IDH1 and IDH2 Mutation Studies in 1473 Patients with Chronic-, Fibrotic- or Blast-Phase Essential Thrombocytethemia, Polycythemia Vera or Myelofibrosis

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Accessibility
In a multi-institutional collaborative project, 1473 patients with myeloproliferative neoplasms (MPN) were screened for isocitrate dehydrogenase 1 (IDH1)/IDH2 mutations: 594 essential thrombocytopenia (ET), 421 polycythemia vera (PV), 312 primary myelofibrosis (PMF), 95 post-PV/ET MF and 51 blast-phase MPN. A total of 38 IDH mutations (18 IDH1-R132, 19 IDH2-R140 and 1 IDH2-R172) were detected: 5 (0.8%) ET, 8 (1.9%) PV, 13 (4.2%) PMF, 1 (1%) post-PV/ET MF and 11 (21.6%) blast-phase MPN (P < 0.01). Mutant IDH was documented in the presence or absence of JAK2, MPL and TET2 mutations, with similar mutational frequencies. However, IDH-mutated patients were more likely to be nullizygous for JAK2 46/1 haplotype, especially in PMF (P = 0.04), and less likely to display complex karyotypic changes, in blast-phase disease (P = 0.01). In chronic-phase PMF, JAK2 46/1 haplotype nullizygosity (P = 0.01; hazard ratio (HR) 2.9, 95% confidence interval (CI) 1.7–5.2), but not IDH mutational status (P = 0.55; HR 1.3, 95% CI 0.5–3.4), had an adverse effect on survival. This was confirmed by multivariable analysis. In contrast, in both blast-phase PMF (P = 0.04) and blast-phase MPN (P = 0.01), the presence of an IDH mutation predicted worse survival. The current study clarifies disease- and stage-specific IDH mutation incidence and prognostic relevance in MPN and provides additional evidence for the biological effect of distinct JAK2 haplotypes.

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Introduction

Despite the seminal discovery of JAK2 or MPL mutations in the majority of patients with BCR-ABL1-negative myeloproliferative neoplasms (MPN), it is becoming increasingly evident that these mutations do not signify either disease-initiating or leukemia-promoting events. It is therefore important to keep looking for additional molecular alterations to clarify the genetic underpinnings of both chronic- and blast-phase MPN. In the last 2 years, mutations involving TET2, ASXL1 and CBL have been described in some patients with BCR-ABL1-negative MPN, including polycythemia vera (PV), essential thrombocytopenia (ET) and primary myelofibrosis (PMF). The precise pathogenetic contribution of these mutations and their clinical relevance are currently under investigation. The glioma-associated isocitrate dehydrogenase 1 (IDH1) and IDH2 mutations are the latest to be added to the ‘MPN mutations list’.

IDH1, located on chromosome 2q33.3, and IDH2, located on chromosome 15q26.1, encode enzymes that catalyze oxidative decarboxylation of isocitrate to α-ketoglutarate. IDH1 (cytoplasm and peroxisome) and IDH2 (mitochondria) use NADPH+ as a co-factor to generate NADPH, which is important in the production of intracellular glutathione. Intact IDH activity is therefore necessary for cellular protection from oxidative stress. Mutant IDH has decreased affinity to isocitrate, but displays neomorphic catalytic activity toward α-ketoglutarate, the net result being decreased supply of α-ketoglutarate and accumulation of 2-hydroxyglutarate. It is currently believed that these intracellular changes facilitate oncogenic pathways including activation of HIF-1α.

IDH1 and IDH2 mutations were first described in low-grade gliomas/secondary glioblastomas and subsequently in acute myeloid leukemia (AML), with respective mutational frequencies of ~70 and 8%. We recently screened 200 patients with either chronic- or blast-phase MPN for IDH1 mutations, and identified 9 patients with either IDH1 (n = 5) or IDH2 (n = 4) mutations. Mutational frequencies were ~21% for blast-phase MPN and ~4% for PMF. In the current study, we expanded our study cohort to include 1473 patients recruited from three MPN centers of excellence, with the intent to accurately describe the prevalence of IDH mutations in chronic-, fibrotic- and blast-phase PV, ET and PMF. In addition, IDH-mutated patients were analyzed for their cytogenetic and molecular (that is, JAK2, MPL and TET2 mutation and JAK2 haplotype status) phenotype, as well as their prognostic relevance.

Materials and methods

This study was approved by the Mayo Clinic institutional review board. All patients provided authorization for use of their medical records for research purposes, and the research was carried out according to the principles of the Declaration of Helsinki. Patient samples were obtained from the Mayo Clinic, Harvard Medical Institute and University of Florence. Mutational analyses were performed on DNA derived from either bone marrow or peripheral blood granulocytes. JAK2 46/1 haplotype analysis on patient samples accrued from Harvard was performed on germline DNA. Diagnoses of MPN, post-PV/ET MF
and AML, in patient samples accrued from the Mayo Clinic and the University of Florence, were according to the World Health Organization and International Working Group criteria. Diagnoses in patients accrued from Harvard were self-reported during an internet-based collection of samples, as previously detailed.

DNA from either bone marrow (Mayo Clinic samples) or granulocytes (samples from Harvard and the University of Florence) was extracted using conventional methods. MPL, JAK2 and TET2 mutation and JAK2 haplotype analyses were performed according to previously published methods. With regard to IDH mutation analysis, Harvard patient samples were analyzed using the following primers for IDH1, which cover amino acid residues 41–138: sense, 5'-TGTGTTGAGATGACGCCTA-3' and anti-sense, 5'-GGTGATCTACAGGCTTCGC-3'. Sequencing of IDH2 used primers that covered amino acid residues 125–226: sense, 5'-CTGCTCTTGTGAGCCCTAG-3' and anti-sense, 5'-ATTCTGTGTTAAGATGGGCGC-3'. Sequence analysis was performed using Mutation Surveyor (SoftGenetics, State College, PA, USA) and all mutations were validated by repeat PCR and sequencing on unamplified DNA from the archival sample.

Mayo Clinic and University of Florence patient samples were screened for IDH1 and IDH2 mutations by direct sequencing.

### Table 1

| MPN center | Diagnosis | N  | Median age in years (range) | Males (%) | JAK2 mutation frequency | MPL mutation frequency | TET2 mutation frequency | JAK2 46/1 nullizygous frequency |
|------------|-----------|----|-----------------------------|-----------|------------------------|------------------------|-------------------------|---------------------------------|
| Florence   | PV        | 150| 62 (16–91)                  | 66        | 83% (123/149)          | NA                     | NA                      | 6% (1/18)                      |
|            | ET        | 199| 56 (13–93)                  | 37        | 63% (124/198)          | 2.2% (4/184)           | NA                      | 52% (25/48)                    |
|            | PMF       | 107| 63 (16–90)                  | 67        | 65% (69/106)           | 4% (4/98)              | NA                      | 39% (37/96)                    |
|            | Post-PV MF| 32 | 62 (48–78)                  | 47        | 100% (32/32)          | 0% (0/28)              | NA                      | 0% (0/16)                      |
|            | Post-ET MF| 26 | 63 (33–82)                  | 50        | 39% (10/26)           | 13% (3/24)             | NA                      | 27% (3/11)                     |
|            | Post-PV AML| 5  | 66                          | 0         | 100% (1/1)           | 0% (0/1)               | NA                      | NA                              |
|            | Post-ET AML| 2 | 65–70                       | 0         | 50% (1/2)            | 0% (0/1)               | NA                      | NA                              |
|            | Post-PMF AML| 5 | 73 (67–83)                  | 80        | 20% (1/5)            | 0% (0/5)               | NA                      | 20% (1/5)                      |
| Harvard    | PV        | 159| 59 (32–85)                  | 48        | 93% (139/150)         | 0% (0/159)             | 9.4% (15/159)           | 23% (29/125)                   |
|            | ET        | 124| 57 (31–84)                  | 26        | 31% (35/114)          | 3.2% (4/124)           | 8% (10/124)             | 42% (41/98)                    |
|            | PMF       | 39 | 64 (50–70)                  | 49        | 42% (16/38)           | 5.1% (2/39)            | 7.7% (3/39)             | 22% (5/23)                     |
| Mayo       | PV        | 112| 66 (21–95)                  | 48        | 95% (106/112)         | 1.8% (1/56)            | 15.7% (14/89)           | 25% (25/99)                    |
|            | ET        | 271| 63 (15–87)                  | 38        | 49% (122/271)         | 4.9% (7/143)           | 5.7% (3/53)             | 34% (91/266)                   |
|            | PMF       | 166| 62 (35–52)                  | 67        | 57% (95/166)          | 10% (11/108)           | 18% (10/57)             | 35% (55/158)                   |
|            | Post-PV MF| 22 | 65 (47–75)                  | 64        | 100% (22/22)          | 7.7% (1/13)            | 7.7% (1/13)             | 5% (1/20)                      |
|            | Post-ET MF| 15 | 63 (39–76)                  | 80        | 47% (7/15)           | 10% (1/10)             | 12.5% (1/8)            | 31% (4/13)                     |
|            | Post-PV AML| 11| 64 (48–57)                  | 64        | 100% (11/11)         | 0% (0/7)               | 20% (1/5)              | 36% (4/11)                     |
|            | Post-ET AML| 5 | 64 (50–75)                  | 60        | 60% (3/5)            | 0% (0/5)               | 25% (1/4)             | 20% (1/5)                      |
|            | Post-PMF AML| 27| 66 (49–83)                  | 74        | 48% (13/27)          | 9% (2/22)              | 0% (0/7)               | 35% (8/23)                     |

Abbreviation: NA, not done or not available.

*Includes JAK2 exon 12 mutations: two cases from Mayo clinic and one case from Harvard.
and/or high-resolution melting assay. Direct sequencing for IDH1 exon 4 mutations was carried out using the following primer sequences: sense, 5'-CGGTCTCAGAAGGACCATT-3' and anti-sense, 5'-ACATTATTGCGCAAATGAC-3'.

IDH2 exon 4 was amplified using sense, 5'-CCACTATATCTCTGCTC-3' and anti-sense, 5'-GCTAGGCAGGAGCTCCAGT-3'.

Both reactions were performed in 25 µl volume containing 100 ng of DNA, 0.25 U Taq polymerase, 0.3 mM each of dATP, dCTP, dGTP and dTTP, 5 µM of 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 30s, annealing at 57°C for 30s and extension at 72°C for 40s. After a final extension at 72°C for 2 min, the products were confirmed by running on 1.3% agarose gel and purified using Qiagen’s PCR Quick Purification Kit. 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'-CCTGAAAGAAGTGTGAAA-3' and anti-sense, 5'-TGATGG GCTCCCGAAGA-3'; R172 sense, 5'-CCAAGCCCATCACCAT TG-3' and anti-sense, 5'-CCACAGTCCAGGATCCC-3' (Figure 1).

Conventional statistical procedures were used (SAS Institute, Cary, NC, USA). All statistically analyzed data were obtained at time of IDH1 mutation analysis. All P-values were two-tailed and statistical significance was set at the level of P<0.05. Categorical variables were described as count and relative frequency and compared by χ² statistics. Comparison of continuous variables between categories was performed by the Mann–Whitney U-test. Survival analysis was performed by the Kaplan–Meier method taking the interval from the date of diagnosis, for chronic-phase disease, or from the date of leukemic transformation, for blast-phase disease, to death or last contact. The log-rank test was used to compare survival data. Cox regression model was used for multivariable analysis.

**Results**

**Disease- and stage-specific IDH mutational frequencies**

A total of 1473 patients with BCR–ABL1-negative MPN were recruited from the Mayo Clinic, Rochester, MN, USA (n = 629), University of Florence, Florence, Italy (n = 532) and Harvard Medical Institute, Boston, Massachusetts, USA (n = 322). Specific diagnoses included ET (n = 594), PV (n = 421), PMF (n = 312), post-PV MF (n = 54), post-ET MF (n = 41), post-PV AML (n = 12), post-ET AML (n = 7) and post-PV AML (n = 32).

Table 1 provides clinical and laboratory details of the study population including age and sex distribution, specific diagnoses and JAK2, MPL and TET2 mutational and JAK2 46/1 haplotype status, stratified by center of patient recruitment. A total of 38 IDH mutations were documented (Table 2): 18 involved IDH1 (10 R132S, 7 R132C and 1 R132G) and 20 IDH2 (18 R140Q, 1 R140W and 1 R172G). IDH mutations were infrequent in chronic- or fibrotic-phase disease and significantly more prevalent in blast-phase disease (P<0.01; Table 3): 5 (0.8%) in ET, 8 (1.9%) in PV, 13 (4.1%) in PMF, 1 (1%) in post-ET/PV MF, none in blast-phase ET, 3 (25%) in blast-phase PV and 8 (25%) in blast-phase PMF.

**Correlation of IDH mutations with other MPN-associated mutations and JAK2 46/1 haplotype**

Considering the preponderance of informative cases with centrally confirmed diagnosis and availability of a more complete laboratory data, the current analysis was limited to patients from the Mayo Clinic cohort (n = 629). IDH mutational frequencies were similar among JAK2- (3.6%), MPL- (4.3%) and TET2 (3.2%)-mutated patients and their respective mutation-negative counterparts (4.2, 5.3 and 6.3%; Table 3). In other words, mutant IDH was shown to co-occur with a JAK2, MPL or TET2 mutation, and mutational frequency did not appear to be influenced by either the type of the coexisting mutation (P=0.96) or the presence or absence of each specific mutation (Table 3). However, IDH-mutated cases were more likely to be nullizygous for JAK2 46/1 haplotype, especially when analysis was restricted to informative (that is, with JAK2 46/1 haplotype information) patients with chronic- (n = 158) or blast (n = 23)-phase PMF, analyzed together (P = 0.007) or separately (P = 0.04; Table 4).

**Clinical correlates and prognostic relevance**

To avoid disease- or stage-specific confounding factors, as well as assure adequate sample size of informative cases, clinical correlates and prognostic studies were limited to PMF. In this patient cohort, detailed clinical information was available in 111 patients with chronic-phase PMF (including 7 IDH-mutated cases) and 27 patients with blast-phase PMF (including 8 IDH-mutated cases), both patient populations were accrued from the Mayo Clinic. In both chronic- and blast-phase PMF, the presence of IDH mutations was not influenced by either age (P = 0.51 and 0.70, respectively) or gender (P = 0.09 and 0.3, respectively). In chronic-phase disease, comparison of prognostically relevant disease variables at diagnosis revealed that cytogenetic findings in IDH-mutated cases often belonged to a low- or intermediate-risk category, although the difference was not statistically significant (Table 4). Similarly, IDH-mutated blast-phase PMF was less likely to display complex karyotype (0 vs 64% in IDH-unmutated cases; P = 0.001).

In addition to biological implications, the aforementioned associations of IDH mutations with favorable cytogenetic profile and JAK2 46/1 haplotype nullizogosity, both which have previously been shown to be prognostically relevant, mandated their inclusion as covariates during multivariable survival analysis. In chronic-phase PMF, univariate analysis showed statistically significant adverse survival effect from JAK2 46/1 haplotype nullizogosity (P = 0.0001; 34 nullizygous vs 74 not nullizygous), high-risk karyotype (P < 0.0001; 13 high-risk vs 98 not high-risk) and higher International Prognostic Scoring System (IPSS; 27 high, 29 intermediate-2, 30 intermediate-1 and 25 low-risk patients) risk score (P < 0.0001), but not from IDH mutational status (P = 0.54; 7 mutated vs 104 unmutated; Figure 2). Multivariable analysis confirmed the independent prognostic value of JAK2 46/1 haplotype status (hazard ratio (HR) 2.2, 95% confidence interval (CI) 1.2–4.2), karyotype (HR 2.8, 95% CI 1.3–5.9) and IPSS risk score (HR 4.8, 95% CI 2.0–11.5).

In blast-phase PMF, despite its association with noncomplex karyotype, the presence of mutant IDH predicted shortened survival, calculated from the time of disease transformation (P = 0.04), and there was a similar trend for JAK2 non-46/1 haplotype (P = 0.14; Figure 3). Significance was lost for both during multivariable analysis, probably because of small sample size. IDH mutation status also predicted worse survival when the analysis included all blast-phase MPN cases from the Mayo

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Leukemia
| Specific diagnosis | Age (years) and sex | IDH mutation variant | JAK2 V617F burden | MPL mutation status | TET2 mutation status | JAK2 haplotype status | Karyotype | Antecedent MPN diagnosis to IDH analysis | IDH analysis to last f/u | Status at last f/u |
|-------------------|---------------------|----------------------|-------------------|-------------------|-------------------|----------------------|----------|----------------------------------------|------------------------|-----------------|
| 1 ET (Mayo)       | 26/F                | IDH2 R140Q           | 0%                | WT                | WT                | Heterozygous         | NN       | 0 month                               | 6 years                | Alive with ET   |
| 2 ET (Mayo)       | 38/F                | IDH2 R140Q           | 5%                | WT                | WT                | Heterozygous         | NA       | 5 years                               | NA                    | Lost to follow-up|
| 3 ET (Florence)   | 80/F                | IDH2 R140Q           | 73%               | WT                | NA                | NA                   | NA       | 1.1 years                             | 4 months               | Alive with ET   |
| 4 ET (Florence)   | 79/M                | IDH2 R140Q           | 64%               | WT                | NA                | Nullizygous          | NA       | 7 months                              | 2 years                | Alive with ET   |
| 5 ET (Florence)   | 65/F                | IDH1 R132C           | 0%                | NA                | NA                | NA                   | NA       | 7.2 years                             | 1 month                | Alive with ET   |
| 6 PV (Harvard)    | 52/F                | IDH2 R140Q           | 58%               | WT                | WT                | Heterozygous         | NA       | 0 month                               | 0 month                | Alive with PV   |
| 7 PV (Harvard)    | 47/M                | IDH2 R140Q           | 90%               | WT                | WT                | Heterozygous         | NA       | 0 month                               | 0 month                | Alive with PV   |
| 8 PV (Florence)   | 79/F                | IDH1 R132S           | 50%               | NA                | ND                | Heterozygous         | NA       | 4 years                               | 5 months               | Dead with AML   |
| 9 PV (Florence)   | 49/M                | IDH2 R140Q           | 25%               | NA                | NA                | NA                   | NA       | 1 month                               | 0 month                | Alive with PV   |
| 10 PV (Mayo)      | 82/M                | IDH1 R132C           | 1%                | WT                | WT                | Heterozygous         | NA       | 4 years                               | 5 months               | Alive with PV   |
| 11 PV (Mayo)      | 50/M                | IDH2 R140Q           | 25%               | WT                | WT                | Heterozygous         | NA       | 4 months                              | 6 months               | Alive with PV   |
| 12 PV (Mayo)      | 75/M                | IDH2 R140Q           | 11%               | WT                | WT                | Heterozygous         | NA       | 0 month                               | 1 month                | Alive with PV   |
| 13 PV (Mayo)      | 82/F                | IDH2 R140Q           | 51%               | WT                | WT                | Heterozygous         | NA       | 29 months                             | 3.4 years              | Dead with PV   |
| 14 PMF (Harvard)  | 76/M                | IDH2 R140Q           | 72%               | WT                | WT                | Heterozygous         | NA       | 0 month                               | 6 years                | Alive with PMF  |
| 15 PMF (Florence) | 57/M                | IDH1 R132S           | 65%               | WT                | ND                | Nullizygous          | NA       | 0 month                               | 6 months               | Alive with PMF  |
| 16 PMF (Florence) | 80/M                | IDH1 R132S           | 50%               | WT                | ND                | Nullizygous          | NA       | 1 year                                | 1 year                 | Alive with PMF  |
| 17 PMF (Florence) | 62/M                | IDH1 R132G           | 10%               | WT                | ND                | Nullizygous          | NA       | 2 months                              | 4 months               | Alive with PMF  |
| 18 PMF (Florence) | 72/M                | IDH2 R140Q           | 70%               | WT                | NA                | Heterozygous         | NA       | 0 month                               | 1.8 years              | Alive with PMF  |
| 19 PMF (Florence) | 50/M                | IDH1 R132S           | 54%               | WT                | NA                | Heterozygous         | NA       | 0 month                               | 5.2 years              | Alive with PMF  |
| 20 PMF (Mayo)     | 74/M                | IDH1 R132S           | 0%                | WT                | Muttered Nullizygous | Nullizygous         | NA       | 0 month                               | 2.8 years              | Dead with PMF   |
| 21 PMF (Mayo)     | 69/M                | IDH2 R140Q           | 22%               | WT                | NA                | Nullizygous          | NN       | 4 months                              | 11 months              | Dead with AML   |
| 22 PMF (Mayo)     | 73/M                | IDH1 R132S           | 26%               | WT                | Nullizygous        | Nullizygous          | NN       | 1 month                               | 6 months               | Dead from unknown cause |
| 23 PMF (Mayo)     | 69/M                | IDH2 R140Q           | 0%                | WT                | WT                | Nullizygous          | NN       | 2 months                              | 3 months               | Dead from unknown cause |
| 24 PMF (Mayo)     | 58/F                | IDH1 R132C           | 0%                | WT                | NA                | Nullizygous          | NN       | 3 years                               | 3.2 years              | Dead with AML   |
| 25 PMF (Mayo)     | 53/M                | IDH2 R140Q           | 0%                | WT                | WT                | Heterozygous         | NN       | 0 month                               | 6 years                | Alive with PMF  |
| 26 PMF (Mayo)     | 50/F                | IDH2 R172G           | 0%                | WT                | WT                | Heterozygous         | +9       | 1 year                                | 5 years                | Alive with PMF  |
| 27 Post-PV MF (Florence) | 56/F | IDH2 R140Q | 84% | WT | NA | Heterozygous | NN | 0 month | 6 years | Alive with PMF |
| 28 Post-PV MF (Florence) | 62/M | IDH2 R140W | 65% | WT | NA | Heterozygous | Nullizygous | +21 | 8 months | 2 months | Dead with AML |
| 29 Post-PV MF (Florence) | 64/M | IDH1 R132C | 0% | WT | WT | Heterozygous | 15q | 5.8 years | 4 months | Dead with AML |
| 30 Post-PV MF (Florence) | 73/M | IDH1 R132C | 0% | WT | WT | Heterozygous | Nullizygous | 0 | 2 years | 2.5 months | Dead with AML |
| 31 Post-PV MF (Florence) | 61/M | IDH2 R140Q | 0% | WT | NA | Nullizygous | -7,20q- | 5 months | 5 months | Dead with AML |
| 32 Post-PV MF (Florence) | 66/M | IDH1 R132S | 96% | WT | WT | Heterozygous | +2 | 4.5 years | 1 month | Dead with AML |
| 33 Post-PV MF (Florence) | 56/F | IDH2 R140Q | 84% | WT | NA | Heterozygous | Nullizygous | +9 | 2 months | <1 month | Dead with AML |
| 34 Post-PV MF (Florence) | 82/F | IDH1 R132S | 1% | WT | NA | Heterozygous | Nullizygous | 0 | 2 months | 1 month | Dead with AML |
| 35 Post-PV MF (Florence) | 80/F | IDH1 R132S | 1% | WT | NA | Heterozygous | Nullizygous | 0 | 2.2 years | 1 month | Dead with AML |
| 36 Post-PV MF (Florence) | 81/M | IDH1 R132C | 0% | MPLW515L | WT | Nullizygous | 15q | 2 years | 2 months | Dead with AML |
| 37 Post-PV MF (Florence) | 82/M | IDH1 R132C | 7% | WT | WT | Heterozygous | -5 and * | NA | 1.5 months | Dead with AML |
| 38 Post-PV MF (Florence) | 65/M | IDH1 R132S | 80% | WT | NA | Heterozygous | der(1;7),+8 | 16 years | 8 months | Dead with AML |

Abbreviations: AML, acute myeloid leukemia; ET, essential thrombocytopenia; IDH, isocitrate dehydrogenase; MPN, myeloproliferative neoplasm; NA, information not available; ND, not done; NN, normal cytogenetics; PMF, primary myelofibrosis; PV, polycythemia vera; WT, wild type.

*Multiple trisomies: +2, +3, +6, +8, +10, +11, +12, +13, +14, +19, +20, +21.
Table 3  IDH mutational frequencies in 1473 patients with polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), post-PV MF, post-ET MF, post-PV acute myeloid leukemia (post-PV AML), post-ET AML or post-PMF AML.

| Variables                          | Number of patients | IDH mutated (IDH1 or IDH2), n (%) | IDH1 mutated, n | IDH2 mutated, n | P-value |
|------------------------------------|--------------------|------------------------------------|----------------|----------------|---------|
| All patients                       | 1473               | 38                                 | 18             | 20             | <0.01   |
| PV                                 | 421                | 8 (1.9%)                           | 2              | 6              |         |
| ET                                 | 594                | 5 (0.8%)                           | 1              | 4              |         |
| PMF                                | 312                | 13 (4.2%)                          | 7              | 6              |         |
| Post-PV MF                         | 54                 | 1 (1.9%)                           | 0              | 1              |         |
| Post-ET MF                         | 41                 | 0                                  | 0              | 0              |         |
| Post-PV AML                        | 12                 | 3 (25%)                            | 2              | 1              |         |
| Post-ET AML                        | 7                  | 0                                  | 0              | 0              |         |
| Post-PMF AML                       | 32                 | 8 (25%)                            | 6              | 2              |         |
| JAK2 mutated vs wild type (n = 629) | 389 vs 240         | 14 (3.6%)                          | 10 (4.2%)      | 0.72            |         |
| MPL mutated vs wild type (n = 364) | 23 vs 341          | 1 (4.3%)                           | 18 (5.3%)      | 0.85            |         |
| TET2 mutated vs wild type (n = 237) | 31 vs 206          | 1 (3.2%)                           | 13 (6.3%)      | 0.5             |         |
| JAK2 46/1 nullizygous vs not nullizygous (n = 596) | 189 vs 407          | 11 (5.8%)                          | 2 (1.9%)       | 0.09            |         |

Abbreviations: AML, acute myeloid leukemia; IDH, isocitrate dehydrogenase; PMF, primary myelofibrosis.

*Analyses limited to Mayo clinic patients only and ‘n’ signifies number of patients evaluated.

Table 4  IDH mutational frequencies in 193 Mayo clinic patients with chronic-phase (n = 166) or blast-phase (n = 27) primary myelofibrosis (PMF) stratified by JAK2 mutational, JAK2 46/1 haplotype or cytogenetic status

| Variables                                  | N     | IDH mutated (IDH1 or IDH2), n (%) | P-value |
|--------------------------------------------|-------|-----------------------------------|---------|
| Chronic-phase PMF (JAK2V617F mutated vs wild type) | 166   | 7 (4.2%) (2 (2.1%) vs 5 (7%))     | 0.12    |
| Blast-phase PMF (JAK2V617F mutated vs wild type) | 158   | 7 (4.4%) (5 (9%) vs 2 (1.3%))    | 0.04    |
| Blast-phase PMF (JAK2 46/1 nullizygous vs not nullizygous) | 23    | 8 (35%) (5 (63%) vs 3 (20%))    | 0.04    |
| Chronic-phase PMF karyotype at diagnosis (high-risk karyotype vs not high-risk) | 111   | 7 (6.3%) (0 (0%) vs 7 (7.1%))    | 0.32    |
| Blast-phase PMF karyotype at transformation (complex karyotype vs not complex) | 22    | 7 (32%) (0 vs 7 (64%))           | 0.001   |

Abbreviations: IDH, isocitrate dehydrogenase; N, number of patients evaluable; PMF, primary myelofibrosis.

Figure 2  Survival curves of 111 patients with chronic-phase primary myelofibrosis stratified by their isocitrate dehydrogenase (IDH) mutational status (a), cytogenetic risk (b), JAK2 46/1 haplotype status (c) or International Prognostic Scoring System risk category (d).
The current study is an extension of the above-mentioned Mayo Clinic study and involves a large number of patients (n = 1473) recruited from three major MPN centers of excellence. The results of the study clarify a number of issues regarding IDH mutations in MPN. First, the study provides robust incidence figures for IDH1 and IDH2 mutations across different disease stages of specific MPN variants. Accordingly, we now show that both IDH1 and IDH2 mutations can occur in chronic-phase ET, PV or PMF, although infrequently. Mutational frequency was equally low in post-PV/ET MF and this fact combined with the significantly higher mutation incidence observed in blast-phase disease suggests a pathogenic contribution to leukemic but not fibrotic disease transformation. Two additional observations support this contention: (i) complex karyotype was infrequently encountered in IDH-mutated blast-phase MPN, which suggests an independent pathogenic contribution that might be tied to distinct molecular alterations, such as, for example, overexpression of the APP (amyloid ß (A4) precursor protein) gene, which has previously been shown in AML to be associated with either complex karyotype or IDH-R172 mutation and (ii) the absence of mutual exclusivity between IDH and other MPN-associated mutations (for example, TET2, MPL), which is consistent with the suggestion that
the former are later-arising cooperating mutations that are more involved in disease progression rather than disease initiation.

The types of IDH mutations seen in our patients with MPN (mostly IDH2R140Q and IDH1R132S/C) are distinctly different than those seen in gliomas (mostly IDH1R132H) and more similar to those seen in AML, although IDH1R132H was significantly more prevalent in AML. Within the context of MPN, IDH2R140Q was over represented in chronic-phase ET and PV, whereas IDH1 mutations were more prevalent in PMF and blast-phase MPN. More studies are needed to confirm this apparent trend. Regardless, there is currently no good explanation for the observed diversity in IDH mutation variants among gliomas and myeloid malignancies and current information suggests similar biological consequences. Whether or not different IDH mutations carry different prognostic relevance in MPN is currently not known and we did not attempt to address the particular issue because of our relatively small number of informative cases. Of note, in a recent study of primary AML with normal karyotype, different types of IDH mutations appeared to variably influence disease-free survival and complete remission rates. One particularly interesting observation from the current study was the significant association between mutant IDH and JAK2 non-46/1 haplotype. The latter phenomenon is further evidence for the JAK2 mutation specificity of the previously described association between the JAK2 46/1 haplotype and MPN. In other words, whereas JAK2 exon 14 or exon 12 mutations have been shown to be associated with JAK2 46/1 haplotype, we did not see the same effect involving MPL mutations (although others have shown otherwise), and now show an association with JAK2 non-46/1 haplotype for IDH mutations. This latter observation is also consistent with our previous report on the prognostically detrimental effect of JAK2 non-46/1 haplotype in PMF; it is possible that patients with PMF who are nullizygous for JAK2 46/1 haplotype are susceptible to additional adverse molecular events, such as IDH mutations, which might lead to biologically more aggressive disease. Consistent with this possible scenario, in the current study, the negative prognostic impact of mutant IDH was accounted for by the JAK2 46/1 genotype in PMF but not in blast-phase MPN, in which risk factors other than JAK2 non-46/1 haplotype might have promoted the development of IDH mutations. It is becoming increasingly evident that there are many more mutations than JAK2 and MPL mutations in BCR-ABL1-negative MPN including those that involve TET2, ASXL1, IDH1, IDH2, CBL, IKZF1, and LNK. Some of these mutations might be later-arising and more prevalent in blast-phase disease. What is currently lacking is a composite evaluation (that is, concurrent analysis of all relevant mutations), which includes paired chronic- and blast-phase samples of a large number of patients with blast-phase MPN. Such an approach is essential for clarifying the individual pathogenetic or prognostic contribution of the aforementioned mutations and their chronological order of appearance. It is very likely that additional mutations in MPN will be described soon, but practical relevance in terms of either disease prognostication or value as drug targets has so far been limited.

Conflict of interest

The authors declare no conflict of interest.

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