Isocitrate dehydrogenase (IDH) mutations are frequent in blast-phase myeloproliferative neoplasms and might therefore contribute to leukemic transformation. We examined this possibility in 301 consecutive patients with chronic-phase primary myelofibrosis (PMF). The mutant IDH was detected in 12 patients (4%): 7 IDH2 (5 R140Q, 1 R140W and 1 R172G) and 5 IDH1 (3 R132S and 2 R132C). In all, 6 (50%) of the 12 IDH-mutated patients also expressed JAK2V617F. Overall, 18 (6%) patients displayed only MPL and 164 (54.3%) only JAK2 mutations. Multivariable analysis that accounted for conventional risk factors disclosed inferior overall survival (OS; \( P < 0.03 \)) and leukemia-free survival (LFS; \( P = 0.003 \)) in IDH-mutated patients: OS hazard ratio (HR) was 0.39 (95% confidence interval (95% CI) 0.2–0.75), 0.50 (95% CI 0.27–0.95) and 0.53 (95% CI 0.23–1.2) for patients with no, JAK2 or MPL mutations, respectively. Further analysis disclosed a more pronounced effect for the mutant IDH on OS and LFS in the presence (\( P = 0.0002 \)) and \( P < 0.0001 \), respectively) as opposed to the absence (\( P = 0.34 \) and \( P = 0.64 \)) of concomitant JAK2V617F. Analysis of paired samples obtained during chronic- and blast-phase disease revealed the presence of both IDH and JAK2 mutations at both time points. Our observations suggest that IDH mutations in PMF are independent predictors of leukemic transformation and raise the possibility of leukemogenic collaboration with JAK2V617F.

Several studies have examined the phenotypic and prognostic effects of both IDH1 and IDH2 mutations in AML, and most have shown a consistent association with normal or intermediate-risk karyotype, sole trisomy 8 and NPM1 mutations.\(^{16,17,23–27}\) The mutant IDH1 was associated with worse prognosis in cytogenetically normal AML with NPM1\(^+/+\)FLT3\(^-\) molecular profile\(^{15,28,29}\) and better prognosis in FLT3\(^+\) AML.\(^{27}\) In some\(^{17,23}\) but not other\(^{19}\) studies, the mutant IDH2 was associated with unfavorable prognosis in cytogenetically normal AML\(^{17}\) whereas a more recent study suggested that the mutant IDH2R140 was prognostically more favorable than the mutant IDH2R172.\(^{31}\)

Unlike the case with AML, there is limited information on the prognostic impact of IDH mutations in chronic myeloid neoplasms, including MDS\(^{19}\) and MPN.\(^{8}\) We recently reported on IDH1 and IDH2 mutational frequencies among 1473 patients with BCR-ABL1-negative MPN:\(^{8}\) 0.8% in essential thrombocythemia, 1.9% in polycythemia vera, 4.1% in PMF, 1% in post-essential thrombocythemia/polycythemia vera MF, 0% in blast-phase essential thrombocythemia, 25% in blast-phase polycythemia vera and 25% in blast-phase PMF. The particular study included only 111 patients with chronic-phase PMF and complete clinical information; therefore, detailed prognostic analysis was limited, especially in terms of clinically relevant mutation interactions. In the current study, we examined the phenotypic and prognostic effects of IDH1 and IDH2 mutations among 301 patients with chronic-phase PMF, in the context of other MPN-associated mutations.

### Materials and methods

This study was approved by the Mayo Clinic Institutional Review Board. All patients provided informed written consent for study sample collection and permission for use in research. Study eligibility criteria included availability of bone marrow histology (IDH2).\(^{8}\) The mutant IDH has decreased affinity for isocitrate but displays catalytic activity in converting \( \alpha \)-ketoglutarate to 2-hydroxyglutarate.\(^{9–12}\) Decreased supply of \( \alpha \)-ketoglutarate or accumulation of 2-hydroxyglutarate is believed to underlie the oncogenic properties of the mutant IDH.\(^{9,13}\)

IDH mutations are prevalent in low-grade gliomas and secondary glioblastomas (mutational frequency ~70%)\(^{14}\) and they have also been described, although at a much lower frequency, in myeloid malignancies including acute myeloid leukemia (AML; 10–20%),\(^{16,18}\) myelodysplastic syndrome (MDS; 3–5%),\(^{19,20}\) MPN (1–4%),\(^{18}\) MDS/MPN including chronic myelomonocytic leukemia (≤9%),\(^{20}\) post-MDS AML (~15%),\(^{19}\) post-MPN AML (~22%),\(^{8}\) post-MDS/MPN AML (~10%),\(^{20}\) del(5q)-associated high-risk MDS or AML (~22%)\(^{21}\) and blast-phase chronic myelogenous leukemia (~4%).\(^{22}\) Single case reports also included angioimmunoblastic lymphoma\(^{23}\) and acute lymphoblastic leukemia.\(^{18}\)

**ORIGINAL ARTICLE**

**IDH mutations in primary myelofibrosis predict leukemic transformation and shortened survival: clinical evidence for leukemogenic collaboration with JAK2V617F**

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and cytogenetic information at the time of referral to the Mayo Clinic. The diagnoses of PMF and leukemic transformation were according to the World Health Organization criteria.32 Patients with blast-phase disease at the time of their referral to the Mayo Clinic were excluded from the study because one of the objectives of the study was to assess mutation impact on leukemic transformation. Unfavorable karyotype designation and DIPSS-plus (Dynamic International Prognostic Scoring System-plus) risk categorization were as described previously.13,34 All study patients were fully characterized for karyotype, JAK2 and MPL mutational status and DIPSS-plus risk category.

DNA from bone marrow or peripheral blood was extracted using conventional methods. MPL and JAK2 mutation analyses were performed according to previously published methods.35–38 IDH1 and IDH2 mutations were analyzed by direct sequencing and/or high-resolution melting assay. Direct sequencing for IDH1 exon 4 mutations was performed using the following primer sequences: sense, 5'-CGGTCCTTCAGAGAAGCCATT-3' and anti-sense, 5' -CATGAC-3'. IDH2 exon 4 was amplified using sense, 5'-CCACATTATCTGCTTCCT-3' and anti-sense, 5'-GCTAGGCAGGACCTGAC-3'.18 Both reactions were performed in 25 μl volume containing 100 ng of DNA, 0.25 Units Taq polymerase, 0.3 mM each of dATP, dCTP, dGTP and dTTP, 5 μl of a 10 × PCR Buffer (Roche Diagnostics, Indianapolis, IN, USA) and 0.2 μM each of sense and anti-sense primers. The reaction was denatured at 94°C for 3 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at 57°C for 30 s and extension at 72°C for 40 s. After a final extension at 72°C for 2 min, the products were confirmed by 1.3% agarose gel and purified using Qiagen's PCR quick purification kit (Qiagen, Santa Clarita, CA, USA). The product was sequenced using the ABI PRISM 3730xl analyzer (Applied Biosystems Inc., Foster City, CA, USA) to screen for the presence of mutations.

High-resolution melting was performed using the LightCycler 480 Real-Time PCR system (Roche Diagnostics), using the above-mentioned primers for IDH1 mutations (R130) and the following primers for IDH2 mutations (R140 and R172): R140 sense, 5'-GCTGAAGAAGATGGGAA-3' and anti-sense, 5'-TGATGGGCTCCGGAGA-3'; R172 sense, 5'-CAGGACCTCACATGTTG-3' and anti-sense, 5'-CAGAGGTCACTGAGGATCC-3'.

All statistical analyses considered clinical and laboratory parameters obtained at the time of first referral to the Mayo Clinic, in which in most instances coincided with the time of bone marrow biopsy at the Mayo Clinic and study sample collection. Differences in the distribution of continuous variables between categories were analyzed by either the Mann–Whitney (for comparison of two groups) or the Kruskal–Wallis (comparison of three or more groups) test. Patient groups with nominal variables were compared by the χ²-test. Overall survival (OS) was calculated from the date of first referral to the date of death (uncensored) or last contact (ensored). Leukemia-free survival (LFS) was calculated from the date of first referral to the date of leukemic transformation (uncensored) or death/last contact (ensored). OS and LFS curves were prepared by the Kaplan–Meier method and compared by the log-rank test. Cox’s proportional hazard regression model was used for multivariable analysis. P-values <0.05 were considered significant. The Stat View (SAS Institute, Cary, NC, USA) statistical package was used for all calculations.

Results

A total of 301 consecutive patients with PMF were included in this study. The median age at the time of study was 63 years (range, 14–82) and 65% were males. DIPSS-plus risk distribution was 11% low, 16% intermediate-1, 36% intermediate-2 and 37% high. Other clinical and laboratory characteristics at the time of Mayo Clinic referral are outlined in Table 1; 40 (13%) patients had received cytoreductive therapy at the time of their first referral at our institution. The study population included 178 patients who were evaluated at or near the time of their diagnosis and their presenting characteristics are separately outlined in Table 2.

The mutant IDH was detected in 12 patients (4%): 7 IDH2 (5 R140Q, 1 R140W and 1 R172G) and 5 IDH1 (3 R132S and 2 R132C). MPL exon 10 was mutated in 18 patients (6.3%) and constituted W515L in 14 patients, W515K in 3 and a novel frameshift mutation in 1 patient. JAK2V617F was detected in 169 (56%) patients. Six patients displayed both JAK2V617F and IDH mutations (IDH2R140Q in two patients, IDH2R140W in one and IDH1R132S in three); JAK2V617F allele burdens in these six patients with concomitant mutant IDH were 1, 7, 22, 27, 30 and 96%, respectively. One patient displayed both IDH1R140Q and MPLW515R. In all, 107 (36%) patients were negative for all three mutations (that is, JAK2V617F, MPL exon 10 and IDH1/2).

The 12 IDH-mutated patients, with or without concomitant JAK2V617F, were clinically compared with patients belonging to the 3 other molecular subgroups: mutated for JAK2 only (n = 164), mutated for MPL only (n = 18) and unmutated for all three (n = 107). As can be seen in Tables 1 and 2, the four molecular subgroups were remarkably similar in their phenotype with few exceptions; IDH-mutated patients were significantly older than those with no mutations (P = 0.04), whereas age distribution was similar between patients with mutant IDH, MPL or JAK2. At the time of this writing, 192 (64%) deaths and 36 (12%) leukemic transformations were documented. The median follow-up time for living patients was 68 months (range 12–296). Treatment over the course of the disease was primarily with conventional drugs, and a total of 53 therapeutic splenectomies and 24 transplants were documented.

In univariate analysis, IDH-mutated patients lived shorter than did those with JAK2 (P = 0.03), MPL (P = 0.047) or no mutations (P = 0.0009). The OS data for the four molecular subgroups are shown in Figure 1. IDH-mutated patients also showed significantly shorter LFS, compared with those with JAK2 (P = 0.0008), MPL (P = 0.02) or no mutations (P = 0.001), as shown in Figure 2. LFS was similar between patients with no mutations and those with either MPL (P = 0.47) or JAK2 (P = 0.99). The OS of patients with no mutations was significantly longer than those with JAK2V617F (P = 0.01), but not than those with MPL mutations (P = 0.41). After accounting for age, the OS difference between patients with JAK2V617F and no mutations became insignificant (P = 0.40), whereas the presence of the mutant IDH remained a significant disadvantage for both OS (P = 0.04) and LFS (P = 0.005).

Multivariable analysis of OS that included risk categorization per DIPSS-plus13 confirmed the independent prognostic relevance of the mutant IDH (P = 0.03): hazard ratio (HR) for patients with no mutations = 0.39, 95% confidence interval (95% CI) 0.2–0.75; HR for JAK2-mutated patients = 0.50, 95% CI, 0.27–0.95; HR for MPL-mutated patients = 0.53, 95% CI, 0.23–1.2. A similar analysis for LFS that included risk factors for leukemic transformation (that is, unfavorable karyotype and platelet count <100 × 10⁹/l) as covariates also confirmed the prognostic relevance of the mutant IDH (P = 0.003): HR for patients with no mutations = 0.16; 95% CI, 0.06–0.46; HR for JAK2-mutated patients = 0.18; 95% CI, 0.06–0.48; HR for MPL-mutated patients = 0.09; 95% CI, 0.01–0.76.33 Further
Circulating blasts X JAK2 and presence of either one of the two risk factors. Prognosis in 10-year incidence of AML was estimated at 12% in the absence of the mutation. These observations suggest that mutated patients who develop AML did not necessarily express JAK2 mutations.

Discussion

The most feared disease complication in MPN is leukemic transformation. In PMF, risk factors for leukemic progression include unfavorable karyotype; thrombocytopenia (platelet count < 100 x 10^9/l) and ≥3% circulating blasts; the 10-year incidence of AML was estimated at 12% in the absence of unfavorable karyotype and thrombocytopenia. The presence of either one of the two risk factors, Prognosis in post-PMF AML is dismal with a median survival of < 3 months and is not favorably affected by conventional chemotherapy. The discovery of JAK2V617F in the majority of patients with PMF raised hopes of better outcome with effective molecularly targeted therapy. However, it has since been realized that the presence or absence of JAK2V617F in PMF did not affect leukemic transformation and that leukemic blasts in JAK2-mutated patients who develop AML did not necessarily express the mutation. These observations suggest that JAK2V617F is neither necessary nor sufficient for leukemic progression in PMF.

This study suggests that the presence of the mutant IDH signifies an increased risk of leukemic transformation in PMF and also raises the intriguing possibility of leukemogenic collaboration between the mutant IDH and JAK2V617F; 4 (67%) of 6 patients with concomitant IDH and JAK2 mutations developed AML as opposed to only 1 (17%) of 6 IDH-mutated patients without concomitant JAK2V617F, 1 (6%) of 18 with MPL mutations, 17 (10%) of 164 with only JAK2 mutation and 13 (12%) of 107 patients with no mutations (P<0.0001; Figure 4). A similar clinical observation was made in a recent report that showed an inferior LFS in IDH-mutated myeloid malignancies with isolated del(5q); in the particular study, two of six patients with IDH mutations also carried JAK2V617F and both had transformed into AML, whereas only two of the remaining four IDH-mutated patients without concomitant JAK2V617F had transformed into AML. The possibility that the mutant IDH collaborates with other oncogenes is further supported by a recent report in which the mutant IDH enhanced growth and mitogen-activated protein kinase and signal transducer and activator of transcription-3 signaling in BRAF-mutated melanoma cells.

Currently known mutations in PMF are believed to represent late genetic events derived from an ancestral abnormal clone the genetic make up of which remains elusive. The fact that many of these mutations are infrequent and lack disease specificity further undermines their pathogenetic contribution to disease initiation. On the other hand, the absence of mutual exclusivity

### Table 1
Comparison of clinical characteristics of patients with primary myelofibrosis stratified by the presence or absence of IDH, MPL and JAK2 mutations

| Variables               | All patients | IDH mutated (n = 301) | MPL mutated (n = 16) | JAK2 mutated (n = 18) | IDH/MPL/JAK2 unmutated (n = 107) | P-value |
|-------------------------|--------------|-----------------------|----------------------|-----------------------|----------------------------------|---------|
| Age (years); median (range) | 63 (14-82)   | 66 (50-74)            | 62 (35-82)           | 65 (28-81)            | 58 (14-79)                       | 0.0002  |
| Age >65 years; n (%)     | 96 (32%)     | 6 (50%)               | 6 (33%)              | 7 (47%)               | 30 (28%)                         | 0.02    |
| Males (%)                | 197 (65%)    | 7 (58%)               | 11 (61%)             | 105 (64%)             | 74 (69%)                         | 0.75    |
| Hemoglobin, g/dl; median (range) | 10 (6-15) | 11 (7-15)             | 10 (6-14)            | 10 (7-15)             | 10 (6-14)                        | 0.97    |
| Leukocyte count, × 10^9/l; median (range) | 9 (1-176) | 9 (4-48)              | 11 (4-50)            | 10 (1-176)            | 7 (1-147)                        | 0.11    |
| Platelet count, × 10^9/l; median (range) | 222 (11-1493) | 141 (66-410)          | 128 (14-662)         | 218 (11-984)           | 257 (13-1493)                    | 0.06    |
| DIPSS-plus risk group (%) |              |                       |                      |                      |                                  |         |
| Low                     | 34 (11%)     | 0                     | 4 (22%)              | 18 (11%)              | 12 (11%)                         |         |
| Intermediate-1          | 47 (16%)     | 2 (16%)               | 2 (11%)              | 19 (12%)              | 24 (22%)                         |         |
| Intermediate-2          | 109 (36%)    | 5 (42%)               | 3 (17%)              | 60 (37%)              | 41 (38%)                         | 0.11    |
| High                    | 111 (37%)    | 5 (42%)               | 9 (50%)              | 67 (41%)              | 30 (28%)                         |         |
| Constitutional symptoms; n (%) | 115 (38%) | 5 (42%)               | 4 (22%)              | 69 (42%)              | 37 (35%)                         | 0.30    |
| Circulating blasts ≥1%; n (%) | 190 (63%) | 10 (83%)              | 11 (61%)             | 101 (62%)             | 68 (64%)                         | 0.50    |
| Hemoglobin <10 g/dl; n (%) | 151 (50%) | 5 (42%)               | 10 (56%)             | 88 (54%)              | 48 (45%)                         | 0.46    |
| Leukocytes ≥25 x 10^9/l; n (%) | 52 (17%) | 2 (17%)               | 4 (22%)              | 33 (20%)              | 13 (12%)                         | 0.36    |
| Platelets <100 x 10^9/l; n (%) | 73 (24%) | 3 (25%)               | 8 (44%)              | 35 (21%)              | 27 (25%)                         | 0.19    |
| Leukocytes <4 x 10^9/l; n (%) | 46 (15%) | 1 (8%)                | 1 (6%)               | 24 (15%)              | 20 (19%)                         | 0.43    |
| Palpable spleen >10 cm; n (%) | 109 (36%) | 2 (17%)               | 5 (28%)              | 67 (41%)              | 35 (33%)                         | 0.20    |
| Splenectomy; n (%)       | 53 (18%)     | 2 (17%)               | 4 (22%)              | 22 (17%)              | 19 (18%)                         | 0.06    |

**Abbreviation:** DIPSS-plus, Dynamic International Prognostic Scoring System-Plus. Bold values indicate significant differences.
and the higher prevalence of some MPN-associated mutations (for example, IDH1, LNK, JAK2, MPL, and TP53 mutations) in blast-phase, as opposed to chronic-phase, disease suggests possible pathogenetic contribution to leukemic transformation. The observations from this study suggest one possibility in which mutations with non-redundant functional consequences collaborate to amplify the development of AML. Alternatively, the presence of mutations of interest (such as the mutant IDH1) in at-risk patients might simply constitute a marker of genomic instability associated with impending leukemic transformation.

Figure 1: Overall survival data for 301 patients with primary myelofibrosis stratified by the presence or absence of IDH, MPL and JAK2 mutations.

Figure 2: Leukemia-free survival data for 301 patients with primary myelofibrosis stratified by the presence or absence of IDH, MPL and JAK2 mutations.

A third possibility considers the distribution of specific mutations in independent clones that arise from a common ancestral clone that is susceptible to both emergence of mutations of interest and leukemic transformation. The prognostic impact of IDH mutations in AML has been studied extensively, and although some studies have reported a preponderance of IDH1 mutations, whereas others have shown the opposite. In the current PMF

Table 2: Comparison of clinical characteristics of patients with primary myelofibrosis, who were evaluated within 1 year of diagnosis, stratified by the presence or absence of IDH, MPL and JAK2 mutations.

| Variables | All patients (n = 178) | IDH mutated (n = 10) | MPL mutated (n = 9) | JAK2 mutated (n = 96) | IDH/MPL/JAK2 un-mutated (n = 63) | P-value |
|-----------|------------------------|----------------------|---------------------|-----------------------|--------------------------------|---------|
| Age (years); median (range) | 63 (14–81) | 66 (50–74) | 60 (35–66) | 65 (28–80) | 58 (14–79) | 0.005 |
| Age >65 years; n (%) | 70 (39%) | 5 (60%) | 1 (11%) | 48 (50%) | 16 (25%) | 0.004 |
| Males; n (%) | 116 (65%) | 7 (70%) | 7 (80%) | 63 (66%) | 39 (62%) | 0.79 |
| Hemoglobin, g/dl; median (range) | 10 (5–15) | 11 (7–15) | 10 (5–14) | 10 (7–15) | 11 (6–14) | 0.50 |
| Leukocyte count, x 10^9/l; median (range) | 8 (1–147) | 10 (4–48) | 11 (4–50) | 9 (1–99) | 7 (2–147) | 0.15 |
| Platelet count, x 10^9/l; median (range) | 253 (12–1493) | 159 (79–410) | 146 (31–662) | 245 (12–984) | 316 (14–1493) | 0.13 |
| DIPSS-plus risk group; n (%) | | | | | | |
| Low | 25 (14%) | 0 | 2 (22%) | 13 (14%) | 10 (16%) | 0.60 |
| Intermediate-1 | 36 (20%) | 2 (20%) | 3 (33%) | 35 (36%) | 20 (32%) | |
| Intermediate-2 | 62 (35%) | 4 (40%) | 3 (33%) | 35 (36%) | 20 (32%) | |
| High | 55 (31%) | 4 (40%) | 3 (33%) | 35 (36%) | 20 (32%) | |
| Constitutional symptoms; n (%) | 65 (37%) | 4 (40%) | 2 (22%) | 38 (40%) | 21 (33%) | 0.68 |
| Circulating blasts ≥1%; n (%) | 101 (57%) | 8 (80%) | 5 (56%) | 54 (56%) | 34(53%) | 0.49 |
| Hemoglobin <10 g/dl; n (%) | 77 (43%) | 4 (40%) | 6 (67%) | 47 (49%) | 20 (32%) | 0.08 |
| Leukocytes ≥25 x 10^9/l; n (%) | 26 (15%) | 2 (20%) | 2 (22%) | 14 (15%) | 8 (13%) | 0.84 |
| Platelets <100 x 10^9/l; n (%) | 27 (16%) | 1 (10%) | 1 (11%) | 12 (13%) | 13 (21%) | 0.51 |
| Palpable spleen >10 cm; n (%) | 44 (25%) | 2 (20%) | 2 (22%) | 28 (29%) | 12 (19%) | 0.45 |
| Splenectomy; n (%) | 24 (13%) | 1 (10%) | 3 (33%) | 12 (13%) | 8 (13%) | 0.35 |
| Cytogenetic categories | | | | | | |
| Normal | 116 (65%) | 7 (70%) | 6 (67%) | 59 (60%) | 45 (71%) | 0.75 |
| Favorable | 48 (27%) | 2 (20%) | 3 (33%) | 30 (31%) | 13 (21%) | |
| Unfavorable | 14 (8%) | 1 (10%) | 0 (0%) | 8 (8%) | 5 (8%) | |
| Transplanted; n (%) | 15 (8%) | 0 (0%) | 2 (22%) | 5 (5%) | 8 (13%) | 0.12 |
| Deaths; n (%) | 107 (60%) | 9 (90%) | 6 (67%) | 59 (61%) | 32 (51%) | 0.09 |
| Leukemic transformations; n (%) | 22 (12%) | 4 (40%) | 1 (11%) | 13 (14%) | 4 (6%) | 0.03 |

Abbreviation: DIPSS-plus, Dynamic International Prognostic Scoring System-Plus. Bold values indicate significant differences.
It would also be interesting to examine the mutant IDH-induced phenotypic modifications of JAK2V617F mouse models. Whether therapeutic targeting of the mutant IDH or interfering with the production/function of its ‘oncogenic’ metabolite (that is, 2-hydroxyglutarate) would favorably affect leukemic progression in PMF remains to be seen.

Conflict of interest
The authors declare no conflict of interest.

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