prognosis adult T-ALLs with N/F mutations but no RAS/PTEN abnormalities that cannot be identified by classical parameters.

**DISCUSSION**

Much progress has been made recently toward the identification of molecular-genetic abnormalities in T-ALL. A number of these genetic events, sometimes defined as type A mutations, act mainly to block T-cell differentiation at a specific developmental stage and delineate T-ALL subgroups displaying specific gene expression profiles. In contrast, type B mutations act by gain-of-function alterations affecting cell cycle, self-renewal, pre-TCR signaling, or constitutive tyrosine kinase activation. RAS and PTEN defects belong to this category and are involved in pre-TCR complex signaling (reviewed in Van Vlierberghe et al), which leads to the downstream activation of both the RAS/MAPK and PI3K/AKT pathways. There is also increasing recognition of the role played by tumor suppressor gene inactivation in T-ALL. PTEN is a lipid and protein phosphatase that negatively regulates the PI3K/AKT/mTOR pathway through de-phosphorylation of thePIP3 lipid second messenger. PTEN plays critical roles in cell growth, survival, and migration. The PTEN expression level can be regulated by multiple mechanisms. In leukemia, PTEN loss promotes self-renewal, stem cell formatio and leukemogenesis. Whether PTEN abnormalities are of prognostic value remains debated in childhood T-ALLs, but in general, PTEN genomic deletions are of poor prognosis, but PTEN mutations were reported to be without significant prognostic impact, albeit in a small series of pediatric T-ALL. We now show that PTEN modification is disproportionately associated with TCR-positive, high WBC, younger adult T-ALLs that demonstrate a relatively low incidence of N/F mutation and poor prognosis.

Several studies have also highlighted the oncogenic role of RAS in leukemogenesis. Oncogenic K-RAS and N-RAS mutations are described in only 2% of pediatric T-ALLs without clinical impact. RAS-mutated adult T-ALLs represent 10% and tend to have more frequently an immature immunophenotype. This association has been recently suggested and, because immature phenotypes are more frequent in adult compared with pediatric T-ALLs, might explain the higher incidence of RAS mutation in our series. As such, RAS- and PTEN-mutated patients have distinct features, in keeping with their virtually mutually exclusive occurrence.

Taken together, we have identified a significant subgroup (40 of 175 patients; 23%) of adult patients with poor prognosis T-ALL with genetic anomalies of either the PI3K/PTEN/Akt/mTOR or the Ras/Raf/MEK/ERK pathway. The intricate links in cell signaling between these pathways and the rationale for targeting both to prevent chemotherapy drug resistance and re-emergence of cancer-initiating cells have led to the development of specific inhibitors of these two pathways. Therefore, it was logical to regroup RAS/PTEN-modified T-ALLs and to develop an oncogenetic classifier of T-ALL as an extension of our previous N/F-based classification. Adults with N/F-mutated, RAS/PTEN germ-line T-ALL compose approximately 50% of patients and have an excellent prognosis. It is important to note that these new risk factors are independent from the two most important classical prognostic factors (ie, WBC count > \(100 \times 10^9/L\) and European Group for the Immunological Classification of Leukemias class). The added value of MRD assessment in these oncogenetically defined subgroups remains to be determined.

At a practical level, increasing availability of high-throughput sequencing strategies will facilitate rapid genotyping (including allelic mutation or deletion of PTEN) of diagnostic samples, thus allowing therapeutic stratification at an earlier stage that is possible with MRD-based stratification. These considerations are currently impacting the design of the next GRAALL T-ALL study.

**AUTHORS’ DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

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