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

Trinquand, Amélie; Tanguy-Schmidt, Aline; Ben Abdelali, Raouf; Lambert, Jérôme; Beldjord, Kheira; Lengliné, Etienne; De Gunzburg, Noémie; Payet-Bornet, Dominique; Lhermitte, Ludovic; Mossafa, Hossein; Lhéritier, Véronique; Bond, Jonathan; Huguet, Françoise; Buzyn, Agnès [**and 12 more**]

VOLUME 31 · NUMBER 34 · DECEMBER 1 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

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

Amélie Trinquand, Aline Tanguy-Schmidt, Raouf Ben Abdelali, Jérôme Lambert, Kheira Beldjord, Etienne Lengliné, Noémie De Gunzburg, Dominique Payet-Bornet, Ludovic Lhermitte, Hossein Mossafa, Véronique Lhéritier, Jonathan Bond, Françoise Huguet, Agnès Buzyn, Thibaud Leguay, Jean-Yves Cahn, Xavier Thomas, Yves Chalandon, André Delannoy, Caroline Bonmati, Sebastien Maury, Bertrand Nadel, Elizabeth Macintyre, Norbert Ifrah, Hervé Dombret, and Vahid Asnafi

Author affiliations appear at the end of this article.

Published online ahead of print at www.jco.org on October 28, 2013.

Support information appears at the end of this article.

Written on behalf of the Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL).

A.T. and A.T.-S. are co-first authors.

H.D. and V.A. are co-director authors.

Presented in part at the 54th Annual Meeting of the American Society of Hematology, Atlanta, GA, December 8-11, 2012; at the 2013 Congress of the Société Française d'Hématologie, Paris, France, March 27-29, 2013; and at the Second European School of Haematology–European Hematology Association Scientific Workshop on T-Cell Acute Lymphoblastic Leukemia, Lisbon, Portugal, March 22-24, 2013.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

Clinical trial information: NCT00327678, NCT00222027.

Corresponding author: Vahid Asnafi, MD, Hôpital Necker-Enfants Malades, Laboratoire d'Hématologie, 149 rue de Sèvres, 75015 Paris, France; e-mail: vahid.asnafi@nck.aphp.fr.

0732-183X/13/3134w-4333w/$20.00

DOI: 10.1200/JCO.2012.48.5292

ABSTRACT

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.

J Clin Oncol 31:4333-4342. © 2013 by American Society of Clinical Oncology

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%.[1] Historical prognostic factors used for therapeutic stratification are predominantly initial clinical features, including age, WBC count, immunophenotype, and CNS involvement.[11] Minimal residual disease (MRD) quantification is a strong prognostic factor[12] 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,[13] which in T-ALL was particularly beneficial for patients harboring *N/F* mutations, compared with unmutated patients.[3] 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,[14,15] 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,[13] of whom 57 had material available for the present study and have been previously reported.[3] 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

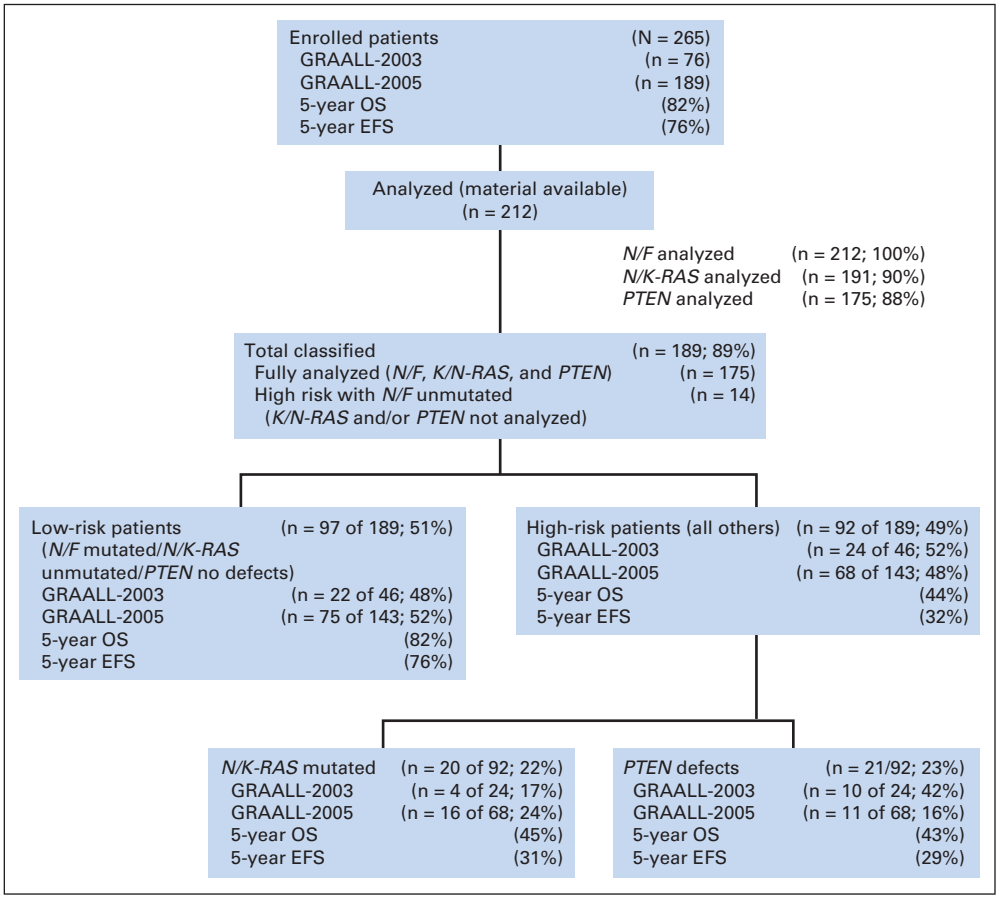

Fig 1. Patient flow diagram. EFS, event-free survival; GRAALL, Group for Research in Adult Acute Lymphoblastic Leukemia; *N/F*, *NOTCH1/FBXW7*; OS, overall survival.

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.[16] Multivariable regressions were performed with the Cox model.[17] 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[18] and then

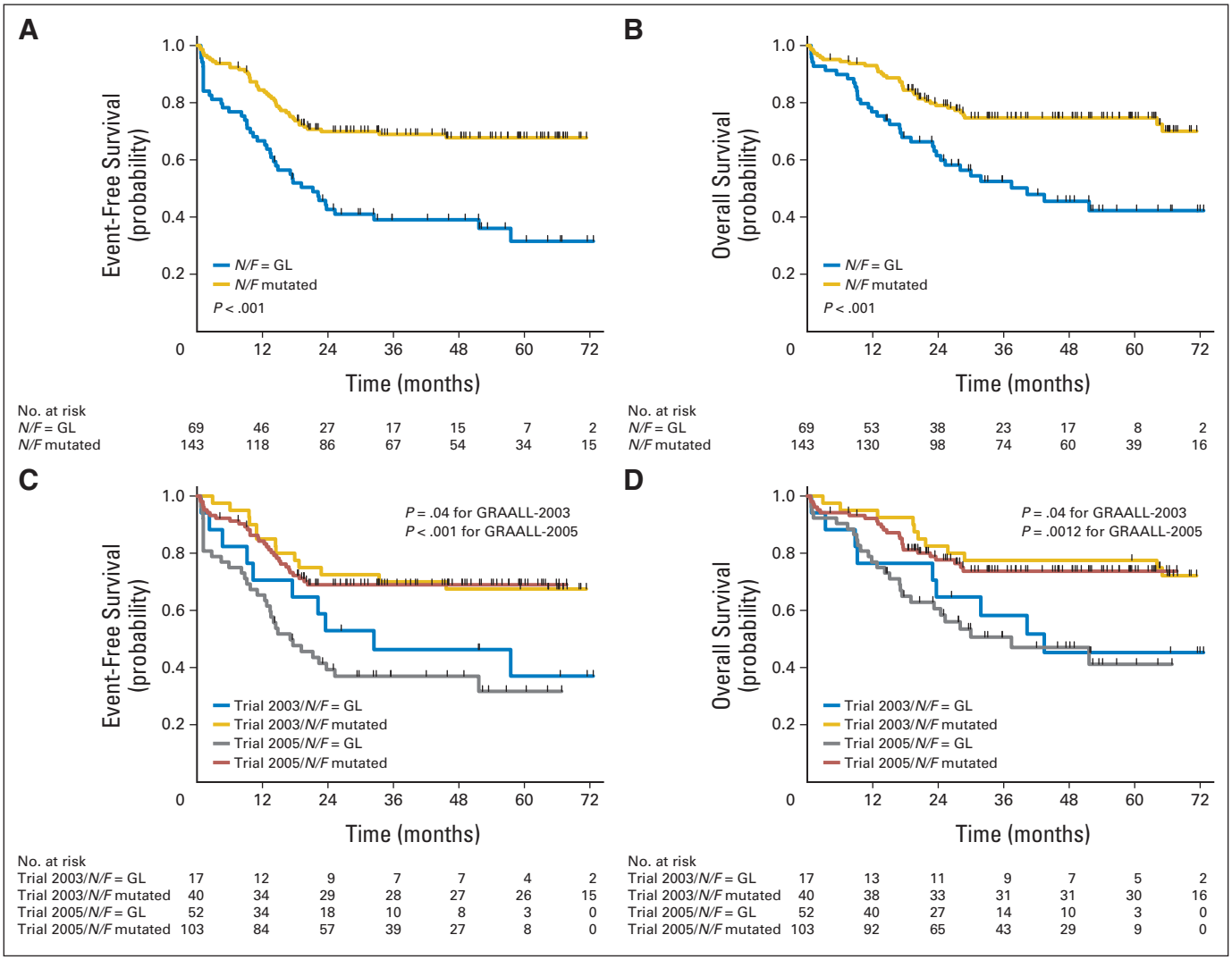

Fig 2. Event-free survival (EFS) and overall survival (OS) by *NOTCH1/FBXW7* (*N/F*) status and trial. (A) EFS by *N/F* status. At 5 years, EFS was estimated at 32% (95% CI, 19% to 45%) in patients with unmutated *N/F*, compared with 69% (95% CI, 60% to 76%) in those with *N/F* mutation. The hazard ratio (HR) for shorter EFS in the former group was 2.6 (95% CI, 1.7 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 75% (95% CI, 66% to 81%) 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 37% (95% CI, 14% to 61%) 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 32% (95% CI, 18% to 47%) in patients with unmutated *N/F*, compared with 69% (95% CI, 59% to 77%) 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 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, 1.01 to 5.9; $P = .04$). For GRAALL-2005 patients, 5-year OS was estimated at 41% (95% CI, 24% to 57%) 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.

compared using the bootstrap method. STATA/SE 10.1 software (STATA, College Station, TX) was used. All tests were two-sided, with a type I error at 5%.

RESULTS

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 100×10^9/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% *v* 50%, respectively; $P = .03$). No significant correlation was found with European Group for the Immunological Classification of Leukemias class or *N/F* status or early sensitivity to corticosteroids and chemotherapy, but *RAS* mutations were notably absent in T-cell receptor (TCR)–positive T-ALLs.

PTEN *Genomic Deletions and Mutations Lead to* PTEN *Loss in 12% of Adult T-ALLs*

PTEN mutations were identified in 17 (10%; 95% CI, 6% to 15%) of 175 patients with available material (all of whom had been tested for *RAS* mutations). All mutations were nonsense or, more frequently, frameshift insertions or insertions/deletions as reported in the Data Supplement. We then analyzed the whole *PTEN* locus by high-resolution comparative genomic hybridization (CGH) array for 100 patients already screened for *PTEN* exon7 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

Table 1. Characteristics of Patients With T-ALL According to Their *RAS* Status

Characteristic	All Patients		*N/K-RAS* Exon 1 Mutation		Wild Type		*P**
	No.	%	No.	%	No.	%	
Total patients	191		20	10	171	90	
TCR subsets analyzed	172						
Immature	44	26	7	41	37	24	.14
Pre-$\alpha\beta$	92	53	10	59	82	53	.8
TCR positive	36	21	0	0	36	23	.025†
EGIL	180						
I-II	70	39	9	50	61	38	.32
III	89	49	9	50	80	49	1.0
IV	21	12	0	0	21	13	.14
Genotype subsets analyzed	183						
CALM-AF10	9	5	0	0	9	5	.6
SIL-TAL1	16	9	0	0	16	10	.37
TLX1	37	20	2	11	35	21	.54
TLX3	25	14	2	11	23	14	1.0
None of above	96	52	14	78	82	50	.03†
N/F mutation	132	69	16	80	116	68	.32
Clinical subsets analyzed							
Male	143	75	14	70	129	76	.59
Age, years							
Median	31		34		31		.23
> 35	77	41	9	45	68	40	.64
WBC count, $\times 10^9$/L							
Median	36.4		47.1		36.4		.99
> 100	53	28	5	25	48	28	1.0
CNS involvement	16	8	5	25	11	6	.02†
CR	176	92	19	95	157	92	.56
Cs	105	55	11	55	94	55	1.0
CHs	108	57	12	60	96	56	.82

Abbreviations: CHs, chemosensitivity; 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.
*Determined using *t* test or Fisher's exact test when appropriate.
†Significant value.

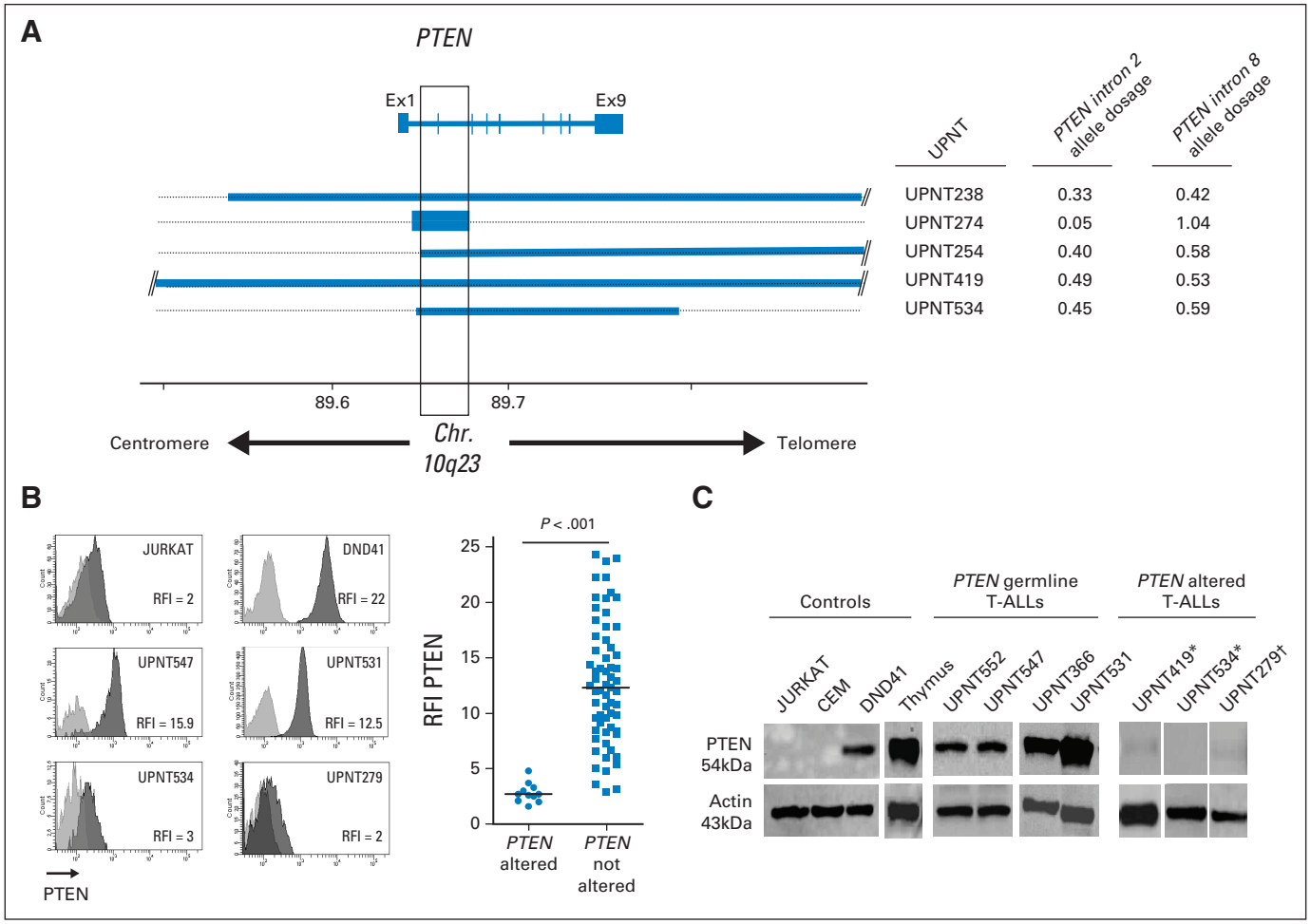

Fig 3. *PTEN* analysis. (A) Schematic representation of *PTEN* deletions (left) and *PTEN intron 2/ALB* and *PTEN intron 8/ALB* genomic quantitative polymerase chain reaction (qPCR) ratios (right) in five T-cell acute lymphoblastic leukemias (T-ALLs). Patient UPNT238 harbors a monoallelic deletion of *PTEN* concordant with gene dosage results (*PTEN intron 2/ALB* and *PTEN intron 8/ALB* qPCR ratios, 0.33 and 0.42, respectively). Patient UPNT274 harbors a biallelic deletion of the exon 2 and intron 2 of *PTEN* concordant with gene dosage results (*PTEN intron 2/ALB* qPCR ratio of 0.05 and *PTEN intron 8/ALB* qPCR ratio of 1.04). (B) Flow cytometry analysis of PTEN expression in T-ALL cell lines and primary T-ALL samples (left) and representation of PTEN ratio of fluorescence intensity (RFI) according to *PTEN* status (right). Lighter gray histograms represent the isotypic control, and the darker gray histograms represent PTEN levels. JURKAT is a *PTEN*-null cell line. DND41 harbors *PTEN* levels similar to germline *PTEN* primary T-ALLs. The two *PTEN*-altered primary T-ALLs show low PTEN protein. RFI was less than 5 in all *PTEN*-altered T-ALLs, whereas RFI ranged from 2.9 and 44.2 (median, 12.6) in *PTEN*-nonaltered T-ALLs. (C) PTEN Western blot analysis in T-ALL cell lines, normal human thymus, and primary T-ALL samples. Tested T-ALLs with germline *PTEN* status harbored higher level of PTEN protein compared with T-ALLs with genomic *PTEN* alterations. Actin was used as a loading control. Western blot data are in concordance with flow cytometric results. (*) Deletion. (†) Mutation.

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* T-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 ($P = .002$). 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 ($P = .001$).

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)

Characteristic	All Patients		*PTEN* Altered		*PTEN* Not Altered		
	No.	%	No.	%	No.	%	*P**
Total patients	175		21	12	154	88	
TCR subsets analyzed	160						
Immature	41	26	1	5	40	28	.046†
Pre-$\alpha\beta$	85	53	9	47	76	54	.6
TCR positive	34	21	9	47	25	18	.006†
EGIL	167						
I-II	65		5	25	60	41	.22
III	82		10	50	72	49	1.0
IV	20		5	25	15	10	.07
Genotype subsets analyzed	170						
CALM-AF10	8	5	1	5	7	5	1.0
SIL-TAL1	16	9	7	33	9	6	< .001†
TLX1	31	18	1	5	30	20	.13
TLX3	24	14	2	10	22	15	.74
None of above	91	54	10	48	81	54	.64
N/F mutated	120	69	8	38	112	73	.002†
Clinical subsets analyzed							
Male	131	75	18	86	113	73	.29
Age, years							
Median	30.8		24.9		31.4		.001†
> 35	69	39	3	14	66	43	.02†
WBC, $\times 10^9$/L							
Median	36.8		110		29.9		.001†
> 100	49	28	13	62	36	23	< .001†
CNS involvement	16	9	4	19	12	8	.11
CR	160	96	20	95	140	96	1.0
Cs	94	54	8	38	86	56	.16
CHs	98	56	15	71	83	54	.16

Abbreviations: CGH, comparative genomic hybridization; CHs, chemosensitivity; 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.

*Determined using *t* test or Fisher's exact test when appropriate.

†Significant value.

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% *v* 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 patients 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 oncogenetic 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* mutations 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 oncogenetic 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 5D, 5E, and 5F show CIR, RFS, and OS according to this new strong oncogenetic 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 oncogenetic risk classification based only on the *N/F* mutational status to this refined oncogenetic 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 oncogenetic classifier remained the only significant prognostic covariate (EFS: HR, 3.2; 95% CI, 1.9 to 5.15; $P < .001$; and OS: HR, 3.2; 95% CI, 1.9 to 5.6; $P < .001$).

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

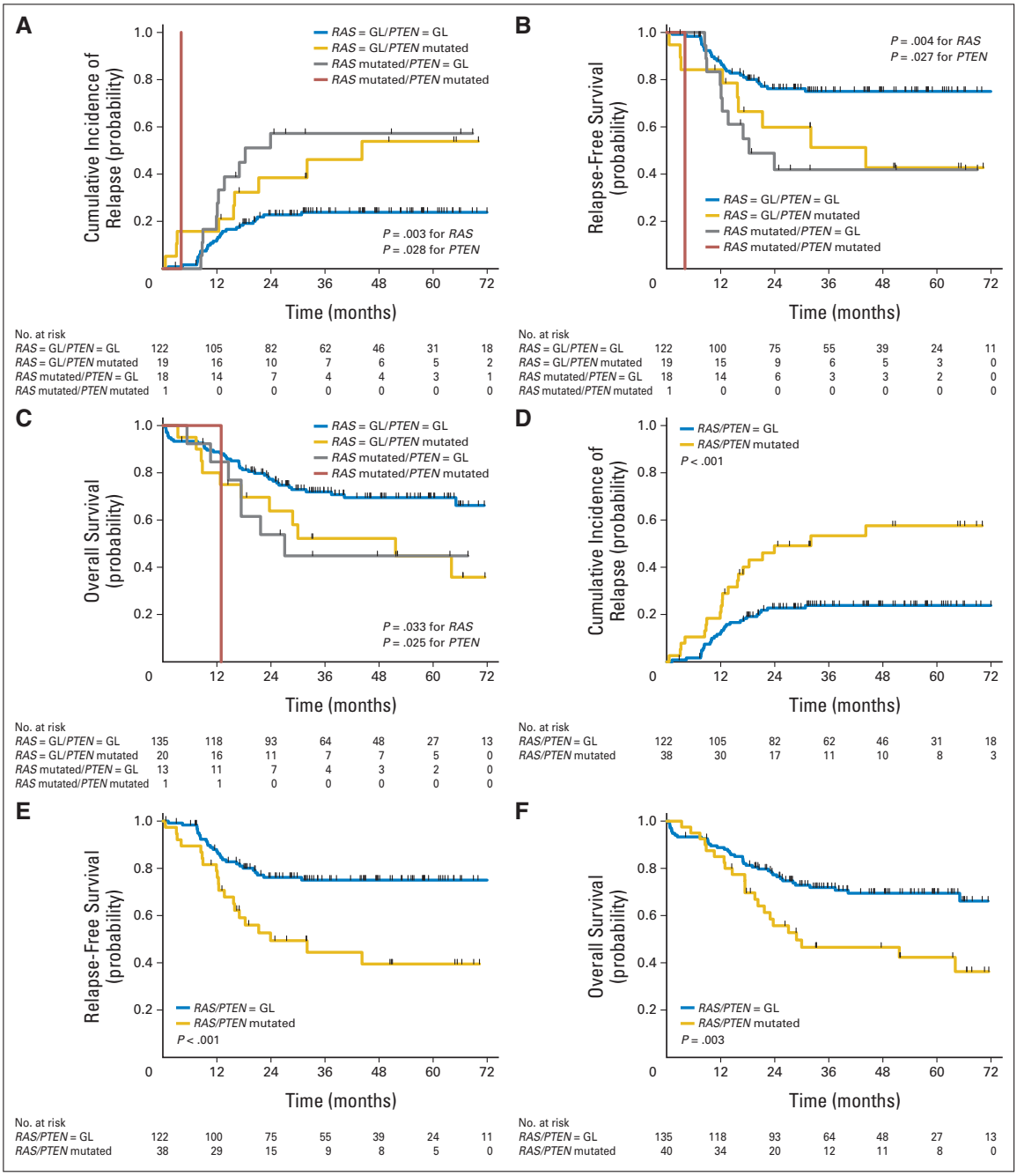

Fig 4. Cumulative incidence of relapse (CIR), relapse-free survival (RFS), and overall survival (OS) by *N/K-RAS* mutation or *PTEN* genomic abnormality. (A) CIR according to the presence of *N/K-RAS* mutation alone, *PTEN* genomic abnormality alone, or both (one single patient). At 5 years, CIR was 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, 36% to 80%) in those with *N/K-RAS* mutation and 54% (95% CI, 32% to 79%) in those with *PTEN* genomic abnormality. In the latter subgroups, the specific hazards of relapse (SHRs) were 2.6 (95% CI, 1.4 to 5.1; $P = .003$) 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, 19% to 64%) in those with *N/K-RAS* mutation and 43% (95% CI, 18% to 66%) 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. (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, HRs 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; $P = .029$), respectively. (D) CIR according to the presence of *N/K-RAS* mutation and/or *PTEN* genomic abnormality. 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 58% (95% CI, 41% to 75%) in those with *N/K-RAS* mutation and/or *PTEN* genomic abnormality. The SHR was 2.8 (95% CI, 1.5 to 4.9) in the latter group ($P < .001$). (E) RFS according to the presence of *N/K-RAS* mutation and/or *PTEN* genomic abnormality. 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 40% (95% CI, 22% to 57%) in those with *N/K-RAS* mutation and/or *PTEN* genomic abnormality. The HR for shorter RFS in the latter group was 2.7 (95% CI, 1.5 to 4.8; $P < .001$). (F) OS according to the presence of *N/K-RAS* mutation and/or *PTEN* genomic abnormality. At 5 years, OS was estimated at 69.5% (95% CI, 60% to 77%) in patients with no *N/K-RAS* mutation or *PTEN* genomic abnormality, compared with 42% (95% CI, 26% to 58%) in those with *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.

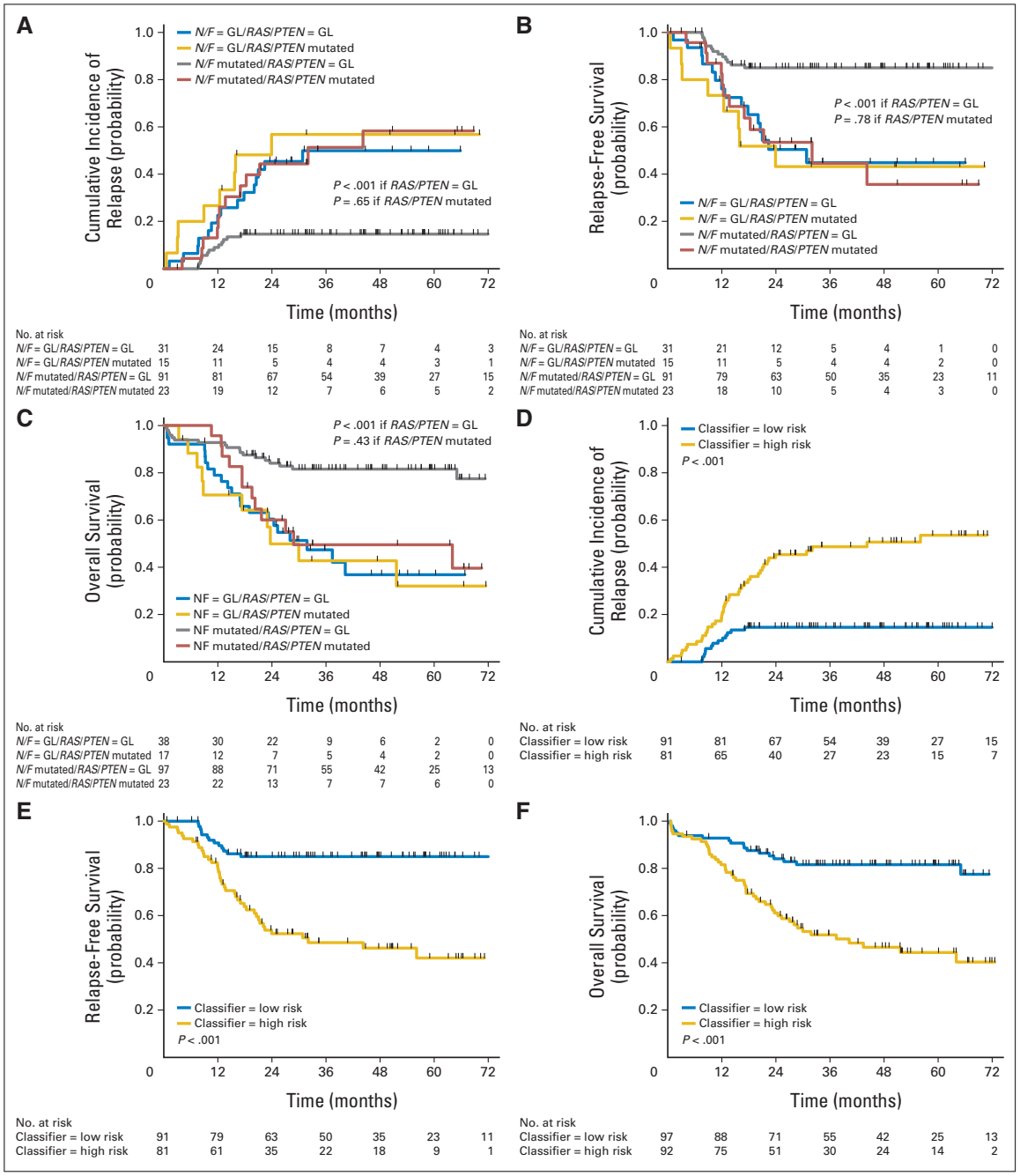

Fig 5. Cumulative incidence of relapse (CIR), relapse-free survival (RFS), and overall survival (OS) by *NOTCH1/FBXW7* (*N/F*) and *RAS/PTEN* mutational status. (A) CIR according to the presence of *N/F* and/or *RAS/PTEN* mutations. In patients with no *N/K-RAS* mutation or *PTEN* genomic abnormality, 5-year CIR was estimated at 15% (95% CI, 9% to 24%) in patients with *N/F* mutation, compared with 50% (95% CI, 34% to 64%) in those without *N/F* 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 and/or *PTEN* genomic abnormality, 5-year CIR was similarly poor in patients with *N/F* mutation and in those without *N/F* 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 ($P = .65$). (B) RFS according to the presence of *N/F* and/or *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 *N/F* mutation, compared with 45% (95% CI, 25% to 63%) in those without *N/F* 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 and/or *PTEN* genomic abnormality, 5-year RFS was similarly poor in patients with *N/F* mutation and in those without *N/F* mutation (36%; 95% CI, 13% to 59% *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 *N/F* and/or *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 *N/F* mutation, compared with 37% (95% CI, 19% to 55%) in those without *N/F* mutation. The HR for shorter OS in the latter group was 3.3 (95% CI, 2.0 to 7.1; $P < .001$). Conversely, in those with *N/K-RAS* mutation and/or *PTEN* genomic abnormality, 5-year OS was similarly poor in patients with *N/F* mutation and in those without *N/F* mutation (49%; 95% CI, 27% to 68% *v* 32%; 95% CI, 10% to 57%, respectively). The HR for longer OS in the former group was 0.7 (95% CI, 0.3 to 1.7; $P = .43$). (D) CIR according to the new *N/F*, *N/K-RAS*, and *PTEN* oncogenetic classifier. At 5 years, CIR was estimated at 15% (95% CI, 9% to 24%) in low-risk patients, compared with 54% (95% CI, 42% to 66%) in high-risk patients. The SHR was 4.1 (95% CI, 2.2 to 7.7) in the latter group ($P < .001$). (E) RFS according to the new *N/F*, *N/K-RAS*, and *PTEN* oncogenetic classifier. At 5 years, RFS was estimated at 85% (95% CI, 76% to 91%) in low-risk patients, compared with 42% (95% CI, 29% to 55%) in high-risk patients. The HR for shorter RFS in the latter group was 4.2 (95% CI, 2.3 to 8.0; $P < .001$). (F) OS according to the new *N/F*, *N/K-RAS*, and *PTEN* oncogenetic 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% *v* 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 100×10^9/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.[7] A number of these genetic events, sometimes defined as type A mutations,[20] act mainly to block T-cell differentiation at a specific developmental stage and delineate T-ALL subgroups displaying specific gene expression profiles.[5,6] 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[20]), which leads to the downstream activation of both the RAS/MAPK and PI3K/AKT pathways.[21] There is also increasing recognition of the role played by tumor suppressor gene inactivation in T-ALL.[7] PTEN is a lipid and protein phosphatase that negatively regulates the PI3K/AKT/mTOR pathway through dephosphorylation of the PIP3 lipid second messenger.[22] PTEN plays critical roles in cell growth, survival, and migration.[23] The *PTEN* expression level can be regulated by multiple mechanisms.[23] In leukemia, *PTEN* loss promotes self-renewable leukemia stem-cell formation and leukemogenesis.[24] Whether *PTEN* abnormalities are of prognostic value remains debated in childhood T-ALLs.[15,25,26] In general, *PTEN* genomic deletions are of poor prognosis, but *PTEN* mutations were reported to be without significant prognostic impact,[15] 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.[27,28] Oncogenic *K-RAS* and *N-RAS* mutations are described in only 2% of pediatric T-ALLs without clinical impact.[29] *RAS*-mutated adult T-ALLs represent 10% and tend to have more frequently an immature immunophenotype. This association has been recently suggested[30] and, because immature phenotypes are more frequent in adult compared with pediatric T-ALLs,[31] 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 chemotherapeutic 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* germline 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).[32,33] 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

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.
Employment or Leadership Position: None **Consultant or Advisory Role:** Françoise Huguet, Amgen (C), ARIAD Pharmaceuticals (C), Bristol-Myers Squibb (C), Novartis (C), Pfizer (C) **Stock Ownership:** None **Honoraria:** Françoise Huguet, ARIAD Pharmaceuticals, Bristol-Myers Squibb, Novartis **Research Funding:** None **Expert Testimony:** None **Patents:** None **Other Remuneration:** None

AUTHOR CONTRIBUTIONS

Conception and design: Hervé Dombret, Vahid Asnafi
Provision of study materials or patients: All authors
Collection and assembly of data: All authors
Data analysis and interpretation: Amélie Trinquand, Vahid Asnafi
Manuscript writing: All authors
Final approval of manuscript: All authors

REFERENCES

1. Pui CH, Evans WE: Treatment of acute lymphoblastic leukemia. N Engl J Med 354:166-178, 2006
2. Aifantis I, Raetz E, Buonamici S: Molecular pathogenesis of T-cell leukaemia and lymphoma. Nat Rev Immunol 8:380-390, 2008
3. Ben Abdelali R, Asnafi V, Leguay T, et al: 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. Blood 118:5099-5107, 2011
4. De Keersmaecker K, Atak ZK, Li N, et al: Exome sequencing identifies mutation in CNOT3 and ribosomal genes RPL5 and RPL10 in T-cell acute lymphoblastic leukemia. Nat Genet 45:186-190, 2013
5. Ferrando AA, Neuberg DS, Staunton J, et al: Gene expression signatures define novel oncogenic pathways in T cell acute lymphoblastic leukemia.

Cancer Cell 1:75-87, 2002

6. Soulier J, Clappier E, Cayuela JM, et al: HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). Blood 106:274-286, 2005

7. Van Vlierberghe P, Ferrando A: The molecular basis of T cell acute lymphoblastic leukemia. J Clin Invest 122:3398-3406, 2012

8. Zhang J, Ding L, Holmfeldt L, et al: The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature 481:157-163, 2012

9. Weng AP, Ferrando AA, Lee W, et al: Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science 306:269-271, 2004

10. 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 204:1813-1824, 2007

11. 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 106:3760-3767, 2005

12. Campana D: Minimal residual disease in acute lymphoblastic leukemia. Hematology Am Soc Hematol Educ Program 2010:7-12, 2010

13. 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 27:911-918, 2009

14. Bonnet M, Loosveld M, Montpellier B, et al: Posttranscriptional deregulation of MYC via PTEN constitutes a major alternative pathway of MYC activation in T-cell acute lymphoblastic leukemia. Blood 117:6650-6659, 2011

15. Gutierrez A, Sanda T, Grebliunaite R, et al: High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. Blood 114:647-650, 2009

16. Kaplan EL, Meier P: Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958

17. Cox D: Regression models and life tables. J R Stat Soc B 34:187-220, 1972

18. Gönen M, Heller G: Concordance probability and discriminative power of proportional hazards regression. Biometrika 92:965-970, 2005

19. 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 113:3918-3924, 2009

20. Van Vlierberghe P, Pieters R, Beverloo HB, et al: Molecular-genetic insights in paediatric T-cell acute lymphoblastic leukaemia. Br J Haematol 143: 153-168, 2008

21. Germain RN, Stefanová I: The dynamics of T cell receptor signaling: Complex orchestration and the key roles of tempo and cooperation. Annu Rev Immunol 17:467-522, 1999

22. Leslie NR, Downes CP: PTEN: The down side of PI 3-kinase signalling. Cell Signal 14:285-295, 2002

23. Song MS, Salmena L, Pandolfi PP: The functions and regulation of the PTEN tumour suppressor. Nat Rev Mol Cell Biol 13:283-296, 2012

24. Guo W, Lasky JL, Chang CJ, et al: Multi-genetic events collaboratively contribute to Pten-null leukaemia stem-cell formation. Nature 453:529-533, 2008

25. Bandapalli OR, Zimmermann M, Kox C, et al: NOTCH1 activation clinically antagonizes the unfavorable effect of PTEN inactivation in BFM-treated children with precursor T-cell acute lymphoblastic leukemia. Haematologica 98:928-936, 2013

26. Zuurbier L, Petricoin EF 3rd, Vuerhard MJ, et al: The significance of PTEN and AKT aberrations in pediatric T-cell acute lymphoblastic leukemia. Haematologica 97:1405-1413, 2012

27. Parikh C, Subrahmanyam R, Ren R: Oncogenic NRAS rapidly and efficiently induces CMML- and AML-like diseases in mice. Blood 108:2349-2357, 2006

28. Zhang J, Wang J, Liu Y, et al: Oncogenic Kras-induced leukemogenesis: Hematopoietic stem cells as the initial target and lineage-specific progenitors as the potential targets for final leukemic transformation. Blood 113:1304-1314, 2009

29. Perentesis JP, Bhatia S, Boyle E, et al: RAS oncogene mutations and outcome of therapy for childhood acute lymphoblastic leukemia. Leukemia 18:685-692, 2004

30. Van Vlierberghe P, Ambesi-Impiombato A, Perez-Garcia A, et al: ETV6 mutations in early immature human T cell leukemias. J Exp Med 208:2571-2579, 2011

31. Asnafi V, Beldjord K, Libura M, et al: Age-related phenotypic and oncogenic differences in T-cell acute lymphoblastic leukemias may reflect thymic atrophy. Blood 104:4173-4180, 2004

32. Digel W, Schultze J, Kunzmann R, et al: Poor prognosis of prethymic phenotype acute lymphoblastic leukemia (pre-T-ALL). Leukemia 8:1406-1408, 1994

33. Thiel E, Kranz BR, Raghavachar A, et al: Prethymic phenotype and genotype of pre-T (CD7+/ER-)-cell leukemia and its clinical significance within adult acute lymphoblastic leukemia. Blood 73:1247-1258, 1989

Affiliations

Amélie Trinquand, Raouf Ben Abdelali, Etienne Lengliné, Noémie De Gunzburg, Ludovic Lhermitte, Jonathan Bond, Agnès Buzyn, Elizabeth Macintyre, and Vahid Asnafi, University Paris Descartes, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR)-8147, and Assistance Publique–Hôpitaux de Paris (AP-HP), Hôpital Necker-Enfants Malades; Jérôme Lambert, UMR-S-717, Hôpital Saint-Louis, AP-HP; Kheira Beldjord, Etienne Lengliné, and Hervé Dombret, University Paris 7, Hôpital Saint-Louis, AP-HP, and Institut Universitaire d'Hématologie, EA3518, Paris; Aline Tanguy-Schmidt and Norbert Ifrah, Pôle de Recherche et d'Enseignement Supérieur L'Université Nantes Angers Le Mans, Centre Hospitalier Universitaire Angers Service des Maladies du Sang et L'Institut National de la Santé et de la Recherche Médicale (INSERM) U892, Angers; Dominique Payet-Bornet and Bertrand Nadel, Center of Immunology of Marseille Luminy, Aix-Marseille University, INSERM U1104 and Centre National de la Recherche Scientifique (CNRS) UMR-7280, Marseille; Hossein Mossafa, Laboratoire Cerba, Cergy-Pontoise; Véronique Lhéritier and Xavier Thomas, Centre Hospitalier Lyon Sud, Lyon; Françoise Huguet, Hôpital Purpan, Toulouse; Thibaud Leguay, Centre Hospitalier du Haut Lévêque, Pessac; Jean-Yves Cahn, UMR-5525 CNRS–Université Joseph Fourier, Grenoble; Caroline Bonmati, Centre Hospitalier Régional Hôpital de Brabois, Vandoeuvre Les Nancy; Sebastien Maury, Hôpital Henry Mondor, Creteil, France; Yves Chalandon, University Hospital of Geneva, Geneva, Switzerland; and André Delannoy, Hopital de Jolimont, La Louviere, Belgium.

Support

Supported by grants to Necker Laboratory from the Association Laurette Fugain, the Comité Départemental de la Ligue Contre le Cancer, and the Institut National du Cancer. The Group for Research in Adult Acute Lymphoblastic Leukemia (GRAALL) was supported by Grants No. P0200701 and P030425/AOM03081 from Le Programme Hospitalier de Recherche Clinique, Ministère de l'Emploi et de la Solidarité, France, and the Swiss Federal Government in Switzerland. Samples were collected and processed by the Assistance Publique–Hôpitaux de Paris Direction de Recherche Clinique Tumor Bank at Necker-Enfants Malades. AT was supported by Soutien pour la formation à la recherche translationnelle en cancérologie dans le cadre du Plan cancer 2009-2013 and Fondation pour la Recherche Médicale. J.B. was supported by a Kay Kendall Leukaemia Fund Intermediate Research Fellowship.