REGULAR ARTICLE

blood advances

Molecular, clinical, and prognostic implications of *PTPN11* mutations in acute myeloid leukemia

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Key Points

- Patients with N-terminal SH2 domain *PTPN11* mutations had an early death (<30 days) more often than those with phosphatase domain mutations.
- *PTPN11* mutations are associated with inferior outcomes in AML patients with wild-type *NPM1*.

Prognostic factors associated with chemotherapy outcomes in patients with acute myeloid leukemia (AML) are extensively reported, and one gene whose mutation is recognized as conferring resistance to several newer targeted therapies is protein tyrosine phosphatase non-receptor type 11 (*PTPN11*). The broader clinical implications of *PTPN11* mutations in AML are still not well understood. The objective of this study was to determine which cytogenetic abnormalities and gene mutations co-occur with *PTPN11* mutations and how *PTPN11* mutations affect outcomes of patients treated with intensive chemotherapy. We studied 1725 patients newly diagnosed with AML (excluding acute promyelocytic leukemia) enrolled onto the Cancer and Leukemia Group B/Alliance for Clinical Trials in Oncology trials. In 140 *PTPN11*-mutated patient samples, *PTPN11* most commonly co-occurred with mutations in *NPM1*, *DNMT3A*, and *TET2*. *PTPN11* mutations were relatively common in patients with an inv(3)(q21q26)/t(3;3)(q21;q26) and a normal karyotype but were very rare in patients with typical complex karyotype and core-binding factor AML. Mutations in the N-terminal SH2 domain of *PTPN11* were associated with a higher early death rate than those in the phosphatase domain. *PTPN11* mutations did not affect outcomes of *NPM1*-mutated patients, but these patients were less likely to have co-occurring kinase mutations (ie, *FLT3*-ITD), suggesting activation of overlapping signaling pathways. However, in AML patients with wild-type *NPM1*, *PTPN11* mutations were associated with adverse patient outcomes, providing a rationale to study the biology and treatment approaches in this molecular group. This trial was registered at www.clinicaltrials.gov as #NCT00048958 (CALGB 8461), #NCT00899223 (CALGB 9665), and #NCT00900224 (CALGB 20202).

Introduction

Acute myeloid leukemia (AML) is the most commonly diagnosed acute leukemia in adults and is best characterized by the aberrant proliferation of clonal myeloid stem or progenitor cells with a differentiation

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Requests for data sharing should be sent to the corresponding author (John C. Byrd; e-mail: byrd2jc@ucmail.uc.edu).

The full-text version of this article contains a data supplement.

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block.[1] Although AML has a common myeloid origin, the pathogenesis is believed to be due to one or more genetic driver events such as chromosome translocations and/or gene mutations followed by the acquisition of mutations that promote the full phenotype of the disease. The complexity of the disease is further amplified by specific, age-associated disease characteristics. Recognition that AML is not one disease, but likely many, may explain why the cure rate remains very low, with similar chemotherapy given to all patients with this disease. Indeed, induction chemotherapy with an anthracycline plus cytarabine regimen followed by intensive consolidation without allogeneic stem cell transplant cures 35% to 40% of patients aged <60 years and 5% to 15% of patients aged ≥60 years.[2] Despite a relatively frequent occurrence, one gene mutation in AML only recently characterized is mutation in the protein tyrosine phosphatase non-receptor type 11 (*PTPN11*) gene.

The *PTPN11* gene encodes the protein Src homology region 2 (SH2)-containing protein tyrosine phosphatase 2 (SHP2). SHP2 is ubiquitously expressed and required for the normal development and function of hematopoietic cells.[3,4] SHP2 is composed of two SH2 domains at the N-terminal (sequentially labeled N- and C-terminal), a phosphatase (PTP) domain, and a C-terminal tail. The N-terminal SH2 (N-SH2) domain self-inhibits the PTP domain.[5,6] Upstream signaling recruits the N-SH2 domain and releases this self-inhibition to induce downstream signaling.[6,7] Oncogenic *PTPN11* mutations induce prolonged SHP2 activation through the removal of self-inhibition.[8]

PTPN11 mutations have been found in various hematologic malignancies, including AML.[9-11] A *PTPN11* mutation is found in ∼7% of patients with de novo AML and ∼12% of patients with therapy-related AML.[12-15] Given the recent emergence of primary resistance to targeted therapy such as ivosidenib, enasidenib, venetoclax, and entospletinib,[16-20] a reassessment of the associations of *PTPN11* mutations with cytogenetic findings, mutations of other genes, clinical characteristics, and outcome features in AML patients treated with standard 7 + 3 chemotherapy is warranted. These analyses are necessary considering that many patients with AML still receive frontline chemotherapy, especially fit, younger patients. There is little information regarding how *PTPN11* mutations affect prognosis of adult patients with AML in response to standard therapy or about associations with co-existing mutations and/or cytogenetic abnormalities. To our knowledge, ours is the largest study of *PTPN11*-mutated patients, in which we examine in detail the exact mutation sites and variant allele frequencies (VAFs) of *PTPN11* mutations, chromosome abnormalities, co-occurring mutations in other genes, clinical features, and outcomes of adult patients with AML treated on clinical studies performed by the Cancer and Leukemia Group B (CALGB)/Alliance for Clinical Trials in Oncology (Alliance).

Methods

Patients and treatment

We analyzed the 1725 adults (≥17 years of age; age range, 17-92 years) with newly diagnosed, de novo AML (excluding acute promyelocytic leukemia) whose pretreatment bone marrow (BM) or blood samples underwent next-generation sequencing analysis.[21] There were 1131 younger patients, defined as those aged <60 years and 594 older patients, defined as those ≥60 years of age. The patients were treated on CALGB trials with standard chemotherapy treatment as described in the supplemental Methods. CALGB is now part of the Alliance. Ninety-five percent of patients received intensive treatment, whereas 5% of patients received nonintensive treatment as described in the supplemental Methods. All patients were considered for outcome analyses, including those who experienced an early death, defined as death within 30 days of starting therapy irrespective of cause.

Patients provided written informed consent to participate in treatment studies and companion protocols. CALGB 8461 (cytogenetic studies), CALGB 9665 (leukemia tissue bank), and/or CALGB 20202 (molecular studies) involved collection of pretreatment BM and blood samples. Treatment protocols were in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards at each center.

Cytogenetic and molecular analyses

Cytogenetic analyses of pretreatment BM and/or blood samples were performed by institutional laboratories approved by CALGB/Alliance using unstimulated short-term (24- or 48-hour) cultures. Normal karyotype was determined in patients for whom at least 20 BM metaphase cells from a short-term culture were analyzed, and no clonal abnormality was found. Cytogenetic results were confirmed by central karyotype review.[22]

Viable cryopreserved BM or blood cells were stored for future analyses before starting treatment. Mononuclear cells from BM or blood were enriched by Ficoll-Hypaque gradient and cryopreserved in liquid nitrogen until thawed at 37°C for analysis. DNA extractions were performed by using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The mutational status of 80 protein-coding genes was determined centrally at The Ohio State University by targeted amplicon sequencing using the MiSeq platform (Illumina, San Diego, CA), as previously described[21] and outlined in the supplemental Methods. Testing for the presence or absence of *FLT3*-ITD was performed as previously described.[23] In addition to the 80 genes analyzed by using the targeted amplicon sequencing panel, testing for *CEBPA* mutations was performed with Sanger sequencing as previously described,[24] thus resulting in a total of 81 genes whose mutational status was assessed in the current study. In accordance with the revision of the World Health Organization classification of myeloid neoplasms and acute leukemia and the European LeukemiaNet guidelines for AML,[25] only patients with biallelic *CEBPA* mutations were considered in the *CEBPA*-mutant category.

Statistical analysis

Definitions of clinical end points are provided in the supplemental Methods. Demographic and clinical features of any 2 patient groups were compared by using the Fisher's exact test for categorical variables and Wilcoxon rank sum tests for continuous variables. The Kaplan-Meier method was used for estimating probabilities of overall survival (OS), disease-free survival (DFS), and event-free survival (EFS) and differences between survival distributions were tested by using the log-rank test.[26] We used logistic regression for modeling complete remission (CR) attainment, Cox proportional hazards regression for modeling DFS and OS for univariable and multivariable outcome analyses, and *P* values adjusted to control for per-family error rate. For the multivariable analysis, a limited backward selection technique was used to build the final model. Variables considered in the multivariable model were significant at the

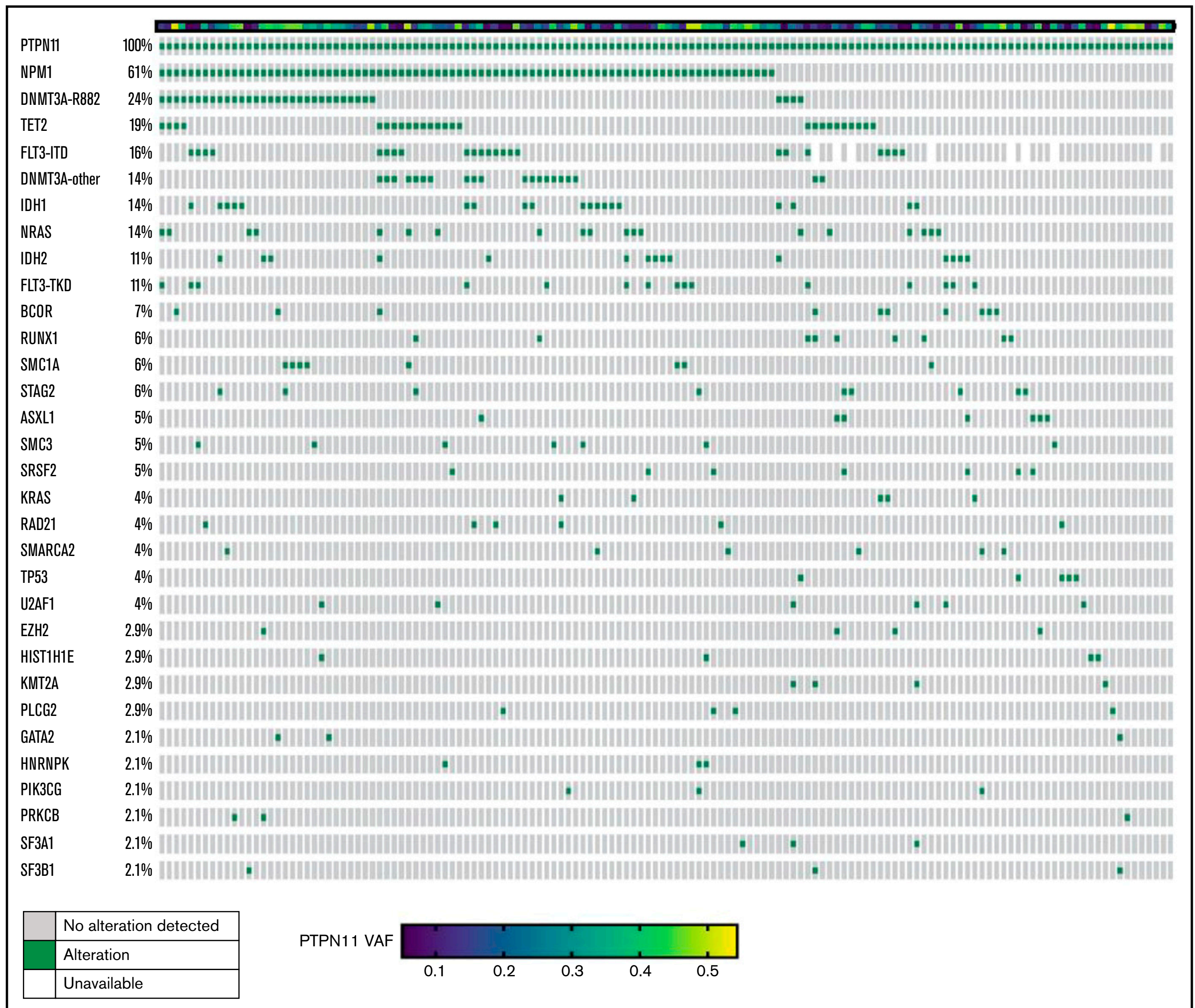

Figure 1. Oncoprint of mutations co-occurring with *PTPN11* mutations and *PTPN11* mutation VAFs in patients with AML. Each column represents an individual patient. The top row represents *PTPN11* VAFs, ranging from 0.05 (blue) to 0.54 (yellow). Each subsequent row represents a gene. Green squares indicate the presence of a mutation, insertion, or deletion; gray squares represent no alteration detected; and white squares represent unavailable gene alteration status.

likelihood ratio test–adjusted P value $< .20$ from the univariable models. All statistical analyses were performed by the Alliance Statistics and Data Center, and SAS 9.4 (SAS Institute, Inc., Cary, NC) was used. The database was locked on June 9, 2020.

Results

Baseline characteristics of patients with *PTPN11* mutations

Of the 1725 patients with AML examined, the presence of a *PTPN11* mutation was detected in 140 (8.1%) patients, which is comparable to the reported mutation frequency in other studies.[12,27] There were 98 younger patients and 42 older patients. The median follow-up of patients still alive was 9.0 years. There was a wide range of VAFs for *PTPN11* mutations among patients, ranging from 0.05 to 0.54, with 59 (42%) patients having a VAF above 0.30 (Figure 1).[28,29] The majority of the mutations (61%) were localized in the N-SH2 domain, a known *PTPN11* mutation hotspot location that is associated with increased SHP2 activity, whereas a minority of mutations were in other portions of the gene, such as the PTP domain (Figure 2).[27-30] With regards to pretreatment clinical characteristics, patients with mutated *PTPN11* (*PTPN11*^{mut}) presented more often with higher platelet counts (median, 72 vs 54 $\times$ 10^9/L; $P < .001$) and were more likely to have extramedullary involvement (33% vs 24%; $P = .03$) compared with *PTPN11* wild-type (*PTPN11*^{wt}) patients (Table 1). All other clinical features of patients with *PTPN11*^{mut} were similar to those of patients with *PTPN11*^{wt}.

Cytogenetic findings at diagnosis are important factors affecting the outcome for patients with AML.[31,32] In our study, patients with *PTPN11*^{mut} more commonly had a normal karyotype (61% vs 45%;

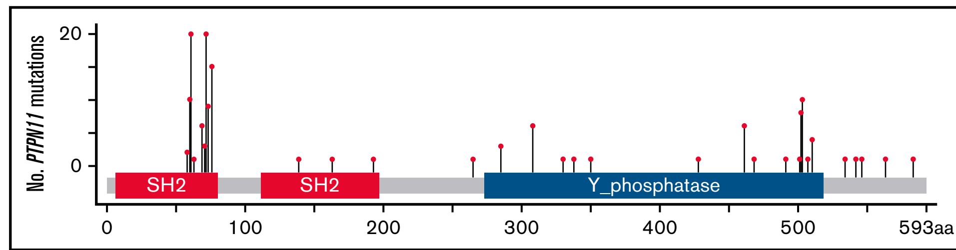

Figure 2. Lollipop plot depicting the location of *PTPN11* mutations. aa, amino acid.

$P < .001$) or inv(3)(q21q26)/t(3;3)(q21;q26) (5% vs 1%; $P = .004$) than patients with *PTPN11*^{wt}. The latter finding was especially striking because as many as 26% (7 of 27) of patients with inv(3)/t(3;3) harbored a *PTPN11* mutation, as previously reported.[33] Moreover, all 7 of these patients also had abnormalities in chromosome 7, including −7 in six and a deletion of the short arm of chromosome 7 [del(7)(p13p15)] in one patient. In contrast, *PTPN11* mutations were less commonly observed in patients with a typical complex karyotype (3% vs 8%; $P = .04$)[34] and in those with core-binding factor AML. There were no *PTPN11*^{mut} patients with t(8;21)(q22;q22) (0% vs 100%; $P = .005$), and only 2% of patients with *PTPN11*^{mut} harbored inv(16)(p13;q22)/t(16;16)(p13;q22) compared with 7% of patients with *PTPN11*^{wt} ($P = .03$) (supplemental Table 1). For other cytogenetic abnormalities, there were no significant associations with *PTPN11* mutations.

Table 1. Clinical characteristics of AML patients with and without *PTPN11* mutations

Characteristic	*PTPN11*^{mut} (n = 140)	*PTPN11*^{wt} (n = 1585)	*P**
Age, y			.74
Median	53	53	
Range	18-84	17-92	
Sex, n (%)			.11
Male	70 (50)	907 (57)	
Female	70 (50)	678 (43)	
Race, n (%)			.59
White	124 (89)	1356 (87)	
Non-white	15 (11)	197 (13)	
Hemoglobin, g/dL			.93
Median	9.1	9.2	
Range	5.7-15.0	2.3-25.1	
Platelet count, ×10^9/L			<.001
Median	72	54	
Range	10-648	4-989	
WBC count, ×10^9/L			.22
Median	29.3	23.3	
Range	1.4-355.0	0.4-560.0	
% Blood blasts			.90
Median	48	53	
Range	0-97	0-99	
% BM blasts			.30
Median	63	67	
Range	12-99	0-99	
Extramedullary involvement, n (%)	45 (33)	363 (24)	.03

BM, bone marrow; WBC, white blood cell.
**P* values are from Fisher's exact test for discrete variables and from the Wilcoxon rank sum test for continuous variables.

In addition to cytogenetic findings at diagnosis, recurrent gene mutations have emerged as important factors affecting the outcome of patients with AML.[25] Previous studies focusing on *PTPN11*-mutant AML mainly included a limited number of recurrently mutated genes, whereas 2 very recently published papers and our own study examined a broader mutation panel relevant to AML.[27,35] We noted that patients with *PTPN11*^{mut} have a higher mutation rate (median number of mutations, 4 vs 3; $P < .001$) than *PTPN11*^{wt} patients, albeit this finding is based on a targeted sequencing panel. An oncoprint of the 140 patients with *PTPN11* mutations shows the co-occurring gene mutations (Figure 1).[28,29] Notably, patients with *PTPN11*^{mut} more frequently harbored *NPM1* (61% vs 31%; $P < .001$), *DNMT3A* (39% vs 22%; $P < .001$), and *STAG2* (6% vs 3%; $P = .04$) mutations than those with *PTPN11*^{wt}. In a similar fashion, patients with *PTPN11*^{mut} less frequently had double-mutated *CEBPA* (1% vs 8%; $P = .003$), *KIT* (1% vs 5%; $P = .04$), *ZRSR2* (1% vs 5%; $P = .04$), and *TP53* (4% vs 8%; $P = .05$) mutations (supplemental Table 2).

Because *PTPN11* mutations tend to cluster in the N-SH2 and PTP domains, which are both involved in SHP2 self-inhibition, we questioned whether mutations in different domains of the *PTPN11* gene resulted in comparable pretreatment clinical characteristics. Eighty-six patients had N-SH2 domain mutations, and 45 patients had PTP domain mutations. We found that the only difference at baseline was that patients with N-SH2 mutations had a higher percentage of blasts in the BM (median, 65% vs 52%; $P = .03$) (Table 2). There were no significant differences in distribution of cytogenetic findings between N-SH2 and PTP *PTPN11*-mutated patients (supplemental Table 3). Patients with N-SH2 mutations were less likely to have *GATA2* (0% vs 7%; $P = .04$) and *PLCG2* (0% vs 7%; $P = .04$) mutations than patients with PTP mutations (supplemental Table 4).

Outcomes of AML patients with *PTPN11* mutations

We compared clinical outcomes of patients with and without *PTPN11* mutations both in the entire patient cohort and, separately, in younger and older patients. There were no significant differences in CR, early death rates, or DFS, OS, and EFS between patients with *PTPN11*^{mut} and *PTPN11*^{wt} in the entire cohort (supplemental Table 5). We then stratified patients into two age groups, those aged <60 years and those aged ≥60 years, because these patients were treated differently on CALGB/Alliance protocols. Although the

Table 2. Pretreatment characteristics of *PTPN11*^{mut} patients according to the location of the mutation within the gene

Characteristic	*PTPN11*^{mut} N-SH2 (n = 86)	*PTPN11*^{mut} phosphatase (n = 45)	*P**
Age, y			.46
Median	54	51	
Range	18-82	23-79	
Sex, n (%)			.46
Male	41 (48)	25 (56)	
Female	45 (52)	20 (44)	
Race, n (%)			1.00
White	76 (88)	39 (89)	
Non-white	10 (12)	5 (11)	
Hemoglobin, g/dL			.98
Median	9.1	9.2	
Range	5.7-13.8	6.0-15.0	
Platelet count, $\times 10^9$/L			.22
Median	72	82	
Range	13-648	17-415	
WBC count, $\times 10^9$/L			.89
Median	31.3	31.6	
Range	1.5-355.0	1.4-135.0	
% Blood blasts			.22
Median	52	42	
Range	0-97	0-88	
% BM blasts			.03
Median	65	52	
Range	12-99	15-90	
Extramedullary involvement, n (%)	27 (33)	15 (34)	1.00

BM, bone marrow; WBC, white blood cell.

**P* values are from Fisher's exact test for discrete variables and from the Wilcoxon rank sum test for continuous variables.

presence of *PTPN11* mutations did not associate with significant differences in early death rates, CR rates, OS, or EFS in either older or younger patients, older patients harboring a *PTPN11*^{mut} had a shorter DFS (3-year rates, 5% vs 15%; $P = .05$) than *PTPN11*^{wt} patients (supplemental Table 6).

We also studied whether mutations in different domains of the *PTPN11* gene affected patients' outcomes. The only difference we detected was that 20% of patients with the *PTPN11* mutations located in the N-SH2 domain died early as opposed to no early deaths among patients with *PTPN11* mutations in the PTP domain ($P < .001$) (supplemental Table 7). There were no significant differences in CR rates or DFS, OS, and EFS between the 2 groups (Figure 3). In addition, higher early death rates ($P = .02$), but no other significant outcome differences, were found in younger patients with N-SH2 domain *PTPN11* mutations compared with those with a mutation in the PTP domain. In the older age group, there were no significant differences in outcome (supplemental Table 8).

PTPN11 mutations result in a different mutational phenotype but do not affect outcomes in *NPM1*^{mut} patients

Given that 85 (61%) of the 140 patients with *PTPN11*^{mut} also harbored an *NPM1* mutation, we next sought to determine if the clinical and molecular features differed between *NPM1*-mutated patients with or without *PTPN11* mutations. With regard to pretreatment characteristics, patients with *NPM1*^{mut}/*PTPN11*^{mut} had a higher baseline platelet counts (median, 78 vs 59 $\times 10^9$/L; $P = .008$) (supplemental Table 9). Distribution of cytogenetic aberrations was similar between the 2 groups (supplemental Table 10). Notably, patients with *NPM1*^{mut}/*PTPN11*^{mut} had a higher frequency of *DNMT3A* mutations (56% vs 43%; $P = .03$), whereas *FLT3*-ITD (19% vs 44%; $P < .001$) was less frequent in this genomic group compared with patients with *NPM1*^{mut}/*PTPN11*^{wt} (supplemental Table 11). This suggests *NPM1*^{mut}/*PTPN11*^{mut} clones are less dependent on additional signaling mutations such as *FLT3*-ITD. Despite these differences in baseline biology, there were no significant differences in any of the outcome end points between *NPM1*-mutated patients with and those without *PTPN11* mutations regardless of age (Figure 4A; supplemental Tables 12 and 13).

PTPN11 mutations negatively influence outcome of patients with *NPM1*^{wt}

We were also interested if *PTPN11* mutations can influence outcomes of patients with *NPM1*^{wt}. A comparison of pretreatment characteristics between patients with *PTPN11*^{wt} and *PTPN11*^{mut} revealed no significant differences (supplemental Table 14). Cytogenetically, patients with *NPM1*^{wt}/*PTPN11*^{mut} were more likely to harbor prognostically unfavorable inv(3)(q21q26)/t(3;3)(q21;q26) (13% vs 2%; $P < .001$), other balanced rearrangements involving 3q26 (4% vs 0.2%; $P = .01$), and t(11;19)(q23;p13.3)/*KMT2A-MLLT1* (4% vs 0.4%; $P = .03$) (supplemental Table 15) compared with patients with *NPM1*^{wt}/*PTPN11*^{wt}. Moreover, patients with *NPM1*^{wt}/*PTPN11*^{mut} had a higher median number of mutations (3 vs 2; $P < .001$) than those with *NPM1*^{wt}/*PTPN11*^{wt} and were more likely to have *KMT2A* (7% vs 1%; $P = .006$) and *NF1* (21% vs 6%; $P = .01$) mutations (supplemental Table 16).

Among combined younger and older patients with *NPM1*^{wt}, those with *PTPN11*^{mut} had a lower CR rate (36% vs 61%; $P < .001$) and shorter EFS (3-year rates, 9% vs 19%; $P = .003$) than patients with *PTPN11*^{wt} (supplemental Table 17). Likewise, younger patients with *NPM1*^{wt}/*PTPN11*^{mut} had a lower CR rate (45% vs 71%; $P = .002$), OS (3-year rates, 30% vs 41%; $P = .04$) (Figure 4B), and EFS (3-year rates, 13% vs 27%; $P = .008$), but not DFS, than those with *NPM1*^{wt}/*PTPN11*^{wt}. Older patients with *NPM1*^{wt}/*PTPN11*^{mut} also had a lower CR rate (18% vs 43%; $P = .04$), DFS (3-year rates, 0% vs 10%; $P = .02$), and EFS (3-year rates, 0% vs 4%; $P = .02$), but not OS, compared with patients with *NPM1*^{wt}/*PTPN11*^{wt} (Table 3).

Multivariable analyses were performed to determine what other factors, including gene mutations, associated with inferior outcomes of AML patients with *NPM1*^{wt}. We could not perform separate multivariable analyses in younger and older patients because there would have been too few patients to obtain meaningful results. In the multivariable modeling for CR attainment, mutations in *PTPN11*, *TP53*, and *FLT3*-ITD and age remained in the final model (Table 4),

Figure 3. Outcomes of patients with mutations in the N-SH2 domain of the *PTPN11* gene vs mutations in the PTP domain. (A) DFS based on the presence of an N-SH2 domain (blue line) or PTP domain (red line) *PTPN11* mutation. (B) OS for patients with an N-SH2 domain (blue line) or PTP domain (red line) *PTPN11* mutation. (C) EFS based on the presence of an N-SH2 domain (blue line) or PTP domain (red line) *PTPN11* mutation.

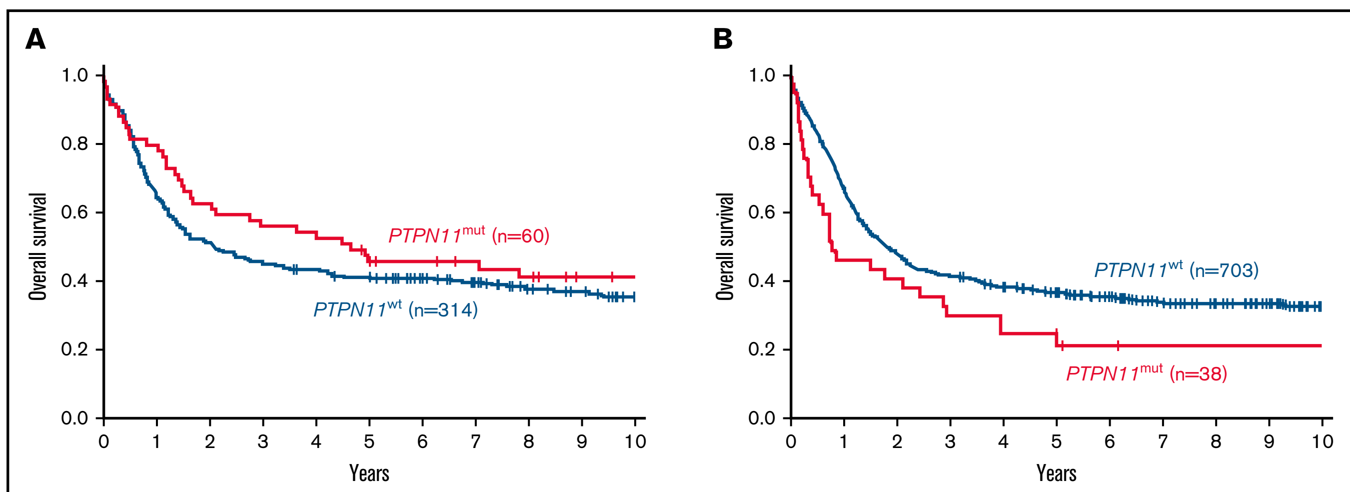

Figure 4. OS of younger (age <60 years) patients. (A) OS in younger patients with *NPM1*^{mut}/*PTPN11*^{wt} (blue line) and *NPM1*^{mut}/*PTPN11*^{mut} (red line). (B) OS for younger patients based on the presence of *NPM1*^{wt}/*PTPN11*^{wt} (blue line) and *NPM1*^{wt}/*PTPN11*^{mut} (red line).

Table 3. **Outcomes of AML patients with wild-type *NPM1* based on the presence of a *PTPN11* mutation**

End point	*PTPN11*^{mut} (n = 38)	*PTPN11*^{wt} (n = 703)	*P**
Younger patients (age <60 y)			
Early death, n (%)	3 (8)	33 (5)	.42
CR, n (%)	17 (45)	498 (71)	.002
DFS			.96
Median, y	2.2	1.2	
% Disease free at 1 y (95% CI)	65 (38-82)	55 (51-60)	
% Disease free at 3 y (95% CI)	29 (11-51)	38 (33-42)	
OS			.04
Median, y	0.8	1.8	
% Alive at 1 y (95% CI)	46 (30-61)	67 (63-70)	
% Alive at 3 y (95% CI)	30 (16-45)	41 (38-45)	
EFS			.008
Median, y	0.2	0.8	
% Event-free at 1 y (95% CI)	29 (16-44)	41 (37-45)	
% Event-free at 3 y (95% CI)	13 (5-26)	27 (24-30)	
End point	***PTPN11*^{mut} (n = 17)**	***PTPN11*^{wt} (n = 371)**	***P***
Older patients (age ≥60 y)			
Early death, n (%)	2 (12)	60 (16)	1.00
CR, n (%)	3 (18)	159 (43)	.04
DFS			.02
Median, y	0.3	0.6	
% Disease-free at 1 y (95% CI)	0	34 (27-42)	
% Disease-free at 3 y (95% CI)	0	10 (6-15)	
OS			.58
Median, y	0.4	0.6	
% Alive at 1 y (95% CI)	24 (7-45)	32 (27-37)	
% Alive at 3 y (95% CI)	12 (2-31)	10 (7-13)	
EFS			.02
Median, y	0.2	0.2	
% Event-free at 1 y (95% CI)	0	18 (14-22)	
% Event-free at 3 y (95% CI)	0	4 (3-7)	

CI, confidence interval.
**P* values are from Fisher's exact test for early death and CR and from the log-rank test for DFS, OS, and EFS.

Table 4. **Multivariable analysis for CR attainment, OS, and EFS in AML patients with wild-type *NPM1* (younger and older patients combined)**

Variable	*P**	CR: Odds ratio (95% CI)
PTPN11, mutated vs wild-type	<.001	0.30 (0.16-0.56)
TP53, mutated vs wild-type	<.001	0.37 (0.24-0.56)
FLT3-ITD, positive vs negative	<.001	0.44 (0.30-0.63)
Age, continuous	<.001	0.70 (0.64-0.76)

Variable	*P**	OS: Hazard ratio (95% CI)
PTPN11, mutated vs wild-type	.86	1.03 (0.73-1.45)
WBC count, continuous	.001	1.12 (1.05-1.20)
Age, continuous	<.001	1.38 (1.32-1.44)
FLT3-ITD, positive vs negative	.002	1.35 (1.12-1.63)
TET2, mutated vs wild-type	.002	1.38 (1.13-1.70)
TP53, mutated vs wild-type	<.001	2.74 (2.23-3.37)
inv(3)(q21q26)/t(3;3)(q21;q26), yes vs no	<.001	2.67 (1.75-4.09)

Varibale	*P**	EFS: Hazard ratio (95% CI)
PTPN11, mutated vs wild-type	.14	1.27 (0.92-1.75)
WBC count, continuous	.004	1.10 (1.03-1.18)
Age, continuous	<.001	1.26 (1.20-1.31)
DNMT3A, mutated vs wild-type	.02	1.27 (1.04-1.54)
FLT3-ITD, positive vs negative	<.001	1.54 (1.28-1.85)
TET2, mutated vs wild-type	.03	1.25 (1.03-1.54)
TP53, mutated vs wild-type	<.001	2.15 (1.75-2.63)
inv(3)(q21q26)/t(3;3)(q21;q26), yes vs no	<.001	2.82 (2.5-5.81)

CI, confidence interval; WBC, white blood cell.
**P* values for logistic and proportional hazard regression are from the likelihood ratio test. An odds ratio <1 (>1) means higher (lower) CR rate for higher values of continuous variables and the first level listed of a dichotomous variable. A hazard ratio >1 (<1) corresponds to a higher (lower) risk for higher values of continuous variables and the first level listed of a dichotomous variable.

indicating that *PTPN11* mutations still affect the probability of CR achievement even when accounting for other variables ($P < .001$). However, in the multivariable analyses of OS and EFS, *PTPN11* mutations did not remain significant in the final models.

Discussion

Herein, we showed that *PTPN11* mutations may affect clinical outcomes dependent on age group and mutation subset analyses in a retrospective study of patients with AML receiving intensive therapy in clinical trials performed by the CALGB/Alliance. Although the presence of a *PTPN11* mutation in addition to an *NPM1* mutation did not associate with poorer outcomes (with the exception of older patients with *PTPN11*^{mut} having a marginally reduced DFS compared with patients with wild-type *PTPN11*), *PTPN11* mutations did associate with inferior outcomes in AML patients with *NPM1*^{wt} regardless of age. We also found that patients with *PTPN11* mutations in the N-SH2 domain had higher BM blast counts and early death rate than those with PTP domain mutations. These results suggest that an N-SH2 mutation might generate a different phenotype. We hypothesize this phenotype could be immunosuppressive, explaining the higher early death rate but no difference in response to chemotherapy induction, DFS, OS, or EFS. Collectively, our study outlines the complex effects of a *PTPN11* mutation in AML and provides evidence that its prognostic impact should be considered in the context of *NPM1* mutation status.

Similar to others, we also found an association between *PTPN11* mutations and inv(3)(q21q26)/t(3;3)(q21;q26), the later aberration being a marker of poor prognosis in AML.[25,33] We also confirmed that *PTPN11* mutations are less likely to occur in patients with typical complex karyotype and those with core-binding factor AML[27]

but are most often found together with *NPM1* mutations.[27,36] We also found an association between *PTPN11* mutations and mutations in *DNMT3A* or *STAG2*. Furthermore, we observed that there were few patients with *PTPN11*^{mut} who also had co-mutations in *CEBPA, KIT*, *TP53*, and *ZRSR2*. Our analysis of co-occurring mutations in patients with *NPM1*^{mut}/*PTPN11*^{mut} revealed that these patients had a lower frequency of co-occurring *FLT3*-ITD mutations, suggesting that *PTPN11* and *FLT3*-ITD mutations result in activation of overlapping signaling pathways.

There are few published data regarding how *PTPN11* mutations affect clinical outcomes. Hou et al[37] and Swoboda et al[35] have shown that patients with *NPM1*^{wt}/*PTPN11*^{mut} had reduced OS compared with patients with *NPM1*^{wt}/*PTPN11*^{wt}, and Alfayez et al[27] reported that *PTPN11* mutations are associated with poor outcomes for both de novo and relapsed/refractory AML. Our current study validates these findings but also goes further by analyzing a larger cohort of patients, which allowed us to stratify the patients according to age. Furthermore, our study analyzed associated mutations and questioned how the location of the mutation within the *PTPN11* gene affected outcome.

A limitation of our study is the time span over which these patients were treated and the fact that, on these clinical trials, patients received only intensive induction followed by consolidation chemotherapy. Supportive care for AML has clearly improved over time with the addition of more effective proton pump inhibitors, antifungal agents, and transfusion support. In addition, patients with *FLT3* mutations on this study typically did not receive midostaurin. Among AML patients with *NPM1*^{wt}, 15% of patients with *PTPN11*^{mut} also harbored *FLT3*-ITD, raising a possibility that inferior outcomes in *PTPN11*-mutated patients could be associated with *FLT3*-ITD. However, both *PTPN11* mutations and *FLT3*-ITD stayed in the multivariable model, suggesting that they negatively affect outcomes independently from each other. Hence, we believe our findings are relevant to the current era of AML therapy, and moving forward, it will be important to study how *PTPN11* mutations affect responses to the newly approved targeted therapies, as early evidence suggests that these patients might be resistant.[16-20,38] More clinical studies and basic science research are needed to understand how SHP2 and NPM1 proteins are interacting and why *PTPN11* mutations are associated with worse outcome in AML patients with *NPM1*^{wt}.

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Authorship

Contribution: S.F., E.H., and J.C.B. conceived and designed the study; S.F., K.M., E.H., and J.C.B. drafted the manuscript; J.K., H.G.O., and D.N. analyzed data; J.C.B. obtained funding for this study; E.H. and J.C.B. supervised this study; and all authors contributed to the acquisition, analysis, and interpretation of these data and were critical in manuscript revision.

Conflict-of-interest disclosure: J.C.B. is a paid consultant for Syndax, Trillium, AstraZeneca, Novartis, and Kronos; and chair of the scientific advisory board and a major stockholder in Vincerx Pharma. J.S.B. consults for AbbVie, AstraZeneca, KITE Pharma, and INNATE Pharma. R.M.S. serves on the advisory board for AbbVie, Actinium, Arog, BMS, Boston Pharmaceuticals, Janssen, Jazz, Novartis, Syros, Takeda, Elevate Bio, Syndax Pharma, Gemoab, Foghorn Thera, GSK, Aprea, and OncoNova; is a part of the Steering Committee for AbbVie and the AML Expert Council for GSK; and serves on the data safety monitoring board for Takeda and Syntrix/ACI Clinical. E.S.W. has received consulting fees from AbbVie, Astellas, BMS, Genentech, GlaxoSmithKline, Jazz, Kite, Kura Oncology, Novartis, Mana Therapeutics, Pfizer, Stemline, and Takeda; serves on the speakers bureau for Stemline, Kura, Pfizer, and Dava Oncology; and serves on the data safety monitoring committee for AbbVie, Rafael Pharmaceuticals. B.L.P. has clinical trial funding from Ambit Biosciences, Hoffmann–La Roche, Jazz Pharmaceuticals, Novartis, Pfizer, and Rafael Pharmaceuticals; and consults for Rafael Pharmaceuticals. The remaining authors declare no competing financial interests.

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