Molecular associations, clinical, and prognostic implications of PTPN11 mutations in acute myeloid leukemia (Alliance)

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Abstract:
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 impact outcomes of patients treated with intensive chemotherapy. We studied 1,725 newly diagnosed AML patients (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 (i.e., 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.

Conflict of interest: COI declared - see note

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Key Points

1. Patients with N-terminal SH2 domain \textit{PTPN11} mutations suffer early death (<30 days) more often than those with phosphatase domain mutations.

2. \textit{PTPN11} mutations are associated with inferior outcomes in AML patients with wild-type \textit{NPM1}.

Abstract

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 impact outcomes of patients treated with intensive chemotherapy. We studied 1,725 newly diagnosed AML patients (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 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 (i.e., 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.
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 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 quite 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 35-40% of patients younger than 60 years of age and 5-15% of patients older than 60 years of age.\(^2\) 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 labelled 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. Oncogenic PTPN11 mutations induce prolonged SHP2 activation through the removal of self-inhibition.

PTPN11 mutations have been found in different hematologic malignancies, including AML. A PTPN11 mutation is found in approximately ~7% of de novo AML patients and ~12% of patients with therapy-related AML. Given the recent emergence of primary resistance to targeted therapy such as ivosidenib, enasidenib, venetoclax, and entospletinib, a reassessment of the associations of PTPN11 mutations with cytogenetic findings, mutations of other genes, transcriptional, clinical, and outcome features in AML patients treated with standard 7 + 3 chemotherapy is warranted. These analyses are necessary considering that many AML patients still receive frontline chemotherapy, especially fit, younger patients. There is little information regarding how PTPN11 mutations affect prognosis of adult AML patients 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 (VAF) 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 1,725 adults (≥17 years of age, range 17-92) with newly diagnosed, de novo AML (excluding acute promyelocytic leukemia) whose pretreatment bone marrow (BM) or
blood samples underwent next generation sequencing analysis.\textsuperscript{21} There were 1,131 younger patients, defined as those under the age of 60 years, and 594 older patients, defined as those \( \geq 60 \) 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 non-intensive treatment as described in the supplemental Methods. All patients were considered for outcome analyses including those who suffered 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 was 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.\textsuperscript{22}

Viable cryopreserved BM or blood cells were stored for future analyses prior to starting treatment. Mononuclear cells from BM or blood were enriched by Ficoll-Hypaque gradient and cryopreserved in liquid nitrogen until thawed at 37\(^\circ\)C for analysis. DNA extractions were
performed 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, USA), as previously described\(^\text{21}\) and outlined in the supplemental Methods. Testing for the presence or absence of \(FLT3\)-ITD was performed as previously described.\(^\text{23}\) In addition to the 80 genes analyzed using the targeted amplicon sequencing panel, testing for \(CEBPA\) mutations was performed with Sanger sequencing as previously described,\(^\text{24}\) thus resulting in a total of 81 genes whose mutational status were assessed in our 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,\(^\text{25}\) only patients with biallelic \(CEBPA\) mutations were considered in \(CEBPA\)-mutant category.

**Statistical analysis**

Definitions of clinical endpoints are provided in the supplemental Methods. Demographic and clinical features of any two patient groups were compared using the Fisher’s exact test for categorical variables and Wilcoxon rank sum tests for continuous variables. For estimating probabilities of overall survival (OS), disease-free survival (DFS), and event-free survival (EFS), we used the Kaplan-Meier method, and differences between survival distributions were tested using the log-rank test.\(^\text{26}\) We used logistic regression for modeling CR, Cox proportional hazard regression for modeling DFS and OS for univariable and multivariable outcome analyses, and adjusted \(P\)-values 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 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 software was used. The database was locked on June 9, 2020.

**Results**

**Baseline characteristics of patients with PTPN11 mutations**

Of the 1,725 patients with AML examined, the presence of a PTPN11 mutation was detected in 140 (8.1%) of patients, which is comparable to the reported mutation frequency in other studies. There were 98 younger 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). The majority of the mutations (61%) were localized in the N-terminal 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 phosphatase domain (PTP) (Figure 2). In regard to pretreatment clinical characteristics, patients with mutated PTPN11 (PTPN11\textsuperscript{mut}) presented more often with higher platelet counts (median: 72 vs 54 x 10\textsuperscript{9}/L, $P$<.001) and were more likely to have extramedullary involvement (33% vs 24%, $P$=.03) compared with PTPN11 wild-type (PTPN11\textsuperscript{wt}) patients (Table 1). All other clinical features of patients with PTPN11\textsuperscript{mut} were similar to those of patients with PTPN11\textsuperscript{wt}.

Cytogenetic findings at diagnosis are important factors affecting the outcome for patients with AML. In our study, patients with PTPN11\textsuperscript{mut} more commonly had a normal karyotype (61% vs 45%, $P$<.001) or inv(3)(q21q26)/t(3;3)(q21;q26) (5% vs 1%, $P$=.004) than
patients with $PTPN11^{wt}$. The latter, novel 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 seven 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.

In addition to cytogenetic findings at diagnosis, recurrent gene mutations have come forth as important factors affecting the outcome of AML patients.$^{25}$ Previous studies focusing on $PTPN11$-mutant AML mainly included a limited number of recurrently mutated genes, whereas two 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).$^{29,30}$ 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).
As *PTPN11* mutations tend to cluster in the N-SH2 and PTP domains, which are both involved in SHP2 self-inhibition, we interrogated whether mutations in different domains of the *PTPN11* gene resulted in comparable pretreatment clinical characteristics. There were 86 patients with N-SH2 domain mutations and 45 patients with 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, DFS, OS, or EFS between patients with *PTPN11* \(^{\text{mut}}\) and *PTPN11* \(^{\text{wt}}\) in the entire cohort (supplemental Table 5). We then stratified patients into two age groups, those younger than 60 years and those aged 60 years or older, because these patients were treated differently on CALGB/Alliance protocols. Although the 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* \(^{\text{mut}}\) had a shorter DFS (3-year rates: 5% vs 15%, \(P=.05\)) than *PTPN11* \(^{\text{wt}}\) patients (supplemental Table 6).
We also studied whether mutations in different domains of the \textit{PTPN11} gene affected patients’ outcomes. The only difference we detected was that 20\% of patients with the \textit{PTPN11} mutations located in the N-SH2 domain died early as opposed to no early death among patients with \textit{PTPN11} mutations in the PTP domain (\textit{P}<.001; supplemental Table 7). There were no significant differences in CR rates, DFS, OS, or EFS between the two groups (Figure 3). Higher early death rates (\textit{P}=.02), but no other significant outcome differences, were also found in younger patients with N-SH2 domain \textit{PTPN11} mutations compared to those with a mutation in the PTP domain. In the older age group, there were no significant differences in outcome (supplemental Table 8).

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

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

**PTPN11 mutations negatively influence outcome of patients with *NPM1*<sup>wt</sup>**

We were also interested if *PTPN11* mutations can influence outcomes of patients with *NPM1*<sup>wt</sup>. A comparison of pretreatment characteristics between patients with *PTPN11*<sup>wt</sup> and *PTPN11*<sup>mut</sup> did not reveal any significant differences (supplemental Table 14). Cytogenetically, patients with *NPM1*<sup>wt</sup>/*PTPN11*<sup>mut</sup> 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 to patients with *NPM1*<sup>wt</sup>/*PTPN11*<sup>wt</sup>. Moreover, patients with *NPM1*<sup>wt</sup>/*PTPN11*<sup>mut</sup> had a higher median number of mutations (3 vs 2, *P*<.001) than those with *NPM1*<sup>wt</sup>/*PTPN11*<sup>wt</sup> 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*<sup>wt</sup>, those with *PTPN11*<sup>mut</sup> 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*<sup>wt</sup> (supplemental Table 17). Likewise, younger patients with *NPM1*<sup>wt</sup>/*PTPN11*<sup>mut</sup> 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*<sup>wt</sup>/*PTPN11*<sup>wt</sup>. Older patients with *NPM1*<sup>wt</sup>/*PTPN11*<sup>mut</sup> 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 those of patients with *NPM1*<sup>wt</sup>/*PTPN11*<sup>wt</sup> (Table 3).
Multivariable analyses were performed to determine what other factors, including gene mutations, associated with inferior outcomes of AML patients with \( NPM1^{\text{wt}} \). 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 \( FLT3-I TD \), and age remained in the final model (Table 4) 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 (Table 4).

**Discussion**

Herein, we demonstrated that \( PTPN11 \) mutations may impact clinical outcomes dependent on age group and mutation subset analyses in a retrospective study of AML patients 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 \( PTPN11^{\text{mut}} \) having a marginally reduced DFS compared to wild-type patients, \( PTPN11 \) mutations did associate with inferior outcomes in AML patients with \( NPM1^{\text{wt}} \) regardless of age. We also found that patients with \( PTPN11 \) mutations in the N-SH2 domain had a 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 the \( PTPN11 \) mutation presence in AML.
and provides evidence that its prognostic impact should be considered in the context of NPM1 mutation status.

Following others, we also show 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 have also confirmed that PTPN11 mutations are less likely to occur in patients with typical complex karyotype and those with core-binding factor AML27 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\textsuperscript{mut} who also had co-mutations in CEBPA, KIT, TP53, and ZRSR2. Our analysis of co-occurring mutations in patients with NPM1\textsuperscript{mut}/PTPN11\textsuperscript{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 is little published data regarding how PTPN11 mutations affect clinical outcomes. Hou et al.37 and Swoboda et al.35 have shown that patients with NPM1\textsuperscript{wt}/PTPN11\textsuperscript{mut} had reduced overall survival compared with patients with NPM1\textsuperscript{wt}/PTPN11\textsuperscript{wt}, and Alfayez et al.27 showed 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 has allowed us to stratify the patients by age. Furthermore, our study analyzed associated mutations and interrogated 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 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, anti-fungal agents, and transfusion support. Additionally, patients with FLT3 mutations on this study typically did not receive midostaurin. Among AML patients with NPM1\textsuperscript{wt}, 15\% of patients with PTPN11\textsuperscript{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 impact 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 impact responses to the newly approved targeted therapies as early evidence suggest that these patients might be resistant.\textsuperscript{16–20}\textsuperscript{,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\textsuperscript{wt}.

**Data Sharing Statement**

Data sharing requests should be sent to the corresponding author (byrd2jc@ucmail.uc.edu).

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Authorship Contributions

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; all authors contributed to the acquisition, analysis, and interpretation of this data and were critical in manuscript revision.

Conflict of Interest Disclosures

J.C.B. is a paid consultant for Syndax, Trillium, AstraZeneca, Novartis, and Kronos. J.C.B. is the 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; 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, Takeda; serves on the speaker bureau for Stemline, Kura, Pfizer, Dava Oncology; serves on the data safety monitoring committee for Abbvie, Rafael Pharmaceuticals. B.L.P. has clinical trial funding from Ambit Biosciences, Hoffman LaRoche, Jazz Pharmaceuticals, Novartis, Pfizer, and Rafael Pharmaceuticals; consults for Rafael Pharmaceuticals. S.F., J.K., H.G.O., K.M., D.N., A.S.M., R.G., S.O., A.J.C., J.E.K., C.C.O., A.K.E., and E.H. have no conflict of interest to declare.

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

| Characteristic                  | PTPN11<sup>mut</sup> n=140 | PTPN11<sup>wt</sup> 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, x10<sup>9</sup>/L |                             |                             | <.001|
| Median                         | 72                          | 54                          |      |
| Range                          | 10-648                      | 4-989                       |      |
| WBC count, x10<sup>9</sup>/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                        |      |
| % Bone marrow blasts           |                             |                             | .30  |
| Median                         | 63                          | 67                          |      |
| Range                          | 12-99                       | 0-99                        |      |
| Extramedullary involvement, n (%) | 45 (33)                    | 363 (24)                    | .03  |

Abbreviations: mut, mutated; wt, wild-type; y, year; n, number; WBC, white blood cells.

*P-values are from Fisher’s exact test for discrete variables and from the Wilcoxon rank sum test for continuous variables.
Table 2. Pretreatment characteristics of $PTPN11^{\text{mut}}$ patients according to the location of the mutation within the gene.

| Characteristic                  | $PTPN11^{\text{mut}}_{\text{N-SH2}}$ n=86 | $PTPN11^{\text{mut}}_{\text{Phosphatase}}$ 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, x$10^9$/L      |                                          |                                               | .22|
| Median                         | 72                                       | 82                                            |    |
| Range                          | 13-648                                   | 17-415                                        |    |
| WBC count, x$10^9$/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                                          |    |
| % Bone marrow blasts           |                                          |                                               | .03|
| Median                         | 65                                       | 52                                            |    |
| Range                          | 12-99                                    | 15-90                                         |    |
| Extramedullary involvement, n (%)| 27 (33)                                  | 15 (34)                                       | 1.00|

Abbreviations: mut, mutated; y, year; n, number; WBC, white blood cells.

*P*-values are from Fisher’s exact test for discrete variables and from the Wilcoxon rank sum test for continuous variables.
Table 3. Outcomes of AML patients with wild-type NPM1 based on the presence of a PTPN11 mutation.

| Endpoint                        | PTPN11<sup>mut</sup> | PTPN11<sup>wt</sup> | P*    |
|--------------------------------|----------------------|---------------------|-------|
| **Younger patients (age <60 years)** |                       |                     |       |
| Early death, n (%)             | 3 (8)                | 33 (5)              | .42   |
| Complete remission, n (%)      | 17 (45)              | 498 (71)            | .002  |
| Disease-free survival          | 2.2                  | 1.2                 | .96   |
| % 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)          |       |
| Overall survival               |                      |                     | .04   |
| Median, years                  | 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)          |       |
| Event-free survival            |                      |                     | .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)          |       |
| **Older patients (age ≥60 years)** |                       |                     |       |
| Early death, n (%)             | 2 (12)               | 60 (16)             | 1.00  |
| Complete remission, n (%)      | 3 (18)               | 159 (43)            | .04   |
| Disease-free survival          | 0.3                  | 0.6                 | .02   |
| Median, y                      | 0                    | 0                   |       |
| % Disease-free at 1 y (95% CI) | 0                    | 34 (27-42)          |       |
| % Disease-free at 3 y (95% CI) | 0                    | 10 (6-15)           |       |
| Overall survival               |                      |                     | .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)           |       |
| Event-free survival            |                      |                     | .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)             |       |

Abbreviations: mut, mutated; wt, wild-type; n, number; y, year; CI, confidence interval.
*P*-values are from Fisher’s exact test for early death and complete remission and from the log-rank test for disease-free, overall, and event-free survival.
Table 4. Multivariable analysis for complete remission attainment, overall survival, and event-free survival in AML patients with wild-type NPM1 (younger and older patients combined).

| Variable | Complete remission | Overall survival | Event-free survival |
|----------|--------------------|------------------|--------------------|
|          | **P**  | **Odds ratio (95% CI)** | **P**  | **Hazard ratio (95% CI)** | **P**  | **Hazard ratio (95% CI)** |
| PTPN11, mutated vs wild-type | <.001 | 0.30 (0.16-0.56) | .86 | 1.03 (0.73-1.45) | .14 | 1.27 (0.92-1.75) |
| TP53, mutated vs wild-type | <.001 | 0.37 (0.24-0.56) | .001 | 1.12 (1.05-1.20) | .004 | 1.10 (1.03-1.18) |
| FLT3-ITD, positive vs negative | <.001 | 0.44 (0.30-0.63) | .002 | 1.35 (1.12-1.63) | <.001 | 1.54 (1.28-1.85) |
| Age, continuous | <.001 | 0.70 (0.64-0.76) | <.001 | 1.38 (1.32-1.44) | <.001 | 1.25 (1.03-1.54) |
| TP53, mutated vs wild-type | <.001 | 0.44 (0.30-0.63) | <.001 | 2.74 (2.23-3.37) | <.001 | 2.15 (1.75-2.63) |
| MLL-AT1, yes vs no | .74 | 1.17 (0.79-1.72) | .002 | 1.38 (1.13-1.70) | .02 | 1.27 (1.04-1.54) |
| MLL-AT2, yes vs no | .01 | 1.39 (1.04-1.88) | .03 | 1.25 (1.03-1.54) | <.001 | 2.15 (1.75-2.63) |
| inv(3)(q21q26)/t(3;3)(q21;q26), yes vs no | <.001 | 0.70 (0.64-0.76) | <.001 | 2.67 (1.75-4.09) | <.001 | 2.82 (2.5-5.81) |

Abbreviations: 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.
Figure Legends

Figure 1. Oncoprint of mutations co-occurring with PTPN11 mutations and PTPN11 mutation VAFs in patients with AML. Each column represents an individual patient, and each row represents a gene. Green squares indicate the presence of a mutation, insertion, or deletion, grey squares represent no alteration detected, and white squares represent unavailable gene alteration status. PTPN11 VAFs ranged from .05 (blue) to .54 (yellow).

Figure 2. Lollipop plot of PTPN11 mutations. Abbreviations: No., number; aa, amino acid.

Figure 3. Outcomes of patients with mutations in the N-SH2 domain of the PTPN11 gene versus mutations in the PTP domain. A. Disease-free survival based on the presence on an N-SH2 domain (blue line) or PTP domain (red line) PTPN11 mutation. B. Overall survival for patients with an N-SH2 domain (blue line) or PTP domain (red line) PTPN11 mutation. C. Event-free survival based on the presence on an N-SH2 domain (blue line) or PTP domain (red line) PTPN11 mutation.

Figure 4. Overall survival of younger (age <60 years) patients. A. Overall survival in younger patients with NPM1\textsuperscript{mut}/PTPN11\textsuperscript{wt} (black line) and NPM1\textsuperscript{mut}/PTPN11\textsuperscript{mut} (blue line). B. Overall survival for younger patients based on the presence of NPM1\textsuperscript{wt}/PTPN11\textsuperscript{wt} (black line) and NPM1\textsuperscript{wt}/PTPN11\textsuperscript{mut} (blue line).
Figure 2
Figure 4

(A) Overall survival of patients with PTPN11<sup>mut</sup> allele (n=60) compared to patients with PTPN11<sup>wt</sup> allele (n=314).

(B) Overall survival of patients with PTPN11<sup>mut</sup> allele (n=38) compared to patients with PTPN11<sup>wt</sup> allele (n=703).