Impact of PTPN11 mutations on clinical outcome analyzed in 1529 patients with acute myeloid leukemia

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Key Points

- PTPN11 mutations are recurrent alterations in patients with AML and are an independent prognostic factor for poor survival.
- The deleterious effect is confined mostly to patients in the ELN favorable risk group and subclonal constellations of PTPN11 mutations.

The tyrosine-protein phosphatase nonreceptor type 11 (PTPN11) is an important regulator of RAS signaling and frequently affected by mutations in patients with acute myeloid leukemia (AML). Despite the relevance for leukemogenesis and as a potential therapeutic target, the prognostic role is controversial. To investigate the prognostic impact of PTPN11 mutations, we analyzed 1529 adult AML patients using next-generation sequencing. PTPN11 mutations were detected in 106 of 1529 (6.93%) patients (median VAF: 24%) in dominant (36%) and subclonal (64%) configuration. Patients with PTPN11 mutations were associated with concomitant mutations in NPM1 (63%), DNMT3A (37%), and NRAS (21%) and had a higher rate of European LeukemiaNet (ELN) favorable cytogenetics (57.8% vs 39.1%; P < .001) and higher white blood cell counts (P = .007) compared with PTPN11 wild-type patients. In a multivariable analysis, PTPN11 mutations were independently associated with poor overall survival (hazard ratio [HR]: 1.75; P < .001), relapse-free survival (HR: 1.52; P = .013), and a lower rate of complete remission (odds ratio: 0.46; P = .008). Importantly, the deleterious effect of PTPN11 mutations was confined predominantly to the ELN favorable-risk group and patients with subclonal PTPN11 mutations (HR: 2.28; P < .001) but not found with dominant PTPN11 mutations (HR: 1.07; P = .775), presumably because of significant differences within the rate and spectrum of associated comutations. In conclusion, our data suggest an overall poor

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Raw data were generated at the University Hospital Dresden and the Deep Sequencing Facility of the DRESDEN-concept Genome Center. Patients did not consent to have data uploaded to a public database, but derived data supporting the findings of this study are available from the corresponding author on reasonable request: christian.thiede@uniklinikum-dresden.de.

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prognostic impact of PTPN11 mutations in AML, which is significantly modified by the underlying cytogenetics and the clonal context in which they occur.

Introduction

Acute myeloid leukemia (AML) is a clonal hematologic malignancy and is heterogeneous with respect to clinical presentation, outcome, and the underlying molecular landscape. To define clinically relevant disease subtypes, several recurrent genetic abnormalities are included in current molecular risk categories summarized in the 2016 World Health Organization classification and the 2017 European LeukemiaNet (ELN) recommendations for diagnosis and management of AML. Beside specific chromosomal aberrations such as t(15;17), t(8;21), and inv(16), molecular groups incorporate gene mutations in NPM1, CEBPA, and provisionally, RUNX1 as full entities. Moreover, the integration of sequencing data from large-scale genomic studies in patients with AML identified a series of frequently mutated genes, functionally linked to epigenetic modification (eg, ASXL1, DNMT3A, IDH1/2, TET2), apoptosis (TP53), or cell signaling (FLT3, NRAS, KIT), which are significant for prognosis and treatment.

In addition to established prognostic markers, the tyrosine-protein phosphatase nonreceptor type 11 (PTPN11) is frequently affected by somatically acquired mutations in AML. PTPN11 comprises 2 N-terminal Src homology 2 (SH2) domains, a protein tyrosine phosphatase (PTP) catalytic domain, and a COOH terminus. The gene encodes for the ubiquitously expressed cytoplasmic phosphatase SHP2, which has a significant role for cell growth and differentiation by mediating cellular response to hormones and cytokines. In normal hematopoiesis, PTPN11 is a positive (signal-enhancing) component downstream of cell surface growth factor receptors and acts as an important regulator of RAS/MAPK signaling pathways. In leukemogenesis, somatic gain-of-function mutations located at the N-SH2/PTP domains of PTPN11 block autoregulation of SHP2 catalytic activity. As a consequence, upregulation of SHP2 was shown to induce hypersensitivity to granulocyte-macrophage colony-stimulating factor and hyperactivation of the RAS signaling axis in hematopoietic progenitor cells, resulting in leukemic transformation.

Beside adult AML, PTPN11 mutations have been implicated in a variety of hematologic malignancies, including juvenile myelomonocytic leukemia, childhood AML, myelodysplastic syndrome, and B-cell acute lymphoblastic leukemia. Moreover, mutations of PTPN11 were detected in diverse solid cancer types (eg, lung, breast, colon cancer, and neuroblastoma) and are associated with developmental pathologies such as Noonan syndrome. Depending on the type of cancer, PTPN11 was identified as tumor suppressor or proto-oncogene, which points at a mutual role of PTPN11 for malignant progression and indicates the potential of SHP2 as therapeutic target.

More recently, the detection of PTPN11 mutations in adult patients with AML was associated with certain clinical features (ie, high rate of concomitant NPM1 mutations) and adverse prognosis (shorter overall survival [OS]). In contrast, other recent reports did not observe a significant effect of PTPN11 mutations on outcome in AML or, vice versa, an improved prognosis from the association with favorable genetic characteristics. Hence, the clinical relevance of PTPN11 mutations in AML still remains controversial and needs further elucidation.

To investigate the prevalence and prognostic impact of PTPN11 mutations in adult patients with AML, we analyzed a large cohort of 1529 patients with newly diagnosed AML. Using next-generation sequencing, we identified an overall prevalence of ~7% PTPN11 mutations in adult AML, with distinct molecular and clinical features. In a multivariable analysis, PTPN11 mutations were an independent prognostic factor for worse outcome, mainly associated with a significant deleterious effect in the ELN favorable risk group. Most importantly, we found a profound and not previously reported association of the clonal rank (dominant vs subclonal) of PTPN11 mutations with the mutational spectrum and clinical outcome, which might help to further refine prognostic implications of PTPN11 mutations in adult AML.

Methods

Patients

We retrospectively screened 1529 adult patients with newly diagnosed AML for the detection of mutations in PTPN11. Patients were included if they had available material (genomic DNA) at diagnosis. All patient samples were obtained and analyzed with written informed consent of the patients. The study was in agreement with the Helsinki declaration and approved by the ethical board of the Technical University Dresden (EK98032010). The protocols were registered for prospective trials of the Study Alliance Leukemia (SAL) with NCT numbers 00180115 (AML96), 00180102 (AML2003), 00180167 (AML60+), and 00893373 (SORAML).

Detailed descriptions of the treatment protocols have been published previously; all protocols included intensive induction chemotherapy and consolidation treatment. AML was defined as de novo when no previous malignancy and no previous treatment were reported. AML was defined as secondary (sAML) or therapy-associated AML (tAML) when myeloid neoplasms or exposure to chemotherapy and/or radiotherapy were documented before AML diagnosis. Early death was defined as death of any cause within 30 days after initial diagnosis (ED30). Remission and survival criteria were defined according to ELN2017 recommendations. Event-free survival (EFS) was defined as the time from diagnosis to death (from any cause), relapse, or failure to achieve a complete remission (CR) after induction.

Patient samples

All molecular studies were performed on DNA isolated from bone marrow aspirates or peripheral blood taken at diagnosis. Genomic DNA from samples was extracted using the DNeasy blood and tissue kit (Qiagen, Hilden, Germany) and quantified with the NanoDrop spectrophotometer. All samples were collected with written informed consent and after approval by the local ethics committee of the Medical Faculty Carl Gustav Carus Dresden, Germany.
Molecular analysis

Profiling of PTPN11 mutational status and associated comutations was done by targeted resequencing using the TruSight Myeloid assay (Illumina, San Diego, CA), according to manufacturer’s recommendations and as described previously. The panel targets 54 genes associated with myeloid neoplasms. For PTPN11, the panel covers all relevant mutational hotspots of the N-terminal SH2 domain (exon 3) and the PTP domain (exon 13) of PTPN11. Briefly, for each reaction, 50 ng genomic DNA was used. Library preparation was done as recommended by the manufacturer (TruSight Myeloid Sequencing Panel Reference Guide 15054779 v02, Illumina). Samples were sequenced paired-end on a NextSeq (150-bp Paired End) or MiSeq (300-bp Paired End) next-generation sequencing instrument (Illumina). Sequence data alignment of demultiplexed FastQ files, variant calling and filtering was done using the Sequence Pilot software package (JSI Medical Systems GmbH, Ettenheim, Germany) with default settings and a 5% variant allele frequency (VAF; dividing the mutant allele by the total alleles.

Figure 1. PTPN11 mutations and associated comutations. (A) Schematic illustration showing the position of acquired PTPN11 mutations in 106 AML patients and the domain structure of PTPN11: 2 Src homology 2 domains (N-SH2 and C-SH2) and a PTP (protein tyrosine phosphatase) domain. Recurrent alterations and their frequency in AML patients are indicated for PTPN11 exons 3 and 13. (B) PTPN11 VAFs, number of comutations (coded by color), PTPN11 clonal rank (dominant vs subclonal), affected functional domains (N-SH2 vs PTP), and the number of PTPN11 single (sm) or double mutated (dm) patients. (C) Associated comutations in PTPN11-mut patients.
Clinical and molecular characteristics of PTPN11-mut and PTPN11-wt patients

| Parameter                     | PTPN11-wt | PTPN11-mut | P     |
|-------------------------------|-----------|------------|-------|
| No of patients (n)            | 1423      | 106        |       |
| Age (y), median (IQR)         | 55 (44-64.5) | 54 (42-63) | 0.377 |
| Sex, n (%)                    |           |            | 0.857 |
| Female                        | 678 (47.6)| 52 (49.1)  |       |
| Male                          | 745 (52.4)| 54 (50.9)  |       |
| Disease status, n (%)         |           |            |       |
| De novo                       | 1198 (85.1)| 89 (85.6)  |       |
| tAML                          | 159 (11.3)| 14 (13.5)  |       |
| sAML                          | 50 (3.6)  | 1 (1)      |       |
| ELN risk 2017, n (%)          |           |            | <.001 |
| Favorable                     | 51 (39.1) | 59 (57.8)  |       |
| Intermediate                  | 543 (41.5)| 23 (22.5)  |       |
| Adverse                       | 253 (19.4)| 20 (19.6)  |       |
| Complex aberrant karyotype, n (%)|       |            | 0.154 |
| No                            | 1139 (87.5)| 92 (92.9)  |       |
| Yes                           | 162 (12.5)| 7 (7.1)    |       |
| FLT3-ITD, n (%)               |           |            | 0.200 |
| No                            | 1089 (77.1)| 88 (83)    |       |
| Yes                           | 323 (22.9)| 18 (17)    |       |
| NPM1 mutation, n (%)          |           |            | <.001 |
| No                            | 984 (70.3)| 38 (36.5)  |       |
| Yes                           | 416 (29.7)| 66 (63.5)  |       |
| ECOG score, n (%)             |           |            | 0.064 |
| 0-1                           | 842 (71.7)| 53 (61.6)  |       |
| 2-4                           | 333 (28.3)| 33 (38.4)  |       |
| Laboratory, median (IQR)      |           |            |       |
| WBC (Gpt/L)                   | 18.8 (4.4-54.37)| 26.76 (13.5-68.17)| 0.007 |
| Hemoglobin (mmol/L)           | 5.9 (5.03-7.01)| 5.77 (5.03-6.77)| 0.408 |
| Platelets (Gpt/L)             | 50 (27-94) | 54 (32-83) | 0.585 |
| Peripheral blasts (%)         | 42 (12-75) | 38 (13.5-64) | 0.477 |
| LDH (U/L)                     | 448 (280-786)| 473.5 (374.25-853.75)| 0.025 |
| BM blasts (%)                 | 63 (44.38-79)| 62.75 (45.5-78) | 0.708 |
| Clinical outcome              |           |            |       |
| CR rate, n (%)                | 1938 (72.9)| 71 (67)    | 0.006*|
| ED30, n (%)                   | 80 (5.6)  | 12 (11.3)  | 0.001*|
| EFS (mo), median (95% CI)     | 7.42 (6.7-8.3) | 7.63 (4.41-10.91) | 0.022*|
| RFS (mo), median (95% CI)     | 18.41 (15.87-23.34)| 14.66 (8.02-28.37)| 0.013*|
| OS (mo), median (95% CI)      | 19.23 (17.16-21.76)| 13.44 (10.13-20.25)| <.001*|

**Table 1.** Clinical and molecular characteristics of PTPN11-mut and PTPN11-wt patients. PTPN11 mutations were found in 106 of 1529 (6.9%) patients. A detailed list of all PTPN11 variants is provided in supplemental Table 1. Most patients (n = 97) carried a single mutation in PTPN11, and 9 patients harbored 2 different PTPN11 variants (double mutated). Mutations exclusively comprised missense single nucleotide variants and were detected predominantly (76%) in the highly conserved N-terminal SH2 domain (24% in PTP domain), relevant for the catalytic function of SHP2 (Figure 1A). PolyPhen score was >0.85 for 94% of variants, predicting a damaging impact on amino acid substitution for most mutations (supplemental Table 1). The most common alterations were A72T and A72V (found in 12 patients each). Other recurrent pathogenic single nucleotide variants, previously associated with rhabdomyosarcoma, Noonan syndrome, and AML, were detected at residues G60, D61, E69, F71, and E76 (N-SH2), as well as S503, G503, and T507 (PTP) (Figure 1A).

Mutations were detected with a median VAF of 24% (range, 5%-52%) in clonal and subclonal configuration (Figure 1B). Most mutations were found at subclonal levels (64%; PTPN11SUB) with significantly lower allele frequency (>10% difference of VAFs) compared with comutated genes (Figure 1B). Dominant PTPN11 variants (PTPN11DOM) with VAFs higher or similar to associated comutations were detected in 38 patients (36%). PTPN11 mutations were significantly associated with concomitant mutations in NPM1 (63% vs 30%; P < .001), and there was a trend for higher rates of DNM1T3A (37% vs 28%; P = .056) and NRAS (21% vs 16%; P = .196) mutations (mut) compared with PTPN11 wild-type (wt) patients (Figure 1C; Table 1). No major difference between PTPN11-mut and -wt patients was detected for other frequently mutated genes in AML, for example, FLT3ITD (17%), FLT3TKD (9%), IDH1/2 (10/13%), and TET2 (12%).

**Results**

Characterization of PTPN11 mutations and associated comutations

PTPN11 mutations were found in 106 of 1529 (6.9%) patients. A detailed list of all PTPN11 variants is provided in supplemental Table 1. Most patients (n = 97) carried a single mutation in PTPN11, and 9 patients harbored 2 different PTPN11 variants (double mutated). Mutations exclusively comprised missense single nucleotide variants and were detected predominantly (76%) in the highly conserved N-terminal SH2 domain (24% in PTP domain), relevant for the catalytic function of SHP2 (Figure 1A). PolyPhen score was >0.85 for 94% of variants, predicting a damaging impact on amino acid substitution for most mutations (supplemental Table 1). The most common alterations were A72T and A72V (found in 12 patients each). Other recurrent pathogenic single nucleotide variants, previously associated with rhabdomyosarcoma, Noonan syndrome, and AML, were detected at residues G60, D61, E69, F71, and E76 (N-SH2), as well as S503, G503, and T507 (PTP) (Figure 1A).

Mutations were detected with a median VAF of 24% (range, 5%-52%) in clonal and subclonal configuration (Figure 1B). Most mutations were found at subclonal levels (64%; PTPN11SUb) with significantly lower allele frequency (>10% difference of VAFs) compared with comutated genes (Figure 1B). Dominant PTPN11 variants (PTPN11DOM) with VAFs higher or similar to associated comutations were detected in 38 patients (36%). PTPN11 mutations were significantly associated with concomitant mutations in NPM1 (63% vs 30%; P < .001), and there was a trend for higher rates of DNM1T3A (37% vs 28%; P = .056) and NRAS (21% vs 16%; P = .196) mutations (mut) compared with PTPN11 wild-type (wt) patients (Figure 1C; Table 1). No major difference between PTPN11-mut and -wt patients was detected for other frequently mutated genes in AML, for example, FLT3ITD (17%), FLT3TKD (9%), IDH1/2 (10/13%), and TET2 (12%).

**Table 1.** Clinical and molecular characteristics of PTPN11-mut and PTPN11-wt patients.
**Associations of PTPN11 mutations with clinical features and outcome**

Descriptive statistics of clinical parameters revealed significant differences between PTPN11-mut and -wt patients (Table 1). Patients with PTPN11 mutations were diagnosed significantly more often with favorable cytogenetics (57.8%) according to the ELN-2017 risk criteria compared with PTPN11-wt patients (39.1%; \( P < .001 \)). Accordingly, PTPN11-mut patients had a lower rate of ELN-2017...
intermediate risk (22.5% vs 41.5% in PTPN11-wt patients). No differences were detected for the relative proportion of ELN-2017 adverse risk between both groups (19.4%-19.6%). In addition, PTPN11-mut patients had significantly (P < 0.007) higher white blood cell (WBC) counts (median 26.7 vs 18.8 Gpt/L; P < 0.007) and higher concentrations of lactate dehydrogenase (LDH; median 473.5 vs 448 U/L; P < 0.025) compared with PTPN11-wt patients. Moreover, there was a trend for a higher prevalence of inferior Eastern Cooperative Oncology Group (ECOG) performance status (score 2-4) for PTPN11-mut patients (38.4% vs 28.3%; P < 0.064).

In contrast, no differences were observed for the age at diagnosis (median, 54 vs 55 years), the presence of a complex karyotype (7.1% vs 12.5%), and platelet counts (median, 54 vs 50 Gpt/L) for PTPN11-mut and -wt patients. Similarly, incidences of de novo AML (85.6%), sAML (13.5%), and tAML (1%) observed in PTPN11-mut patients virtually resembled rates of AML types found in PTPN11-wt patients. Also, bone marrow (and blood) blast counts were similar in both groups.

With respect to clinical outcome, PTPN11-mut patients were significantly associated with poor OS (median, 13.44 vs 19.23 months; P = 0.026; Figure 2A). In univariate analyses, no significant differences between PTPN11-mut and -wt patients were observed for EFS (P = .381; Figure 2B) and RFS (P = .183; Figure 2C). However, the multivariable analysis with clinical variable such as age, cytogenetics, AML type, and ECOG performance status using Cox proportional hazard regression revealed that PTPN11 mutations are an independent prognostic factor for worse OS (hazard ratio [HR], 1.75; 95% confidence interval [CI], 1.34-2.29; P < .001), RFS (HR, 1.52; 95% CI, 1.09-2.12; P = .013), EFS (HR, 1.35; 95% CI, 1.04-1.75; P = .022), and lower rates of complete remission (CR1; odds ratio, 0.46; 95% CI, 0.26-0.82; P = .008; Table 1; Figure 2G). Moreover, PTPN11 mutations were significantly associated with elevated rates of early death (ED30) in univariate (11.3% vs 8.6%; P = .007) and multivariable analyses (HR, 3.61; 95% CI, 1.71-7.59; P = .001; Figure 2G). However, causes of early death were predominantly caused by infectious diseases. The deleterious effect of PTPN11 mutations on outcome was similar for patients with (OS HR, 1.848; 95% CI, 0.726-4.704; P = .198; n = 10) or without (OS HR, 1.798; 95% CI, 1.351-2.393; P < .001; n = 96) allogeneic stem cell transplantation performed in first remission. Also, no differential effect for clinical outcome was detected for the affected functional domains of PTPN11, with a median OS of 12.9 (N-SH2) vs 13.6 months (PTP).

Figure 3. Association of PTPN11 clonal rank with clinical outcome. Kaplan-Meier analysis showing the probability of (A) OS, RFS, and EFS for AML patients without (wt; black) or with PTPN11 mutation in subclonal (PTPN11^sub; blue) or dominant (PTPN11^dom; red) configuration, as well as OS in ELN-2017 favorable (D), intermediate (E), and adverse (F) risk groups. Numbers at risk are presented below each figure.
Figure 4. Correlations of *PTPN11* clonal hierarchy with molecular features. (A) Rates of associated comutations in patients with *PTPN11* mutations in subclonal (*PTPN11*\textsuperscript{sub}; blue) and dominant (*PTPN11*\textsuperscript{dom}; red) configuration. Significant differences are indicated. (B) VAFs of subclonal (n = 68) and dominant (n = 38) *PTPN11* mutations. (C) Overall number of comutations associated with subclonal (n = 68) and dominant (n = 38) *PTPN11* mutations. (D-E) Schematic illustration of representative clonal hierarchies (depicted from VAFs of *PTPN11* mutations and comutations) in *PTPN11*\textsuperscript{dom} (D) and *PTPN11*\textsuperscript{sub} (E) AMLs of 2 *PTPN11*-mut patients (overlapping circles, top) and potential sequential acquisition of *PTPN11* mutations (and comutations) as early (*PTPN11*\textsuperscript{dom}) or late (*PTPN11*\textsuperscript{sub}) event during clonal evolution (grid of circles, bottom).
The comparison of survival times across each ELN risk category demonstrated a deleterious effect of PTPN11 mutations predominantly within the favorable risk group (HR, 1.97; 95% CI, 1.42-2.74; \( P < .001 \)); Figure 2D). No significant effects of PTPN11 mutations for OS were observed in the intermediate (HR, 1.41; 95% CI, 0.87-2.26; \( P = .153 \); Figure 2E) and adverse risk groups (HR, 0.96; 95% CI, 0.59-1.56; \( P = .897 \); Figure 2F). Similar associations of poor outcome in PTPN11-mut patients within the favorable ELN group were detected for EFS, CR1, and ED30 (supplemental Table 2).

**Impact of PTPN11 clonal rank on clinical outcome**

To address the impact of PTPN11 mutations on clinical outcome in more detail and because of the high prevalence of subclonal PTPN11 variants, survival was next analyzed related to the clonal rank (dominant vs subclonal) of detected PTPN11 variants (Figure 3). Compared with patients with dominant PTPN11 mutations (PTPN11\(^{\text{DOM}}\)), the detection of PTPN11 mutations in subclonal configuration (PTPN11\(^{\text{SUB}}\)) was associated with poorer median OS (10.38 vs 29.88 months; \( P = .0018 \); Figure 3A), RFS (8.62 vs 39.48 months; \( P = .0061 \); Figure 3B), and EFS (5.39 vs 11.94 months; \( P = .13 \); Figure 3C), as well as higher rates of ED30 (14.7% vs 5.3%; \( P = .003 \)) and lower rates of CR1 (61.8% vs 76.3%; \( P = .046 \)). In line, univariate (supplemental Table 3) and multivariable analyses (Figure 2G) to assess the specific effect of PTPN11\(^{\text{SUB}}\) demonstrated an inferior outcome compared with PTPN11\(^{\text{wt}}\) (and PTPN11\(^{\text{DOM}}\)) patients. In contrast, no significant differences for clinical outcome were observed between PTPN11\(^{\text{DOM}}\) and PTPN11\(^{\text{wt}}\) cases. Similar to the overall effect of PTPN11-mut on survival, PTPN11\(^{\text{SUB}}\) configuration was associated with poor outcome, in particular within favorable (Figure 3D) and intermediate cytogenetic contexts (Figure 3E), whereas no differences between PTPN11\(^{\text{DOM}}\) and PTPN11\(^{\text{DOM}}\) (and PTPN11\(^{\text{wt}}\)) were detected in the ELN adverse risk group (Figure 3F).

In addition to clinical outcome, PTPN11\(^{\text{SUB}}\) patients were associated with distinct molecular features (Figure 4). PTPN11\(^{\text{SUB}}\) mutations were detected with significantly lower VAFs (median, 16.5%; interquartile range [IQR], 9%-25.75%; \( P < .001 \)), compared with PTPN11\(^{\text{DOM}}\) variants (median, 42.5%; IQR, 34%-48%; Figure 4B) and had a significantly (\( P < .001 \)) higher rate of associated comutations (median, 3; range, 1-7) compared with PTPN11\(^{\text{DOM}}\) (median, 2; range, 0-5; Figure 4C). Moreover, the mutational spectrum differed between both groups, with significantly higher rates of concomitant mutations in TET2 (17.6% vs 2.6%; \( P = .023 \)), NDRG1 (27.9% vs 7.8%; \( P = .014 \)), and RUNX1 (10.3% vs 0%; \( P = .041 \)) for PTPN11\(^{\text{SUB}}\) vs PTPN11\(^{\text{DOM}}\) (Figure 4A). Also, molecular lesions in ASXL1 and FLT3\(^{\text{ITD}}\) were almost exclusively detected within the PTPN11\(^{\text{SUB}}\) group. No differences were observed for the association with mutations in DNMT3A (36.7% vs 36.8%), NPM1 (66.1% vs 57.9%), and FLT3\(^{\text{ITD}}\) (16.2% vs 18.4%). A schematic representation of typical clonal configurations for PTPN11\(^{\text{DOM}}\) and PTPN11\(^{\text{SUB}}\) AMLs is shown in Figure 4D-E.

With respect to clinical characteristics, no major differences between PTPN11\(^{\text{DOM}}\) and PTPN11\(^{\text{SUB}}\) were observed. As an exception, patients with PTPN11\(^{\text{SUB}}\) were significantly (\( P = .003 \)) older (median, 56.5 years; IQR, 46.75-68.25 years) compared with PTPN11\(^{\text{DOM}}\) cases (median, 50.2 years; IQR, 31.5-56.5 years). Also, LDH concentrations (U/L) were significantly (\( P = .027 \)) elevated in the group of PTPN11\(^{\text{DOM}}\) (median, 544.5 U/L; IQR, 445.75-957.5 U/L), whereas LDH levels of PTPN11\(^{\text{SUB}}\) patients (median, 442.7 U/L; IQR, 326.75-780.5 U/L) were comparable to values measured in PTPN11\(^{\text{wt}}\) patients.

**Discussion**

In the present study, we analyzed a large cohort of 1529 adult AML patients for the prevalence of PTPN11 mutations. We confirm that PTPN11 mutations are recurrent alterations in patients with AML, which are independently associated with poor clinical outcome and distinct molecular and clinical features. Importantly, a more detailed analysis of PTPN11 clonal hierarchy and ELN risk categories revealed that the deleterious effect of PTPN11 mutations is mostly confined to subclonal constellations of PTPN11 mutations and patients with a favorable cytogenetic context.

Generally, PTPN11 mutations were detected with a frequency of 7%, which is in the range of prevalences (4%-12%) previously reported for AML.\(^{1,25,26,28-30}\) In line with published data,\(^{26,28-30}\) mutations predominantly affected the N-terminal SH2 domain of PTPN11, involved in basal inhibition of SHP2,\(^{12-15}\) and were found at subclonal levels in most patients.\(^{26}\) This is consistent with a predominance of RAS signaling gene mutations (eg, FLT3\(^{\text{ITD}}, \)NRAS, and KIT) as secondary or subclonal lesions in AML that either emerge or are lost during clonal disease evolution.\(^{1,37,38}\) The importance of individual PTPN11 variants (eg, E69K, G503A, and E76K), recurrently detected in our cohort, for RAS signaling hyperactivation, leukemic transformation, and chemoresistance in AML, has been summarized recently.\(^{26}\)

Similar to previous findings on clinical phenotype in AML, PTPN11-mut patients had significantly higher WBC counts\(^{26,30}\) and were associated with a characteristic mutational spectrum (ie, NPM1 and NRAS) frequently found comutated in PTPN11-mut AML.\(^{25,26,28-30}\) which is suggestive for a distinct pathway of pathogenesis. Interestingly, the association with NRAS mutations in cases with dominant PTPN11 mutations contradicts a presumed mutual exclusivity of activating mutations in PTPN11 (or FLT3 and KIT) with downstream RAS mutations (NRAS/KRAS) initially observed in hematologic malignancies.\(^{38-40}\)

However, except for general similarities with respect to PTPN11 prevalences and some phenotypic and molecular features, no consensus has been reached for prognostic implications of PTPN11-mut AML in the literature.\(^{26-30}\) In part, this might be attributed to considerable differences in patients’ characteristics (and numbers) of the analyzed cohorts. For example, in our study, PTPN11-mut patients largely (57.8%) clustered within the ELN 2017 favorable risk category, mainly because of a high rate of associated NPM1 mutations (63%), which confirms recent reports on rates of NPM1 mutations (60.5%-61%) and the underlying cytogenetics in PTPN11-mut AML.\(^{29,30}\) This is in conflict with other documentations, with a low rate (18%)\(^{26}\) or absence (0%)\(^{30}\) of favorable cytogenetics in PTPN11-mut AML, likely because of a lower frequency of concomitant NPM1 mutations (29% and 22%)\(^{26,27}\), in addition to the cytogenetic context, previous work on PTPN11-mut AML varied considerably with respect to the age of PTPN11-mut patients at diagnosis (eg, 67-70\(^{25-27}\) vs <60 years\(^{29,30}\)) and the respective treatment regimes (eg, high vs low intensity).

In our large cohort of newly diagnosed and intensively treated AML patients, the median age at diagnosis was 55 years (54 years for...
PTPN11-mut patients). For this clinically relevant setting, multivariable analysis revealed that PTPN11 mutations are an independent prognostic factor for worse outcome. In addition, we show that the overall unfavorable prognostic effect is mainly substantiated by an adverse impact of PTPN11-mut in the ELN 2017 favorable (and to some extent in the intermediate) risk group, whereas no such association was observed in patients with adverse cytogenetics. This is in disagreement with recent published data for AML patients (n = 410) treated with high-intensity therapy, where PTPN11 mutations were associated with poor survival, mainly in the ELN intermediate and adverse groups.26

The latter work also reported on an (insignificant) effect of VAFs >40% for worse outcome. Here, we could not confirm an association of high allelic fraction PTPN11 variants with poor survival. In contrast, we observed an inferior impact on outcome for PTPN11 mutations in subclonal configuration (PTPN11SUB), which were associated with significantly lower allelic fraction, higher rates of comutations, and different comutational patterns (ie, NRAS and TET2) compared with AMLs with dominant PTPN11 clones, suggesting a potential impact of cooperating mutant proteins. TET2 mutations were recently shown to confer a poor outcome in patients with myelodysplastic syndrome and concomitant secondary SF3B1 mutations, whereas no adverse effects of TET2 mutations were detected for the association with dominant SF3B1 variants in the same cohort.41 Similar associations of distinct dominant/subclonal gene–gene interactions with specific clinical features were reported previously and are especially pronounced for NPM1-mutated AML.1,42 A higher overall rate of associated comutations in PTPN11SUB patients (compared with PTPN11DOM) may also coincide with a higher clonal diversity and the presence of various treatment escape mechanisms because of the deregulation of multiple cell signaling pathways and a higher degree of genomic instability.137,38 If confirmed, these results would suggest that the acquisition of a subclonal mutation as a late event, occurring with multiple other adverse features, may lead to poor outcome. In contrast, when PTPN11 was a dominant driver mutation, clinical outcomes were similar (or even more favorable) compared with PTPN11-wt patients, indicating a less aggressive disease in these patients. Thus, considering these substantial differences, the overall deleterious effect of PTPN11 mutations in AML might be an epiphenomenon, depending on the clonal constellation in which they occur. The impact of this effect is presumably diminished in PTPN11-mut AML with ELN adverse cytogenetics, where no prognostic difference between PTPN11SUB and PTPN11DOM patients was detected. However, to fully understand the prognostic role of PTPN11 clonal hierarchy and to discriminate between true subclonal and secondary PTPN11 mutations, future investigations may track clonal progression at disease recurrence in PTPN11-mut AML. Furthermore, single-cell sequencing will potentially provide a higher resolution to address the dynamics of individual clones present in the same pathway (eg, PTPN11 and NRAS) and their impact for relapse development.43

Taken together, our data suggest that PTPN11 mutations are recurrent alterations in patients with AML and are an independent prognostic factor for poor survival, with worse clinical outcome in patients within the ELN favorable risk group. The association with distinct clinical and molecular features, points to a specific genesis in this subset of AML. Importantly, the deleterious outcome in PTPN11-mut patients was found to be confined to subclonal configurations of PTPN11 mutations, which are associated with a higher rate of concomitant mutations and a different mutational spectrum, as compared with PTPN11 variants in dominant clonal rank.

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Authorship

Contribution: C.T., M.M., J.N.E., and S.S. conceived the work, acquired and analyzed the data, and interpreted the data; S.S. performed bioinformatic analysis and drafted the manuscript; M.K. provided statistical analysis; G.E. and M.B. provided administrative support; and all authors provided samples, collected data, and have seen and approved the manuscript.

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Marburg GmbH, Marburg, Andreas Neubauer; Universitätsklinikum Münster, Münster, Wolfgang E. Berdel; Klinikum Nürnberg Nord, Nürnberg, Martin Wilhelm; Elblandklinikum Riesa, Riesa, Jörk Schubert; Agaplesion Diakonieklinikum Rotenburg GmbH, Rotenburg (Wümme), Achim Meinhardt; Diakonie-Krankenhaus, Schwäbisch-Hall, Thomas Geer; Klinikum Sindelfingen-Böblingen, Sindelfingen, Markus Ritter; Robert-Bosch-Krankenhaus, Stuttgart, Walter Erich Aultzy; HSK Wiesbaden, Wiesbaden, Natalia Heinz; Reims-Murr-Klinikum Winnenden, Winnenden, Markus Schaich; Universitätsklinikum Würzburg, Würzburg, Hermann Einsele.

References

1. Papaemmanuil E, Gerstung M, Bullinger L, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209-2221.

2. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia [published correction appears in Blood. 2016;128(3):462-463]. Blood. 2016;127(20):2391-2405.

3. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424-447.

4. Patel JP, Gloor M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079-1089.

5. Loh ML, Reynolds MG, Vattikuti S, et al; Children’s Cancer Group. PTPN11 mutations in pediatric patients with acute myeloid leukemia: results from the Children’s Cancer Group. Leukemia. 2004;18(11):1831-1834.

6. Loh ML, Vattikuti S, Schubbert S, et al. Mutations in PTPN11 imply the SHP-2 phosphatase in leukemogenesis. Blood. 2004;103(6):2325-2331.

7. Tartaglia M, Niemeyer CM, Fragale A, et al. Somatic mutations in PTPN11 in juvenile myelomonocytic leukemia, myelodysplastic syndromes and acute myeloid leukemia [published correction appears in Nat Genet. 2003;34:464]. Nat Genet. 2003;34(2):148-150.

8. Chen L, Chen W, Mysliwski M, et al. Mutated Ptpn11 alters leukemic stem cell frequency and reduces the sensitivity of acute myeloid leukemia cells to Mcl1 inhibition. Leukemia. 2015;29(6):1290-1300.

9. Bentires-Alj M, Paez JG, David FS, et al. Activating mutations of the noonan syndrome-associated SHP2/PTPN11 gene in human solid tumors and acute myeloid leukemia [published correction appears in Nat Genet. 2003;23:862-866]. Nat Genet. 2003;34(2):148-150.

10. Tonks NK. Protein tyrosine phosphatases: from genes, to function, to disease. Nat Rev Mol Cell Biol. 2003;4(2):148-150.

11. Pandey R, Williams IR, Dearolf CR, et al. Prognostic, therapeutic, and mechanistic implications of a mouse model of leukemia evoked by Shp2 hyperproliferation and malignant disease in vivo. Blood. 2012;6(3):275-279.

12. Chen YN, LaMarche MJ, Chan HM, et al. Allosteric inhibition of SHP2 phosphorylates inhibits cancers driven by receptor tyrosine kinases. Nature. 2016;535(7610):148-152.

13. Prahlad A, Heynen GJ, Germano G, et al. PTPN11 is a central node in intrinsic and acquired resistance to targeted cancer drugs. Cell Rep. 2015;12(12):1978-1985.

14. Nabinger SC, Li XJ, Ramdas B, et al. The protein tyrosine phosphatase, Shp2, positively contributes to FLT3-ITD-induced hematopoietic progenitor hyperproliferation and malignant disease in vivo. Leukemia. 2013;27(2):398-408.

15. Stevens BM, Jones CL, Winters A, et al. PTPN11 mutations confer unique metabolic properties and increase resistance to venetoclax and azacitidine in acute myelogenous leukemia [abstract]. Blood. 2018;132(suppl 1). Abstract 909.

16. Chou A, Lin M, Chang Y, et al. Prognostic and therapeutic implications of PTPN11 mutations in patients with acute myeloid leukemia. Cancer Cell. 2017;20(6):1290-1300.

17. Schubbert S, Lieuw K, Rowe SL, et al. Human somatic PTPN11 mutations induce hematopoietic-cell hypersensitivity to granulocyte-macrophage colony-stimulating factor. Blood. 2005;105(9):3737-3742.

18. Chan RJ, Feng GS. PTPN11 is the first identified proto-oncogene that encodes a tyrosine phosphatase. Blood. 2007;109(3):862-867.

19. Bard-Chapeau EA, Liew K, Rowe SL, et al. Functional analysis of leukemia-associated PTPN11 mutations in primary hematopoietic cells. Blood. 2005;106(1):311-317.

20. Dong L, Yu WM, Zheng H, et al. Leukaemogenic effects of Ptpn11 activating mutations in the stem cell microenvironment. Nature. 2016;539(7628):304-308.

21. Chan G, Kalaitzidis D, Neel BG. The tyrosine phosphatase Shp2 (PTPN11) in cancer. Cancer Metastasis Rev. 2008;27(2):179-192.

22. Chou A, Lin M, Chang Y, et al. Prognostic and therapeutic implications of PTPN11 mutations in patients with acute myeloid leukemia. Cancer Cell. 2017;20(6):1290-1300.

23. Chou A, Lin M, Chang Y, et al. Prognostic and therapeutic implications of PTPN11 mutations in patients with acute myeloid leukemia. Cancer Cell. 2017;20(6):1290-1300.

24. Stevens BM, Jones CL, Winters A, et al. PTPN11 mutations confer unique metabolic properties and increase resistance to venetoclax and azacitidine in acute myelogenous leukemia [abstract]. Blood. 2018;132(suppl 1). Abstract 909.

25. Kaner JD, Mencia-Trinchant N, Schaap A, et al. Acute myeloid leukemia (AML) with somatic mutations in PTPN11 is associated with treatment resistance and poor overall survival [abstract]. Blood. 2018;132(suppl 1). Abstract 2760.
26. Alfayez M, Issa GC, Patel KP, et al. The clinical impact of PTPN11 mutations in adults with acute myeloid leukemia. *Leukemia*. 2020;35(3):691-700.

27. Swoboda DM, Al Ali NE, Padron E, et al. PTPN11 mutations are associated with poor outcomes across myeloid malignancies. *Blood*. 2020;136(suppl 1):8-9.

28. Hou HA, Chou WC, Lin LI, et al. Characterization of acute myeloid leukemia with PTPN11 mutation: the mutation is closely associated with NPM1 mutation but inversely related to FLT3/ITD. *Leukemia*. 2008;22(5):1075-1078.

29. Fobare S, Kohlschmidt J, Gulcin Ozer H, et al. Clinical and prognostic implications of PTPN11 mutations in acute myeloid leukemia (Alliance). *Blood*. 2020;136(suppl 1):20-21.

30. Metzeler KH, Rothenberg-Thurley M, Görlich D, et al. PTPN11 mutations and outcomes in adult patients with acute myeloid leukemia. *Blood*. 2020;136(suppl 1):4-5.

31. Schaich M, Röllig C, Soucek S, et al. Cytarabine dose of 36 g/m² compared with 12 g/m² within first consolidation in acute myeloid leukemia: results of patients enrolled onto the prospective randomized AML96 study. *J Clin Oncol*. 2011;29(19):2696-2702.

32. Röllig C, Kramer M, Gabrecht M, et al; Study Alliance Leukemia (SAL). Intermediate-dose cytarabine plus mitoxantrone versus standard-dose cytarabine plus daunorubicin for acute myeloid leukemia in elderly patients. *Ann Oncol*. 2018;29(4):973-978.

33. Schetelig J, Schaich M, Schäfer-Eckart K, et al; Study Alliance Leukemia. Hematopoietic cell transplantation in patients with intermediate and high-risk AML: results from the randomized Study Alliance Leukemia (SAL) AML 2003 trial. *Leukemia*. 2015;29(5):1060-1068.

34. Röllig C, Serve H, Hüttmann A, et al; Study Alliance Leukaemia. Addition of sorafenib versus placebo to standard therapy in patients aged 60 years or younger with newly diagnosed acute myeloid leukaemia (SORAML): a multicentre, phase 2, randomised controlled trial. *Lancet Oncol*. 2015;16(16):1691-1699.

35. Stasik S, Middeke JM, Kramer M, et al; Study Alliance Leukemia (SAL). EZH2 mutations and impact on clinical outcome: an analysis in 1,604 patients with newly diagnosed acute myeloid leukemia. *Haematologica*. 2020;105(5):e228-e231.

36. Thiede C, Steudel C, Mohr B, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002;99(12):4326-4335.

37. Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012;150(2):264-278.

38. Ward AF, Braun BS, Shannon KM. Targeting oncogenic Ras signaling in hematologic malignancies. *Blood*. 2012;120(17):3397-3406.

39. Tartaglia M, Martinelli S, Cazzangia G, et al. Genetic evidence for lineage-related and differentiation stage-related contribution of somatic PTPN11 mutations to leukemogenesis in childhood acute leukemia. *Blood*. 2004;104(2):307-313.

40. Yamamoto T, Isomura M, Xu Y, et al. PTPN11, RAS and FLT3 mutations in childhood acute lymphoblastic leukemia. *Leuk Res*. 2006;30(9):1085-1089.

41. Awada H, Kerr CM, Adema V, et al. The clonal trajectories of SF3B1 mutations in myeloid neoplasia. *Blood*. 2020;136(suppl 1):8.

42. Nagata Y, Makishima H, Kerr CM, et al. Invariant patterns of clonal succession determine specific clinical features of myelodysplastic syndromes. *Nat Commun*. 2019;10(1):5386.

43. Morita K, Wang F, Jahn K, et al. Clonal evolution of acute myeloid leukemia revealed by high-throughput single-cell genomics [published correction appears in *Nat Commun*. 2020;11:5996]. *Nat Commun*. 2020;11(1):5327.