

ORIGINAL ARTICLE

IDH mutations in primary myelofibrosis predict leukemic transformation and shortened survival: clinical evidence for leukemogenic collaboration with JAK2V617FA Tefferi¹, T Jimma¹, NH Sulai¹, TL Lasho¹, CM Finke¹, RA Knudson², RF McClure³ and A Pardanani¹¹Division of Hematology, Department of Medicine, Mayo Clinic, Rochester, MN, USA; ²Division of Cytogenetics, Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA and ³Division of Hematopathology, Department of Laboratory Medicine, Mayo Clinic, Rochester, MN, USA

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.

Leukemia (2012) 26, 475–480; doi:10.1038/leu.2011.253; published online 13 September 2011

Keywords: IDH1; IDH2; myeloproliferative; prognosis; cytogenetics

Introduction

Among the three *BCR-ABL1*-negative myeloproliferative neoplasms (MPNs), including polycythemia vera, essential thrombocythemia and primary myelofibrosis (PMF), the latter is by far the worst in terms of both survival and quality of life.^{1,2} The more aggressive disease biology in PMF is also manifest by the higher prevalence of cytogenetic abnormalities³ and somatic mutations.⁴ The latter involve *JAK2*, *MPL*, *TET2*, *ASXL1*, *CBL*, isocitrate dehydrogenase (*IDH1*), *IDH2*, *IKZF1*, *LNK*, *EZH2* and *DNMT3A*.^{4,5} Recent studies have reported higher frequencies of *IDH1/IDH2* and *LNK* mutations in blast-phase MPN,^{6,7} suggesting a pathogenetic contribution to disease progression.

Isocitrate dehydrogenase-1 is located on chromosome 2q33.3 and *IDH2* on chromosome 15q26.1. Both genes encode enzymes that catalyze oxidative decarboxylation of isocitrate to α -ketoglutarate. *IDH* mutations involve exon 4 and affect three specific arginine residues: R132 (*IDH1*), R172 (*IDH2*) and R140

(*IDH2*).⁸ The mutant *IDH* has decreased affinity for isocitrate but displays catalytic activity in converting α -ketoglutarate to 2-hydroxyglutarate.^{9–12} Decreased supply of α -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 $\sim 70\%$)¹⁴ and they have also been described, although at a much lower frequency, in myeloid malignancies including acute myeloid leukemia (AML; 10–20%),^{15–18} myelodysplastic syndrome (MDS; 3–5%),^{19,20} MPN (1–4%),^{8,18} MDS/MPN including chronic myelomonocytic leukemia ($\sim 9\%$),²⁰ post-MDS AML ($\sim 15\%$),¹⁹ post-MPN AML ($\sim 22\%$),⁸ post-MDS/MPN AML ($\sim 10\%$),²⁰ del(5q)-associated high-risk MDS or AML ($\sim 22\%$)²¹ and blast-phase chronic myelogenous leukemia ($\sim 4\%$).²² Single case reports also included angioimmunoblastic lymphoma²³ and acute lymphoblastic leukemia.¹⁸

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^{17,28,29} and better prognosis in *FLT3*⁺ AML.²⁷ In some¹⁷ but not other³⁰ studies, the mutant *IDH2* was associated with unfavorable prognosis in cytogenetically normal AML,¹⁷ whereas a more recent study suggested that the mutant *IDH2*R140 was prognostically more favorable than the mutant *IDH2*R172.³¹

Unlike the case with AML, there is limited information on the prognostic impact of *IDH* mutations in chronic myeloid neoplasms, including MDS¹⁹ 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

Correspondence: Professor A Tefferi, Division of Hematology, Department of Medicine, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905, USA.

E-mail: tefferi.ayalew@mayo.edu

Received 6 July 2011; accepted 12 July 2011; published online 13 September 2011

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.^{33,34} 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'-CGGTCTTCAGAGAAGCCATT-3' and anti-sense, 5'-CACATTATTGCCAACTGAC-3'.¹⁸ *IDH2* exon 4 was amplified using sense, 5'-CCACTATTATCTGTCTCCTC-3' and anti-sense, 5'-GCTAGGCGAGGAGCTCCAGT-3'.¹⁹ Both reactions were performed in 25 μ l volume containing 100 ng of DNA, 0.25 Units Taq polymerase, 0.3 mM each of dATP, dCTP, dGTP and dTTP, 5 μ l of a 10 \times PCR Buffer (Roche Diagnostics, Indianapolis, IN, USA) and 0.2 μ M each of sense and anti-sense primers. The reaction was denatured at 94 $^{\circ}$ C for 3 min, followed by 35 cycles of denaturing at 94 $^{\circ}$ C for 30 s, annealing at 57 $^{\circ}$ C for 30 s and extension at 72 $^{\circ}$ C for 40 s. After a final extension at 72 $^{\circ}$ 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'-GCTGAAGAAGATGTGGAA-3' and anti-sense, 5'-TGATGGGCTCCCGGAAGA-3'; R172 sense, 5'-CCAAGCCCATCACCATTG-3' and anti-sense, 5'-CCCAGGTCAGTGGATCCC-3'.

All statistical analyses considered clinical and laboratory parameters obtained at the time of first referral to the Mayo Clinic, 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 χ^2 -test. Overall survival (OS) was calculated from the date of first referral to the date of death (uncensored) or last contact (censored). Leukemia-free survival (LFS) was calculated from the date of first referral to the date of leukemic transformation (uncensored) or death/last contact (censored). 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. *JAK2*V617F was detected in 169 (56%) patients. Six patients displayed both *JAK2*V617F and *IDH* mutations (*IDH2*R140Q in two patients, *IDH2*R140W in one and *IDH1*R132S in three); *JAK2*V617F allele burdens in these six patients with concomitant mutant *IDH* were 1, 7, 22, 27, 30 and 96%, respectively. One patient displayed both *IDH*R140Q and *MPL*W515R. In all, 107 (36%) patients were negative for all three mutations (that is, *JAK2*V617F, *MPL* exon 10 and *IDH1/2*).

The 12 *IDH*-mutated patients, with or without concomitant *JAK2*V617F, 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 *JAK2*V617F (*P* = 0.01), but not than those with *MPL* mutations (*P* = 0.41). After accounting for age, the OS difference between patients with *JAK2*V617F 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-plus³³ 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 \times 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

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 (n = 301)	IDH mutated (n = 12)	MPL mutated (n = 18)	JAK2 unmutated (n = 164)	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%)	77 (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
<i>DIPSS-plus risk group (%)</i>						
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 × 10 ⁹ /l; n (%)	52 (17%)	2 (17%)	4 (22%)	33 (20%)	13 (12%)	0.36
Platelets < 100 × 10 ⁹ /l; n (%)	73 (24%)	3 (25%)	8 (44%)	35 (21%)	27 (25%)	0.19
Leukocytes < 4 × 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%)	28 (17%)	19 (18%)	0.96
<i>Cytogenetic categories</i>						
Normal	181 (60%)	9 (75%)	12 (67%)	92 (56%)	68 (64%)	
Favorable	89 (30%)	2 (17%)	6 (33%)	53 (32%)	28 (26%)	0.55
Unfavorable	31 (10%)	1 (8%)	0 (0%)	19 (12%)	11 (10%)	
Transplanted; n (%)	24 (8%)	0 (0%)	2 (11%)	10 (6%)	13 (12%)	0.31
Deaths; n (%)	192 (64%)	11 (92%)	12 (67%)	109 (66%)	58 (54%)	0.04
Leukemic transformations; n (%)	36 (12%)	5 (42%)	1 (6%)	17 (11%)	13 (12%)	0.01

Abbreviation: DIPSS-plus, Dynamic International Prognostic Scoring System-Plus. Bold values indicate significant differences.

analysis disclosed that the negative OS (Figure 3) and LFS (Figure 4) effect of the mutant *IDH* was most pronounced in the presence ($P=0.0002$ and $P<0.0001$, respectively) as opposed to the absence ($P=0.34$ and $P=0.64$, respectively) of concomitant *JAK2V617F* expression. Analysis of paired samples obtained during the chronic and blast phases of the disease was possible in two *IDH*-mutated patients and showed the presence of both *IDH* and *JAK2* mutations at both time points.

Discussion

The most feared disease complication in MPN is leukemic transformation.³⁹ In PMF, risk factors for leukemic progression include unfavorable karyotype,³ thrombocytopenia (platelet count < 100 × 10⁹/l) and ≥ 3% circulating blasts,^{33,40} the 10-year incidence of AML was estimated at 12% in the absence of unfavorable karyotype and thrombocytopenia and 31% in the presence of either one of the two risk factors.³³ Prognosis in post-PMF AML is dismal with a median survival of < 3 months and is not favorably affected by conventional chemotherapy.³⁹ The discovery of *JAK2V617F* in the majority of patients with PMF raised hopes of better outcome with effective molecularly targeted therapy.⁴¹ However, it has since been realized that the presence or absence of *JAK2V617F* in PMF did not affect leukemic transformation³⁵ and that leukemic blasts in *JAK2*-mutated patients who develop AML did not necessarily express the mutation.⁴² 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);⁴³ 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.⁴³ 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.⁴⁴

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.⁴ On the other hand, the absence of mutual exclusivity

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 (28–81)	58 (14–79)	0.005
Age > 65 years; n (%)	70 (39%)	5 (50%)	1 (11%)	48 (50%)	16 (25%)	0.004
Males (%)	116 (65%)	7 (70%)	7 (80%)	63 (66%)	39 (62%)	0.79
Hemoglobin, g/dl; median (range)	10 (6–15)	11 (7–15)	10 (6–14)	10 (7–15)	11 (6–14)	0.50
Leukocyte count, $\times 10^9/l$; median (range)	8 (1–147)	10 (4–48)	11 (4–50)	9 (1–99)	7 (2–147)	0.15
Platelet count, $\times 10^9/l$; median (range)	253 (12–1493)	159 (79–410)	146 (31–662)	245 (12–984)	316 (14–1493)	0.13
<i>DIPSS-plus</i> risk group (%)						
Low	25 (14%)	0	2 (22%)	13 (14%)	10 (16%)	0.60
Intermediate-1	36 (20%)	2 (20%)	1 (11%)	15 (16%)	18 (29%)	
Intermediate-2	62 (35%)	4 (40%)	3 (33%)	35 (36%)	20 (32%)	
High	55(31%)	4 (40%)	3 (33%)	33 (34%)	15 (24%)	
Constitutional symptoms; n (%)	65 (37%)	4 (40%)	2 (22%)	38 (40%)	21 (33%)	0.68
Circulating blasts $\geq 1\%$; n (%)	101 (57%)	8 (80%)	5 (56%)	54 (56%)	34(53%)	0.49
Hemoglobin < 10g/dl; n (%)	77 (43%)	4 (40%)	6 (67%)	47 (49%)	20 (32%)	0.08
Leukocytes > $25 \times 10^9/l$; n (%)	26 (15%)	2 (20%)	2 (22%)	14 (15%)	8 (13%)	0.84
Platelets < $100 \times 10^9/l$; n (%)	36 (20%)	1 (10%)	4 (44%)	17 (18%)	14 (22%)	0.22
Leukocytes < $4 \times 10^9/l$; n (%)	27 (15%)	1 (10%)	1 (11%)	12 (13%)	13 (21%)	0.51
Palpable spleen > 10cm; 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
<i>Cytogenetic categories</i>						
Normal	116 (65%)	7 (70%)	6 (67%)	58 (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.

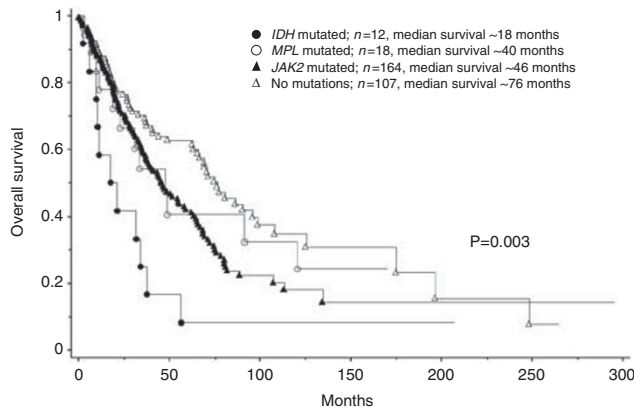


Figure 1 Overall survival data for 301 patients with primary myelofibrosis stratified by the presence or absence of *IDH*, *MPL* and *JAK2* mutations.

and the higher prevalence of some MPN-associated mutations (for example, *IDH*,⁶ *LNK*,⁷ *IKZF1*⁴⁵ and *TP53*⁴⁶ 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 *IDH*) in at-risk patients might simply constitute a marker of genomic instability associated with impending leukemic transformation.

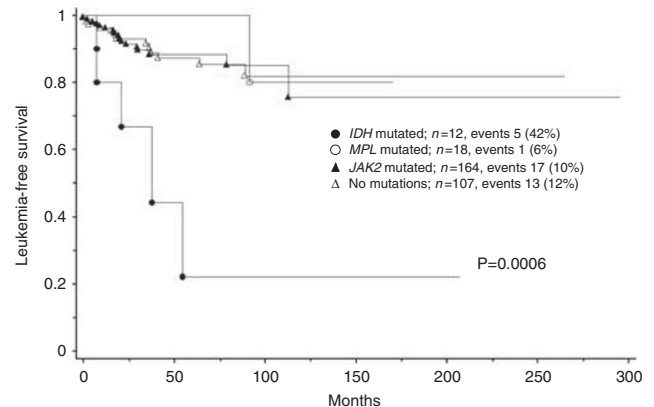


Figure 2 Leukemia-free survival data for 301 patients with primary myelofibrosis stratified by the presence or absence of *IDH*, *MPL* and *JAK2* mutations.

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.⁴⁷

The prognostic impact of *IDH* mutations in AML has been studied extensively.^{16,17,23–30} In contrast, very few studies have looked into this matter in chronic myeloid malignancies. Both *IDH1* and *IDH2* mutations occur in MDS, although some studies¹⁹ have reported a preponderance of *IDH1* mutations, whereas others have shown the opposite.²⁰ In the current PMF

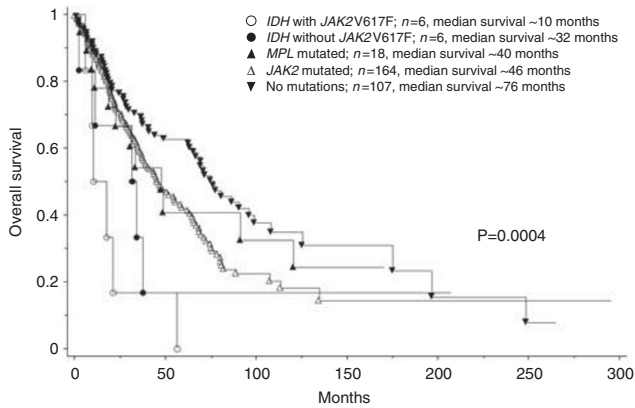


Figure 3 Overall survival data for 301 patients with primary myelofibrosis stratified by the presence or absence of *MPL*, *JAK2* or *IDH* mutations with or without concomitant *JAK2V617F* expression.

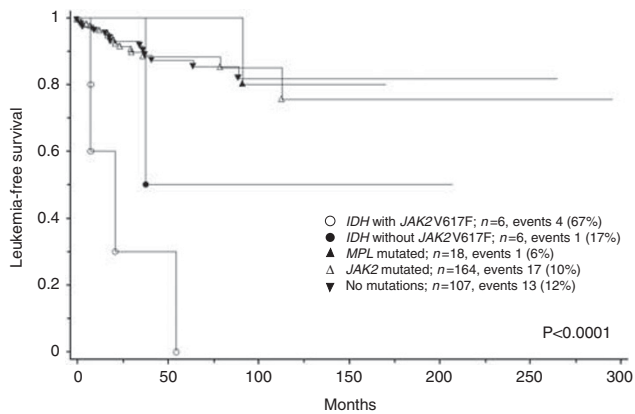


Figure 4 Leukemia-free survival data for 301 patients with primary myelofibrosis stratified by the presence or absence of *MPL*, *JAK2* or *IDH* mutations with or without concomitant *JAK2V617F* expression.

study, 7 of the 12 *IDH* mutations involved *IDH2*. In MDS and other myeloid neoplasms associated with sole del(5q), the presence of mutant *IDH* has been associated with inferior OS and LFS.^{19,21,43} It is noteworthy that the MDS study showing a detrimental prognostic effect of the mutant *IDH* involved only *IDH1* mutations.¹⁹ In this study, there was no evidence to suggest that *IDH1* and *IDH2* mutations were prognostically different (data not shown). Regardless, the number of cases with *IDH* mutations in this study ($n = 12$) was too small to accurately determine the individual prognostic contribution of *IDH1* vs *IDH2* mutations in PMF.

JAK2V617F in PMF and other MPN is associated with advanced age.⁴⁸ This study suggests that *IDH* mutations in PMF also cluster with older age. A similar observation has been made in AML as well²⁸ and underscores the importance of accounting for age in evaluating the prognostic significance of *IDH* mutations. Another characteristic feature of *IDH*-mutated PMF in this study was the relative paucity of abnormal or unfavorable karyotype. This particular observation has also been noted in the context of AML, MDS, MDS/MPN and post-MDS/MPN AML^{8,18,20} and suggests that *IDH* mutations are not simply markers of genomic instability.

Our clinical observations underscore the potential relevance of looking for other mutations or epigenetic abnormalities that functionally mimic the mutant *IDH*, in *JAK2*-mutated PMF.¹³

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.

Acknowledgements

This study is supported in part by grants from the ‘Myeloproliferative Disorders Foundation, Chicago, IL, USA’ and ‘The Henry J Predolin Foundation for Research in Leukemia, Mayo Clinic, Rochester, MN, USA’.

References

- Tefferi A, Vainchenker W. Myeloproliferative neoplasms: molecular pathophysiology, essential clinical understanding, and treatment strategies. *J Clin Oncol* 2011; **29**: 573–582.
- Mesa RA, Niblack J, Wadleigh M, Verstovsek S, Camoriano J, Barnes S et al. The burden of fatigue and quality of life in myeloproliferative disorders (MPDs): an international Internet-based survey of 1179 MPD patients. *Cancer* 2007; **109**: 68–76.
- Caramazza D, Begna KH, Gangat N, Vaidya R, Siragusa S, Van Dyke DL et al. Refined cytogenetic-risk categorization for overall and leukemia-free survival in primary myelofibrosis: a single center study of 433 patients. *Leukemia* 2011; **25**: 82–88.
- Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: *JAK2*, *MPL*, *TET2*, *ASXL1*, *CBL*, *IDH* and *IKZF1*. *Leukemia* 2010; **24**: 1128–1138.
- Abdel-Wahab O, Pardanani A, Rampal R, Lasho TL, Levine RL, Tefferi A. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. *Leukemia* 2011; **25**: 1219–1220.
- Pardanani A, Lasho TL, Finke CM, Mai M, McClure RF, Tefferi A. *IDH1* and *IDH2* mutation analysis in chronic- and blast-phase myeloproliferative neoplasms. *Leukemia* 2010; **24**: 1146–1151.
- Pardanani A, Lasho T, Finke C, Oh ST, Gotlib J, Tefferi A. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with *TET2*, *IDH*, *JAK2* or *MPL* mutations. *Leukemia* 2010; **24**: 1713–1718.
- Tefferi A, Lasho TL, Abdel-Wahab O, Guglielmelli P, Patel J, Caramazza D et al. *IDH1* and *IDH2* mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. *Leukemia* 2010; **24**: 1302–1309.
- Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P et al. Glioma-derived mutations in *IDH1* dominantly inhibit *IDH1* catalytic activity and induce HIF-1 α . *Science* 2009; **324**: 261–265.
- Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM et al. Cancer-associated *IDH1* mutations produce 2-hydroxyglutarate. *Nature* 2009; **462**: 739–744.
- Gross S, Cairns RA, Minden MD, Driggers EM, Bittinger MA, Jang HG et al. Cancer-associated metabolite 2-hydroxyglutarate accumulates in acute myelogenous leukemia with isocitrate dehydrogenase 1 and 2 mutations. *J Exp Med* 2010; **207**: 339–344.
- Ward PS, Patel J, Wise DR, Abdel-Wahab O, Bennett BD, Collier HA et al. The common feature of leukemia-associated *IDH1* and *IDH2* mutations is a neomorphic enzyme activity converting α -ketoglutarate to 2-hydroxyglutarate. *Cancer Cell* 2010; **17**: 225–234.
- Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A et al. Leukemic *IDH1* and *IDH2* mutations result in a hypermethylation phenotype, disrupt *TET2* function, and impair hematopoietic differentiation. *Cancer Cell* 2010; **18**: 553–567.

- 14 Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P *et al*. An integrated genomic analysis of human glioblastoma multiforme. *Science* 2008; **321**: 1807–1812.
- 15 Mardis ER, Ding L, Dooling DJ, Larson DE, McLellan MD, Chen K *et al*. Recurring mutations found by sequencing an acute myeloid leukemia genome. *N Engl J Med* 2009; **361**: 1058–1066.
- 16 Chou WC, Lei WC, Ko BS, Hou HA, Chen CY, Tang JL *et al*. The prognostic impact and stability of isocitrate dehydrogenase 2 mutation in adult patients with acute myeloid leukemia. *Leukemia* 2011; **25**: 246–253.
- 17 Boissel N, Nibourel O, Renneville A, Gardin C, Reman O, Contentin N *et al*. Prognostic impact of isocitrate dehydrogenase enzyme isoforms 1 and 2 mutations in acute myeloid leukemia: a study by the Acute Leukemia French Association group. *J Clin Oncol* 2010; **28**: 3717–3723.
- 18 Abbas S, Lugthart S, Kavelaars FG, Schelen A, Koenders JE, Zeilemaker A *et al*. Acquired mutations in the genes encoding IDH1 and IDH2 both are recurrent aberrations in acute myeloid leukemia: prevalence and prognostic value. *Blood* 2010; **116**: 2122–2126.
- 19 Thol F, Weissinger EM, Krauter J, Wagner K, Damm F, Wichmann M *et al*. IDH1 mutations in patients with myelodysplastic syndromes are associated with an unfavorable prognosis. *Haematologica* 2010; **95**: 1668–1674.
- 20 Kosmider O, Gelsi-Boyer V, Slama L, Dreyfus F, Beyne-Rauzy O, Quesnel B *et al*. Mutations of IDH1 and IDH2 genes in early and accelerated phases of myelodysplastic syndromes and MDS/myeloproliferative neoplasms. *Leukemia* 2010; **24**: 1094–1096.
- 21 Pardanani A, Patnaik MM, Lasho TL, Mai M, Knudson RA, Finke C *et al*. Recurrent IDH mutations in high-risk myelodysplastic syndrome or acute myeloid leukemia with isolated del(5q). *Leukemia* 2010; **24**: 1370–1372.
- 22 Soverini S, Score J, Iacobucci I, Poerio A, Lonetti A, Gnani A *et al*. IDH2 somatic mutations in chronic myeloid leukemia patients in blast crisis. *Leukemia* 2011; **25**: 178–181.
- 23 Caramazza D, Lasho TL, Finke CM, Gangat N, Dingli D, Knudson RA *et al*. IDH mutations and trisomy 8 in myelodysplastic syndromes and acute myeloid leukemia. *Leukemia* 2010; **24**: 2120–2122.
- 24 Thol F, Damm F, Wagner K, Gohring G, Schlegelberger B, Hoelzer D *et al*. Prognostic impact of IDH2 mutations in cytogenetically normal acute myeloid leukemia. *Blood* 2010; **116**: 614–616.
- 25 Chou WC, Lei WC, Ko BS, Hou HA, Chen CY, Tang JL *et al*. The prognostic impact and stability of isocitrate dehydrogenase 2 mutation in adult patients with acute myeloid leukemia. *Leukemia* 2011; **25**: 246–253.
- 26 Schnittger S, Haferlach C, Ulke M, Alpermann T, Kern W, Haferlach T. IDH1 mutations are detected in 6.6% of 1414 AML patients and are associated with intermediate risk karyotype and unfavorable prognosis in adults younger than 60 years and unmutated NPM1 status. *Blood* 2010; **116**: 5486–5496.
- 27 Green CL, Evans CM, Hills RK, Burnett AK, Linch DC, Gale RE. The prognostic significance of IDH1 mutations in younger adult patients with acute myeloid leukemia is dependent on FLT3/ITD status. *Blood* 2010; **116**: 2779–2782.
- 28 Paschka P, Schlenk RF, Gaidzik VI, Habdank M, Kronke J, Bullinger L *et al*. IDH1 and IDH2 mutations are frequent genetic alterations in acute myeloid leukemia and confer adverse prognosis in cytogenetically normal acute myeloid leukemia with NPM1 mutation without FLT3 internal tandem duplication. *J Clin Oncol* 2010; **28**: 3636–3643.
- 29 Marcucci G, Maharry K, Wu YZ, Radmacher MD, Mrozek K, Margeson D *et al*. IDH1 and IDH2 gene mutations identify novel molecular subsets within *de novo* cytogenetically normal acute myeloid leukemia: a Cancer and Leukemia Group B study. *J Clin Oncol* 2010; **28**: 2348–2355.
- 30 Wagner K, Damm F, Gohring G, Gorlich K, Heuser M, Schafer I *et al*. Impact of IDH1 R132 mutations and an IDH1 single nucleotide polymorphism in cytogenetically normal acute myeloid leukemia: SNP rs11554137 is an adverse prognostic factor. *J Clin Oncol* 2010; **28**: 2356–2364.
- 31 Green CL, Evans CM, Zhao L, Hills RK, Burnett AK, Linch DC *et al*. The prognostic significance of IDH2 mutations depends on the location of the mutation. *Blood* 2011; **118**: 409–412.
- 32 Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A *et al*. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. *Blood* 2009; **114**: 937–951.
- 33 Gangat N, Caramazza D, Vaidya R, George G, Begna K, Schwager S *et al*. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. *J Clin Oncol* 2011; **29**: 392–397.
- 34 Passamonti F, Cervantes F, Vannucchi AM, Morra E, Rumi E, Pereira A *et al*. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood* 2010; **115**: 1703–1708.
- 35 Tefferi A, Lasho TL, Huang J, Finke C, Mesa RA, Li CY *et al*. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. *Leukemia* 2008; **22**: 756–761.
- 36 Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M *et al*. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood* 2006; **108**: 3472–3476.
- 37 Tefferi A, Levine RL, Lim KH, Abdel-Wahab O, Lasho TL, Patel J *et al*. Frequent TET2 mutations in systemic mastocytosis: clinical, KITD816V and FIP1L1-PDGFRα correlates. *Leukemia* 2009; **23**: 900–904.
- 38 Tefferi A, Lasho TL, Patnaik MM, Finke CM, Hussein K, Hogan WJ *et al*. JAK2 germline genetic variation affects disease susceptibility in primary myelofibrosis regardless of V617F mutational status: nullizygosity for the JAK2 46/1 haplotype is associated with inferior survival. *Leukemia* 2010; **24**: 105–109.
- 39 Mesa RA, Li CY, Ketterling RP, Schroeder GS, Knudson RA, Tefferi A. Leukemic transformation in myelofibrosis with myeloid metaplasia: a single-institution experience with 91 cases. *Blood* 2005; **105**: 973–977.
- 40 Huang J, Li CY, Mesa RA, Wu W, Hanson CA, Pardanani A *et al*. Risk factors for leukemic transformation in patients with primary myelofibrosis. *Cancer* 2008; **112**: 2726–2732.
- 41 James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C *et al*. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature* 2005; **434**: 1144–1148.
- 42 Theocharides A, Boissinot M, Girodon F, Garand R, Teo SS, Lippert E *et al*. Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. *Blood* 2007; **110**: 375–379.
- 43 Patnaik MM, Lasho TL, Finke CM, Knudson RA, Ketterling RP, Chen D *et al*. Isolated del(5q) in myeloid malignancies: clinicopathologic and molecular features in 143 consecutive patients. *Am J Hematol* 2011; **86**: 393–398.
- 44 Shibata T, Kokubu A, Miyamoto M, Sasajima Y, Yamazaki N. Mutant IDH1 confers an *in vivo* growth in a melanoma cell line with BRAF mutation. *Am J Pathol* 2011; **178**: 1395–1402.
- 45 Jager R, Gisslinger H, Passamonti F, Rumi E, Berg T, Gisslinger B *et al*. Deletions of the transcription factor Ikaros in myeloproliferative neoplasms. *Leukemia* 2010; **24**: 1290–1298.
- 46 Harutyunyan A, Klampfl T, Cazzola M, Kralovics R. p53 lesions in leukemic transformation. *N Engl J Med* 2011; **364**: 488–490.
- 47 Green A, Beer P. Somatic mutations of IDH1 and IDH2 in the leukemic transformation of myeloproliferative neoplasms. *N Engl J Med* 2010; **362**: 369–370.
- 48 Vannucchi AM, Antonioli E, Guglielmelli P, Pardanani A, Tefferi A. Clinical correlates of JAK2V617F presence or allele burden in myeloproliferative neoplasms: a critical reappraisal. *Leukemia* 2008; **22**: 1299–1307.



This work is licensed under the Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>