HHS Public Access

Author manuscript

Leukemia. Author manuscript; available in PMC 2015 November 20.

Published in final edited form as:

Leukemia. 2009 May ; 23(5): 905–911. doi:10.1038/leu.2009.47.

TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis

A Tefferi[1], **A Pardanani**[1], **K-H Lim**[1,2], **O Abdel-Wahab**[3,4], **TL Lasho**[1], **J Patel**[3], **N Gangat**[1], **CM Finke**[1], **S Schwager**[1], **A Mullally**[5], **C-Y Li**[1], **CA Hanson**[1], **R Mesa**[1], **O Bernard**[6,7,8], **F Delhommeau**[9], **W Vainchenker**[9], **DG Gilliland**[5,10], and **RL Levine**[3,4]

[1]Divisions of Hematology and Hematopathology, Departments of Medicine and Laboratory Medicine, Mayo Clinic, Rochester, MN, USA

[2]Division of Hematology-Oncology, Mackay Memorial Hospital and Mackay Medicine, Nursing and Management College, Taipei, Taiwan

[3]Human Oncology and Pathogenesis Program, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

[4]Leukemia Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

[5]Division of Hematology, Department of Medicine, Brigham and Women's Hospital, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA

[6]INSERM E0210, Hopital Necker

[7]APHP Laboratoire d'Hématologie, Hôpital Cochin

[8]Université Paris V, Paris, France

[9]INSERM U790, Institut Gustave Roussy, Villejuif, France

[10]The Howard Hughes Medical Institute, Boston, MA, USA

Abstract

High-throughput DNA sequence analysis was used to screen for *TET2* mutations in bone marrow-derived DNA from 239 patients with *BCR-ABL*-negative myeloproliferative neoplasms (MPNs). Thirty-two mutations (19 frameshift, 10 nonsense, 3 missense; mostly involving exons 4 and 12) were identified for an overall mutational frequency of ~13%. Specific diagnoses included polycythemia vera (PV; *n*=89), essential thrombocythemia (ET; *n*=57), primary myelofibrosis (PMF; *n*=60), post-PV MF (*n*=14), post-ET MF (*n*=7) and blast phase PV/ET/MF (*n*=12); the corresponding mutational frequencies were ~16, 5, 17, 14, 14 and 17% (*P*=0.50). Mutant *TET2* was detected in ~17 and ~7% of *JAK2*V617F-positive and -negative cases, respectively (*P*=0.04). However, this apparent clustering of the two mutations was accounted for by an independent

Correspondence: Professor A Tefferi, Departments of Medicine and Laboratory Medicine, Divisions of Hematology and Hematopathology, Mayo Clinic, Rochester, MN 55905, USA. tefferi.ayalew@mayo.edu or Dr RL Levine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA. leviner@mskcc.org.

Conflict of interest

The authors declare no conflict of interest.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

association between mutant *TET2* and advanced age; mutational frequency was ~23% in patients 60 years old versus ~4% in younger patients (*P*<0.0001). The presence of mutant *TET2* did not affect survival, leukemic transformation or thrombosis in either PV or PMF; a correlation with hemoglobin <10 g per 100 ml in PMF was noted (*P*=0.05). We conclude that *TET2* mutations occur in both *JAK2*V617F-positive and -negative MPN, are more prevalent in older patients, display similar frequencies across MPN subcategories and disease stages, and hold limited prognostic relevance.

Keywords

JAK2; *MPL*; myeloproliferative; polycythemia; thrombocythemia; myelofibrosis

Introduction

The recent descriptions of *JAK2*V617F, *JAK2* exon 12 and *MPL*515 mutations in myeloproliferative neoplasms (MPNs) underline JAK-STAT as the signaling pathway of interest in these diseases.[1–9] However, despite the fact that the expression of these mutations in mouse hematopoietic stem cells induces MPN phenotype,[10–14] their precise pathogenetic contribution in human disease has not been clarified. First, neither *JAK2* nor *MPL* mutations are always found in patients with MPN and, when present, they are not necessarily specific to a particular clinicopathological entity.[15–17] Second, although *JAK2/MPL* mutations contribute to MPN pathogenesis and phenotypic determination, there is growing evidence that suggests an additional role for mutant allele burden, host genetic factors and for the presence or absence of other coexisting mutations.[11,18–22] For example, the presence of a *JAK2* mutation appears to be essential for, but not specific to, the polycythemia vera (PV) phenotype, which is also characterized by a higher mutant allele burden, compared with that seen in other MPNs, including essential thrombocythemia (ET).[23,24] Similarly, mutant *MPL* often overshadows *JAK2*V617F when the two coexist, and this scenario is usually associated with primary myelofibrosis (PMF) or ET.[19,22] These observations underscore the complexity of pathogenetic mechanisms in MPN and the need for identifying additional molecular alterations, in tandem with clinical correlative studies.

In a recent communication, Delhommeau *et al*.[25] showed that *TET2* (TET oncogene family member 2), a putative tumor suppressor gene located in the minimal loss-of-heterozygosity (LOH) region of chromosome 4q24 in patients with myeloid malignancies, harbored the loss-of-function mutations that coexisted with *JAK2*V617F and sometimes predated its acquisition. The authors screened 181 *JAK2*V617F-positive patients with PV, ET or PMF, and documented an overall mutational frequency of ~14%. They also showed clonal involvement of both multipotent and committed myeloid progenitors and increased engrafting capacity, observed in nonobese diabetic–severe combined immunodeficient (NOD–SCID) mice, of *TET2*-mutated human hematopoietic stem cells from MPN patients. Following this seminal observation, we have reported an even higher *TET2* mutational frequency of 29% in patients with systemic mastocytosis (SM),[26] and also documented the mutation in a spectrum of myeloid neoplasms, Including myelodysplastic syndrome and chronic myelomonocytic leukemia.[27] In SM, mutant *TET2* segregated with *KIT*D816V,

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

occurred in both indolent and aggressive SM variants, was significantly associated with monocytosis, and did not appear to affect survival.[26] The purpose of this study was to confirm the prevalence of *TET2* mutations in *JAK2*V617F-positive MPN, estimate mutational frequency in the absence of *JAK2*V617F and during different disease stages and establish clinical and laboratory correlates in the context of PV, ET or PMF.

Materials and methods

After approval by the institutional review board, the Mayo Clinic database of adult MPN patients (age 18 years) was reviewed to select consecutive patients with *BCR-ABL*-negative classic MPN representing different disease stages and in whom stored bone marrow (BM) cells were available for DNA extraction and mutation analysis. As such, our study population included patients with PV, ET, PMF, post-PV MF, post-ET MF and acute myeloid leukemia (AML) arising from antecedent PV, ET or MF. Diagnoses were established on the basis of the 2001 World Health Organization (WHO) criteria.[28] Mutation screening for *JAK2*V617F (reverse transcriptase PCR), *JAK2* exon 12 mutations and *MPL*W515L/K were performed using BM-derived cells, according to previously published methods.[29,30] In patients who were alive, every attempt was made to update follow-up information by means of a questionnaire/telephone call sent to both patients and their primary doctors, and the 'date of last contact' reflected this time point, and not the last time they were seen at the Mayo Clinic. All BM specimens were either collected or reviewed at our institution to ensure accuracy of diagnosis. The management mostly consisted of what was considered at the time as 'standard' therapy and reflected the individual physician's best clinical judgment. All parameters used for statistical analysis, except for those addressing prognosis (for example, survival, leukemic or fibrotic transformation and thrombosis), were those obtained at the time of diagnosis and before any therapeutic intervention.

High-throughput DNA sequence analysis was used to screen for *TET2* mutations in BM-derived DNA. M13-appended gene-specific primers were designed to amplify and sequence all coding exons of all isoforms of *TET2*, including TET2 isoform A (NM_001127208–2002 amino acids) and TET2 isoform B (NM_017628–1165 amino acids). PCR primers were designed to amplify and sequence <500 bp amplicons, and overlapping PCR amplicons were designed for all exons >400 bp to ensure complete coverage. For each PCR reaction, 20 ng of genomic DNA was used for PCR amplification using a Duncan Water Bath Thermal Cycler, followed by magnetic bead purification (SPRI, Agencourt Bioscience, Beverly, MA, USA). Bidirectional sequencing was performed using ABI 3730 capillary sequencers (Agencourt Bioscience) as described previously.[31] Missense and nonsense mutations were detected in bidirectional sequence traces using a Mutation Surveyor (Softgenetics Inc., State College, PA, USA). All traces were reviewed manually and with the Mutation Surveyor for the presence of frameshift mutations. Frameshift and nonsense mutations were annotated according to the predicted effects on TET2-coding sequence using TET2 isoform A as the predominant isoform.

All frameshift and nonsense mutations were scored as pathogenic TET2 mutations on the basis of the observation that we have not observed germline frameshift/nonsense mutations in >500 paired tumor and normal samples. Nonsynonymous mutations were first compared

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

with published SNP data (dbSNP, http://www.ncbi.nlm.nih.gov/projects/SNP), such as previously annotated SNPs, were not considered pathogenic mutations. Missense mutations that were not included in the published SNP database were annotated as somatic mutations on the basis of published data showing that these are somatic mutations[25] or sequence analysis of paired tumor/normal MPN/MDS samples that showed that these mutations are present in tumor and not in matched normal DNA. Samples with missense alleles that were neither in dbSNP nor could be shown to be somatic in paired samples were censored from analysis. We, therefore, classify samples as 'TET2-wt' if the sample was wild-type in all coding exons of TET2, and as 'TET2-mutant' if the sample contained a frameshift, nonsense or proven somatic missense mutation.

The statistical procedures used were conventional, and all data were analyzed by using StatView (SAS Institute, Cary, NC, USA). All *P*-values were two-tailed and statistical significance was set at the level of $P<0.05$. Continuous variables were summarized as medians and ranges. Categorical variables were described as count and relative frequency (%). Comparison between categorical variables was performed by Chi-squared statistics. Comparison between categorical and continuous variables was performed by either the Mann–Whitney *U*-test or the Kruskal–Wallis test. The association of variables selected from univariate analysis was explored using logistic regression models. Survival curves for patients with and without *TET2* mutation were constructed by Kaplan–Meier method taking, the interval from the date of diagnosis to death or last contact, and compared using the Log-rank test.

Results

A total of 239 patients with *BCR-ABL*-negative classic MPN were included in this study: PV ($n=89$), ET ($n=57$), PMF ($n=60$), post-PV MF ($n=14$), post-ET MF ($n=7$) and blast-phase PV/ET/MF ($n=12$). Among the latter were four patients with post-PV AML, four with post-ET AML and four with post-PMF AML. The overall ($n=239$) *TET2* mutational frequency was ~13% and included 32 mutations that most frequently involved exons 4 and 12: 19 frameshift, 10 nonsense and 3 missense (Table 1). None of the mutations were associated with a karyotypically apparent 4q24 rearrangement. Disease-specific mutational frequencies were ~16% in PV, ~5% in ET, ~17% in PMF, ~14% in post-PV MF, ~14% in post-ET MF and ~17% in blast-phase PV/ET/PMF ($P=0.50$). All 239 study patients were also screened for *JAK2*V617F; *TET2* mutations occurred in 5 (~7%) of 75 *JAK2*V617F-negative versus 27 (~17%) of 164 *JAK2*V617F-positive cases ($P=0.04$). The corresponding figures after excluding chronic and advanced phases of PV (excluded because of their almost invariable association with a *JAK2* mutation) were 8 and 15% ($P=0.17$). However, the *JAK2*V617F allele burden did not correlate with the presence of mutant *TET2* ($n=108$; $P=0.95$). Information on *MPL*W515L/K status was available in 73 patients, and the five mutation-positive cases as well as two other patients with *JAK2* exon 12 mutations did not carry mutant *TET2*.

Considering all 239 study patients, both mutant *TET2* ($P<0.0001$) and *JAK2*V617F ($P=0.008$) clustered with older age. This was also true when analysis was restricted to ET, PMF or their advanced stages ($n=132$; $P=0.001$ and 0.05, respectively); as pointed out in the

Author Manuscript

above paragraph, chronic and advanced phases of PV were excluded in the latter analysis because of their almost perfect association with a *JAK2* mutation. The association between *JAK2*V617F and older age remained significant or near significant, when *TET2*-mutated patients were excluded from either the overall analysis (n=207; P=0.03) or the analysis restricted to ET, PMF or their advanced stages (n=117; P=0.08). However, a multivariable analysis of all 239 study patients, which included mutational status, age and sex as covariates, showed an independent association only between the presence of mutant *TET2* and advanced age; mutational frequency was 23% in patients 60 years of age versus 4% in younger patients (P<0.0001).

Accurate clinical correlative studies of novel mutations in MPN require separate analysis of patients with 'chronic phase' PV, ET or PMF. Accordingly, the clinical features (Table 2) and events presented during the course of disease (Table 3) for PV, ET and PMF were reviewed in the context of their *TET2* mutational status. As the number of *TET2*-mutated patients with ET was small (n=3), we did not attempt a formal comparison between mutated and unmutated ET cases. However, as is evident from Tables 2 and 3, it is reasonable to mention that the three *TET2*-mutated ET patients were older compared with their unmutated counterparts, and were followed up for 19 to 144 months without fibrotic or leukemic transformation. Table 4 lists the P-values during statistical comparison of relevant clinical and laboratory features in PV and PMF, and shows the lack of convincing evidence for specific genotype–phenotype correlates, with the one exception of a significant association between the presence of mutant *TET2* and a hemoglobin level of <10 g per 100 ml in PMF. Table 5 provides a comprehensive account of pre- and post-disease transformation details in PV, ET or MF patients who underwent conversion into the fibrotic or leukemic phase of their disease. Once again, there was no overt evidence to suggest that mutant *TET2* influenced disease duration before or after fibrotic or leukemic transformation. Finally, Kaplan–Meier survival curves between *TET2*-mutated and -unmutated patients with either PV (Figure 1) or PMF (Figure 2) were not significantly different.

Discussion

The TET family of proteins share highly conserved regions, but their biological function is not known. *TET1* is short for 'ten–eleven translocation 1′ and was the name given to a novel gene located at chromosome 10q22, which was identified as the fusion partner of *MLL* during an AML-associated chromosomal translocation, t(10;11)(q22;q23).[32] Subsequent genomic database exploration identified two homologous human proteins that were accordingly designated as TET2 and TET3. It is interesting that *TET2* is located at chromosome 4q24, which is a breakpoint that is also involved in other AML-associated translocations: t(3;4)(q26;q24), t(4;5)(q24;p16), t(4;7)(q24;q21) and del(4)(q23q24). In 2005, Viguie *et al*.[33] suggested that these 4q24 rearrangements occurred in multipotent myeloid–lymphoid progenitor cells, and by using BAC clones and fluorescent *in situ* hybridization, were able to show a 0.5-Mb commonly deleted region that accompanied the 4q24 breakpoint.[33] In 2008, Delhommeau *et al*.[25] reported acquired LOH/deletions at chromosome 4q24 in patients with MPN, and identified *TET2* as the gene of interest contained within the minimally deleted/homozygosed region.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

TET2 has multiple isoforms, including *TET2* isoform A (NM 001127208–2002 amino acids) and *TET2* isoform B (NM 017628–1165 amino acids); notably, many of the somatic mutations identified to date are observed in coding sequence specific to isoform A. *TET2* isoform A spans ~133 kb, including 12 exons, and is predicted to encode a protein of 2002 amino acids. At the time of this study, the function of TET2 in normal and malignant hematopoiesis has not been fully elucidated; however, the enhanced engraftment of *TET2*-mutant MPN patient cells in NOD–SCID mice suggests that TET2 might be involved in self-renewal pathways relevant to hematopoietic transformation.[25] Delhommeau *et al*.[25] subsequently sequenced *TET2* in 181 *JAK2*V617F-positive patients with PV, ET and PMF, and discovered frameshift, nonsense and missense mutations in ~14% of the patients.[25]

The *TET2* mutational frequency observed in this study (~17%) among *JAK2*V617F-positve MPNs is remarkably similar to that reported by Delhommeau *et al*.[25] In addition, our study shows the occurrence of the mutation in *JAK2*V617F-negative MPN, perhaps at a lesser frequency (~7%). This apparent segregation of mutant *TET2* with *JAK2*V617F is similar to the association between mutant *TET2* and *KIT*D816 V, which was seen in our recent report involving SM patients.[26] However, the latter observation is confounded by the fact that *KIT*D816V is present in more than 90% of patients with SM and that *KIT*D816V negativity in our above-cited report probably resulted from the use of inadequately sensitive mutation screening assay (PCR sequencing), and therefore represented a low mutant allele burden rather than a true absence of the mutation.[34] Similarly, in this study, multivariable analysis revealed that the association between mutant *TET2* and *JAK2*V617F was fully accounted for by a strong and independent association between the former and advanced age. In this regard, it is to be recalled that both *JAK2*V617F and mutant *MPL*515 are also more prevalent in older patients with MPN.[22,30,35,36] Furthermore, this study showed that the higher prevalence of *JAK2*V617F in older patients was not accounted for by the presence of mutant *TET2*. Collectively, these observations support the concept of 'genetic instability' in MPN, and underscore the importance of accounting for age during clinical correlative studies concerning MPN-associated mutations. Whether or not differences in the mutational profile of young and older patients with MPN will translate into varied responses during targeted therapy remains to be seen.

In this study, *TET2* mutational frequencies were not significantly different across MPN subcategories or specific disease stages. In this regard, as discussed above, the apparently lower incidence of *TET2* mutation in ET patients (~5%) may have been related to their younger age range (*vide supra*). Furthermore, although the numbers involved were too low to allow making statistically valid conclusions, it is reasonable to mention that the pre- and post-disease transformation time intervals among informative patients with ET, PV or PMF were not overtly different to suggest an influence from mutant *TET2* (Table 5). These observations lessen the possibility that mutant *TET2* emerges or disappears over time and, coupled with the absence of significant correlations between the presence of mutant *TET2* and survival or leukemic transformation, at least in PV and PMF, undermine the potential value of the mutation as a prognostic tool. These data also suggest the possibility that there are additional genetic or epigenetic events that target *TET2* or other genes in related pathways in *TET2* wild-type patients.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

There were no evident mutational hotspots detected either in this study or in our earlier report on SM.[26] The *TET2* mutations were spread over several exons and regions of the gene, and most resulted in either frameshift or stop codon alterations. Such changes usually result in truncated translation, and therefore inadequate production of a potentially tumor suppressor protein. Such a scenario would be consistent with the aforementioned observations from Delhommeau *et al.*,[25] which indicated, in a subset of MPN/MDS patients, biallelic inactivation of *TET2* observed because of biallelic mutations, deletion of the remaining wild-type copy or somatic isodisomy for mutant *TET2*. When we compared the results from our two successive *TET2* mutation studies involving PV/ET/MF and SM patients, respectively, we noticed that none of the former, but 5 of the 12 *TET2*-mutated SM patients displayed more than one *TET2* mutation.[26] Multiple *TET2* mutations in the same patient were also infrequent in our other patients with chronic myelomonocytic leukemia, myelodysplastic syndrome or acute leukemia.[27] These observations need to be extended through an analysis of copy number and zygosity using SNP arrays to assess the relative frequency of homozygous *TET2* inactivation because of mutation, deletion and LOH in the different myeloid malignancies. If this observation was to be validated by SNP array analysis and through an analysis of additional patient cohorts, it would suggest that biallelic inactivation of *TET2* is more common in some diseases, such as SM, than in others, such as *JAK2*V617F-positive MPN.

Unlike the case with *JAK2*V617F,[37] the presence of mutant *TET2* did not appear to significantly influence leukocyte or platelet count in patients with PV or PMF. Hemoglobin level in PV or *JAK2*V617F allele burden in mutation-positive MPN patients was also not affected. Similarly, unlike the case with SM,[26] the presence of mutant *TET2* in PMF did not correlate with monocytosis. At the same time, the association between mutant *TET2* and anemia in PMF did not escape our attention. However, because of the relatively small number of patients studied, we prefer to postpone additional comments regarding genotype–phenotype correlates, and instead recommend that these data be treated as preliminary. For now, we can reasonably conclude that *TET2* mutations are even less specific than *JAK2*V617F in the context of myeloid malignancies, occur in the presence or absence of other MPN relevant mutations and are more likely to be detected in older patients. Larger studies including an adequate number of *TET2*-mutated patients are needed to properly assess not only the prognostic relevance of mutant *TET2* by itself, but also its potential prognostic interaction with other MPN-associated molecular markers. In the latter, it is interesting to glean from Table 5 that all *TET2*-mutated patients who have undergone fibrotic or leukemic transformation also carried *JAK2*V617F.

Acknowledgements

Adriana Heguy and Igor Dolgalev from Memorial Sloan-Kettering Cancer Center, New York, NY, USA are acknowledged for their assistance with sequence analysis. This study was supported in part by grants from the Myeloproliferative Disorders Foundation, Chicago, IL, USA. DGG is an investigator of the Howard Hughes Medical Institute and is a Doris Duke Charitable Foundation Distinguished Clinical Scientist. RLL is an Early Career Award recipient of the Howard Hughes Medical Institute, a Clinical Scientist Development Award recipient of the Doris Duke Charitable Foundation and is the Geoffrey Beene Junior Chair at Memorial Sloan Kettering Cancer Center.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

References

1. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005; 7:387–397. [PubMed: 15837627]
2. 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. [PubMed: 15793561]
3. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005; 352:1779–1790. [PubMed: 15858187]
4. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, et al. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. Lancet. 2005; 365:1054–1061. [PubMed: 15781101]
5. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, et al. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006; 3:e270. [PubMed: 16834459]
6. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, et al. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. N Engl J Med. 2007; 356:459–468. [PubMed: 17267906]
7. 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. [PubMed: 16868251]
8. Pardanani A, Lasho TL, Finke C, Hanson CA, Tefferi A. Prevalence and clinicopathologic correlates of JAK2 exon 12 mutations in JAK2V617F-negative polycythemia vera. Leukemia. 2007; 21:1960–1963. [PubMed: 17597810]
9. Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Theocharides A, et al. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. Blood. 2008; 111:1686–1689. [PubMed: 17984312]
10. Wernig G, Mercher T, Okabe R, Levine RL, Lee BH, Gilliland DG. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood. 2006; 107:4274–4281. [PubMed: 16478879]
11. Tiedt R, Hao-Shen H, Sobas MA, Looser R, Dirnhofer S, Schwaller J, et al. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. Blood. 2008; 111:3931–3940. [PubMed: 18160670]
12. Shide K, Shimoda HK, Kumano T, Karube K, Kameda T, Takenaka K, et al. Development of ET, primary myelofibrosis and PV in mice expressing JAK2 V617F. Leukemia. 2008; 22:87–95. [PubMed: 18033315]
13. Lacout C, Pisani DF, Tulliez M, Gachelin FM, Vainchenker W, Villeval JL. JAK2V617F expression in murine hematopoietic cells leads to MPD mimicking human PV with secondary myelofibrosis. Blood. 2006; 108:1652–1660. [PubMed: 16670266]
14. Zaleskas VM, Krause DS, Lazarides K, Patel N, Hu Y, Li S, et al. Molecular pathogenesis and therapy of polycythemia induced in mice by JAK2 V617F. PLoS ONE. 2006; 1:e18. [PubMed: 17183644]
15. Steensma DP, Dewald GW, Lasho TL, Powell HL, McClure RF, Levine RL, et al. The JAK2 V617F activating tyrosine kinase mutation is an infrequent event in both 'atypical' myeloproliferative disorders and myelodysplastic syndromes. Blood. 2005; 106:1207–1209. [PubMed: 15860661]
16. Vizmanos JL, Ormazabal C, Larrayoz MJ, Cross NC, Calasanz MJ. JAK2 V617F mutation in classic chronic myeloproliferative diseases: a report on a series of 349 patients. Leukemia. 2006; 20:534–535. [PubMed: 16408096]
17. Jones AV, Kreil S, Zoi K, Waghorn K, Curtis C, Zhang L, et al. Widespread occurrence of the JAK2 V617F mutation in chronic myeloproliferative disorders. Blood. 2005; 106:2162–2168. [PubMed: 15920007]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

18. Pardanani A, Fridley BL, Lasho TL, Gilliland DG, Tefferi A. Host genetic variation contributes to phenotypic diversity in myeloproliferative disorders. Blood. 2008; 111:2785–2789. [PubMed: 18006699]
19. Lasho TL, Pardanani A, McClure RF, Mesa RA, Levine RL, Gary Gilliland D, et al. Concurrent MPL515 and JAK2V617F mutations in myelofibrosis: chronology of clonal emergence and changes in mutant allele burden over time. Br J Haematol. 2006; 135:683–687. [PubMed: 17107350]
20. Kralovics R, Teo SS, Li S, Theocharides A, Buser AS, Tichelli A, et al. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. Blood. 2006; 108:1377–1380. [PubMed: 16675710]
21. Pardanani A, Lasho TL, Finke C, Mesa RA, Hogan WJ, Ketterling RP, et al. Extending Jak2V617F and MplW515 mutation analysis to single hematopoietic colonies and B and T lymphocytes. Stem Cells. 2007; 25:2358–2362. [PubMed: 17540852]
22. Vannucchi AM, Antonioli E, Guglielmelli P, Pancrazzi A, Guerini V, Barosi G, et al. Characteristics and clinical correlates of MPL 515W>L/K mutation in essential thrombocythemia. Blood. 2008; 112:844–847. [PubMed: 18519816]
23. Levine RL, Belisle C, Wadleigh M, Zahrieh D, Lee S, Chagnon P, et al. X-inactivation-based clonality analysis and quantitative JAK2V617F assessment reveal a strong association between clonality and JAK2V617F in PV but not ET/MMM, and identifies a subset of JAK2V617F-negative ET and MMM patients with clonal hematopoiesis. Blood. 2006; 107:4139–4141. [PubMed: 16434490]
24. Lippert E, Boissinot M, Kralovics R, Girodon F, Dobo I, Praloran V, et al. The JAK2-V617F mutation is frequently present at diagnosis in patients with essential thrombocythemia and polycythemia vera. Blood. 2006; 108:1865–1867. [PubMed: 16728702]
25. Delhommeau F, Dupont S, James C, Masse A, le Couedic JP, Valle VD, et al. TET2 is a novel tumor suppressor gene inactivated in myeloproliferative neoplasms: identification of a pre-JAK2 V617F event. ASH Annu Meet Abstr. 2008; 112:lba–lb3. Late-breaking abstract.
26. Tefferi A, Levine RL, Lim K-H, Abdel-Wahab O, Lasho TL, Patel J, et al. Frequent TET2 mutations in systemic mastocytosis: Clinical, KITD816V and FIP1L1-PDGFRA correlates. Leukemia. 2009 (in press).
27. Tefferi A, Lim K-H, Abdel-Wahab O, Lasho TL, Patel J, Patnaik MM, et al. Detection of mutant *TET2* in myeloid malignancies other than myeloproliferative neoplasms: CMML, MDS, MDS/MPN and AML. Leukemia. 2009 (in press).
28. Jaffe, ES.; Harris, NL.; Stein, H.; Vardiman, JW. World Health Organization Classification of Tumours of Hematopoietic and Lymphoid Tissues. IARC Press; Lyon, France: 2001. p. 1-351.
29. Pardanani A, Reeder TL, Kimlinger TK, Baek JY, Li CY, Butterfield JH, et al. Flt-3 and c-kit mutation studies in a spectrum of chronic myeloid disorders including systemic mast cell disease. Leuk Res. 2003; 27:739–742. [PubMed: 12801532]
30. 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. [PubMed: 18216871]
31. Loriaux MM, Levine RL, Tyner JW, Frohling S, Scholl C, Stoffregen EP, et al. High-throughput sequence analysis of the tyrosine kinome in acute myeloid leukemia. Blood. 2008; 111:4788–4796. [PubMed: 18252861]
32. Lorsbach RB, Moore J, Mathew S, Raimondi SC, Mukatira ST, Downing JR. TET1, a member of a novel protein family, is fused to MLL in acute myeloid leukemia containing the t(10;11)(q22;q23). Leukemia. 2003; 17:637–641. [PubMed: 12646957]
33. Viguie F, Aboura A, Bouscary D, Ramond S, Delmer A, Tachdjian G, et al. Common 4q24 deletion in four cases of hematopoietic malignancy: early stem cell involvement? Leukemia. 2005; 19:1411–1415. [PubMed: 15920487]
34. Garcia-Montero AC, Jara-Acevedo M, Teodosio C, Sanchez ML, Nunez R, Prados A, et al. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. Blood. 2006; 108:2366–2372. [PubMed: 16741248]

35. Tefferi A, Lasho TL, Schwager SM, Steensma DP, Mesa RA, Li CY, et al. The JAK2 tyrosine kinase mutation in myelofibrosis with myeloid metaplasia: lineage specificity and clinical correlates. Br J Haematol. 2005; 131:320–328. [PubMed: 16225651]

36. Wolanskyj AP, Lasho TL, Schwager SM, McClure RF, Wadleigh M, Lee SJ, et al. JAK2 mutation in essential thrombocythaemia: clinical associations and long-term prognostic relevance. Br J Haematol. 2005; 131:208–213. [PubMed: 16197451]

37. 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. [PubMed: 18496562]

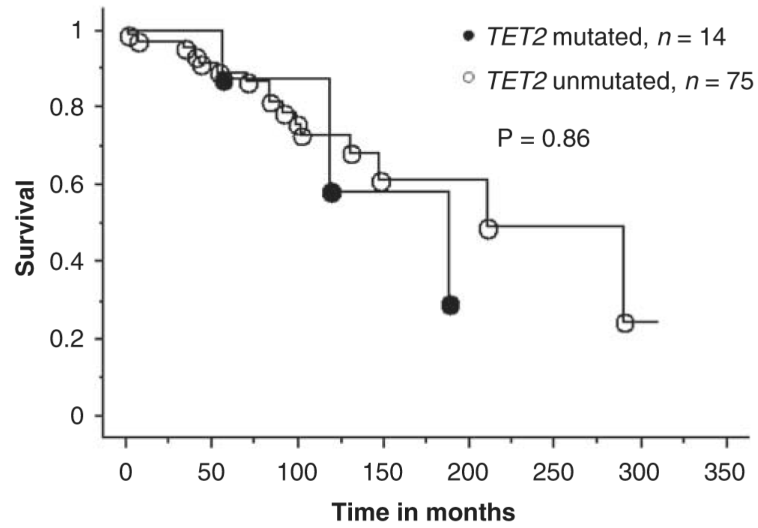

Figure 1.

Survival curves for 89 patients with polycythemia vera stratified by their *TET2* mutational status (14 mutated and 75 unmutated patients).

Author Manuscript
Author Manuscript
Author Manuscript
Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

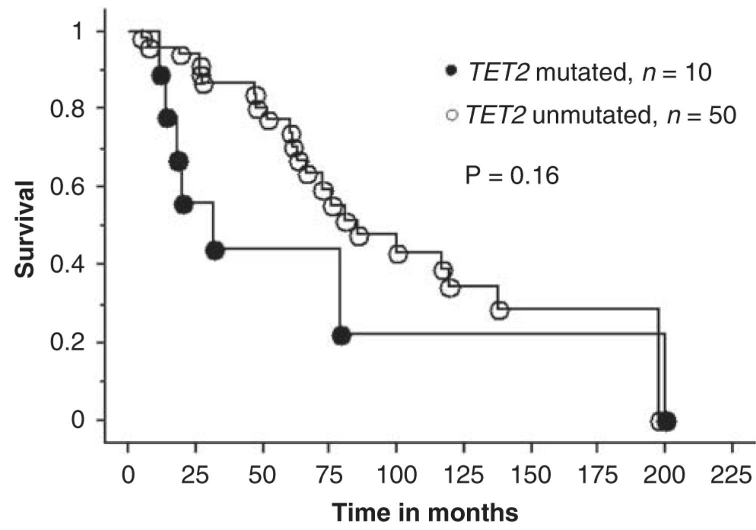

Figure 2.

Survival curves for 60 patients with primary myelofibrosis stratified by their *TET2* mutational status (10 mutated and 50 unmutated patients).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

TET2 mutation details in 32 patients with myeloproliferative neoplasms

Exon	Nucleotide change	*Consequence*	*Mutation type*	*Diagnosis*	*JAK2V617F mutational status*	*Age*	*Sex*
4	370_delC	Frameshift	Frameshift	PV	Pos.	69	F
4	3990_4000delAAGTCGTTAT	Frameshift	Frameshift	PV	Pos.	65	F
4	1726 C>G	S509X	Nonsense	PV	Pos.	76	M
4	2568 C>T	Q790X	Nonsense	PV	Pos.	66	M
4	3081_3082insacagcagcaaacacagcaaccccaa	Frameshift	Frameshift	PV	Pos.	51	M
4	2946 C>T	Q916X	Nonsense	PV	Pos.	52	F
8	266 C>A	S1291X	Nonsense	PV	Pos.	60	M
11	411 C>T	R1465X	Nonsense	PV	Pos.	61	F
12	699_delA	Frameshift	Frameshift	PV	Pos.	74	F
12	1233_1243delCTGACATTGGG	Frameshift	Frameshift	PV	Pos.	77	M
12	484_485insT	Frameshift	Frameshift	PV	Pos.	84	F
12	209 C>T	R1516X	Nonsense	PV	Pos.	72	M
12	537_538delAG	Frameshift	Frameshift	PV	Pos.	81	M
12	722 C>T	Q1687X	Nonsense	PV	Pos.	66	M
4	2036_2037insG	Frameshift	Frameshift	PMF	Pos.	63	M
4	3509_3510delTT	Frameshift	Frameshift	PMF	Pos.	58	M
4	1142_1145delTTCC	Frameshift	Frameshift	PMF	Pos.	78	M
7	331 A>T	D1242 V	Missense	PMF	Neg.	60	F
7	370_371insA	Frameshift	Frameshift	PMF	Neg.	64	M
10	231 C>T	R1358C	Missense	PMF	Pos.	69	M
11	301_delA	Frameshift	Frameshift	PMF	Neg.	74	M
12	640 T>G	Y1659X	Nonsense	PMF	Neg.	56	M
12	1423_1424inscatggcttggctctttgggaagccaaa	Frameshift	Frameshift	PMF	Pos.	60	F
12	272 C>T	Q1537X	Nonsense	PMF	Pos.	70	M
4	438 C>T	Q80X	Nonsense	ET	Neg.	80	M
7	384_delA	Frameshift	Frameshift	ET	Pos.	77	M
12	1044_1048delCTAAT	Frameshift	Frameshift	ET	Pos.	51	F
4	1171_delA	Frameshift	Frameshift	Post-PV MF	Pos.	69	M
12	1281 T>C	I1873T	Missense	Post-PV MF	Pos.	74	M
4	1671_delC	Frameshift	Frameshift	Post-ET MF	Pos.	66	M
4	1137_1140delACCT	Frameshift	Frameshift	Post-ET AML	Pos.	68	M
4	2655_2656insA	Frameshift	Frameshift	Post-PV AML	Pos.	62	F

Abbreviations: AML, acute myeloid leukemia; ET, essential thrombocythemia; Neg., negative; PMF, primary myelofibrosis; Pos., positive; PV, polycythemia vera.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Presenting clinical and laboratory features of 206 *TET2*-mutated or -unmutated patients with PV, ET or PMF

	PV (all patients)	TET2 mutated	TET2 unmutated	ET (all patients)	TET2 mutated	TET2 unmutated	PMF (all patients)	TET2 mutated	TET2 unmutated
N (%)	89 (100)	14 (16)	75 (84)	57 (100)	3 (5)	54 (95)	60 (100)	10 (17)	50 (83)
Males, *n* (%)	43 (48)	9 (64)	34 (45)	20 (35)	2 (67)	18 (33)	38 (63)	8 (80)	30 (60)
Age in years, median (range)	63 (21–93)	68 (51–84)	60 (21–93)	49 (18–88)	77 (51–80)	47 (18–88)	59 (35–78)	62 (48–78)	57 (35–76)
Palpable splenomegaly, *n* (%)	28 (33)	5 (36)	23 (32)	15 (27)	0	15 (29)	36 (71)	5(63)	31(72)
Hemoglobin (g per 100 ml), median (range)	18.3 (15–23)	18.6 (16.6–20.5)	18.2 (15–23)	14 (10.7–16.4)	12.6 (12–16.3)	14 (10.7–16.4)	11.6 (6.7–14.9)	10.5 (6.7–12)	11.6 (8.6–14.9)
Leukocyte count ($\times 10^9$/l), median (range)	11.5 (4.3–120)	12.3 (4.3–24.8)	11.3 (4.6–120)	9.7 (3.4–29.8)	8 (6–10.9)	9.7 (3.4–29.8)	8.3 (1.8–77.1)	9.3 (8.2–20.2)	6.7 (1.8–77.1)
Monocyte count ($\times 10^9$/l), median (range)	NA	NA	NA	NA	NA	NA	0.4 (0–3.1)	0.6 (0–1.6)	0.4 (0–3.1)
Platelet count ($\times 10^9$/l), median (range)	493 (132–2088)	499 (160–787)	486 (132–2088)	1041 (623–3300)	983 (840–1000)	1046 (623–3300)	246 (12–768)	211 (81–524)	248 (12–768)
Pruritus, *n* (%)	27 (38)	6 (43)	21 (36)	NA	NA	NA	NA	NA	NA
Arterial thrombosis, *n* (%)	7 (8)	0	7 (10)	11 (20)	1 (33)	10 (19)	NA	NA	NA
Venous thrombosis, *n* (%)	12 (14)	1 (7)	11 (15)	3 (5)	0	3 (6)	NA	NA	NA
*JAK2*V617F, *n* (%)	80 (90)	14 (100)	66 (88)	26 (46)	2 (67)	24 (44)	33 (55)	6 (60)	27 (54)

Abbreviations: ET, essential thrombocythemia; NA, not available or not applicable; PMF, primary myelofibrosis; PV, polycythemia vera.

Among the nine PV patients in whom JAK2V617F was not detected, two carried *JAK2* exon 12 mutations.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 3

Post-diagnosis clinical events in 206 *TET2*-mutated or -unmutated patients with PV, ET or PMF

	PV (all patients)	**TET2 mutated**	**TET2 unmutated**	**ET (all patients)**	**TET2 mutated**	**TET2 unmutated**	**PMF (all patients)**	**TET2 mutated**	**TET2 unmutated**
N (%)	89 (100)	14 (16)	75 (84)	57 (100)	3 (5)	54 (95)	60 (100)	10 (17)	50 (83)
Disease duration in months, median (range)	60 (1–310)	57 (1–191)	60 (1–310)	163 (0.3–398)	89 (19–144)	170 (0.3–398)	48 (5–200)	26 (6–200)	55 (5–197)
Arterial thrombosis after diagnosis, *n* (%)	15 (18)	1 (7)	14 (20)	16 (29)	2 (67)	14 (27)	NA	NA	NA
Venous thrombosis after diagnosis, *n* (%)	9 (11)	1 (7)	8 (11)	7 (13)	1 (33)	6 (12)	NA	NA	NA
Leukemictransformation, *n* (%)	4 (5)	1 (7)	3 (4)	2 (4)	0	2 (4)	3 (5)	1 (10)	2 (4)
Fibrotic transformation, *n* (%)	6 (7)	1 (7)	5 (7)	3 (5)	0	3 (6)	NA	NA	NA
Deaths, *n* (%)	19 (21)	3 (21)	16 (21)	13 (23)	2 (67)	11 (20)	29 (48)	7 (70)	22 (44)

Abbreviations: ET, essential thrombocythemia; NA, not available or not applicable; PMF, primary myelofibrosis; PV, polycythemia vera.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 4

P-values during univariate analysis correlating the presence of *TET2* mutations with clinical and laboratory features at presentation and follow-up

	Polycythemia vera (total n=89; TET2 mutated=14)	**Primary myelofibrosis (total n=60; TET2 mutated=10)**
Older age	0.04	0.15
Sex	0.19	0.23
*JAK2*V617F mutational status	0.17	0.72
Hemoglobin level	0.96 (*n*=86)	NA
Hemoglobin <10 g per 100 ml	NA	0.05 (*n*=45)
Leukocyte count	0.83 (*n*=85)	0.16 (*n*=45)
Leukocyte count <4 or $>30\times10^9$/l	NA	0.27 (*n*=45)
Leukocyte count $>25\times10^9$/l	NA	0.53 (*n*=45)
Platelet count	0.79 (*n*=86)	0.89 (*n*=45)
Platelet count $<100\times10^9$/l	NA	0.64 (*n*=45)
Monocyte count $<1\times10^9$/l	NA	0.64 (*n*=45)
Circulating blast count 1%	NA	0.83 (*n*=45)
Hypercatabolic symptoms	NA	0.56 (*n*=45)
Spleen size	0.78 (*n*=86)	0.86 (*n*=45)
Pruritus	0.64 (*n*=72)	NA
Arterial thrombosis at diagnosis	0.23 (*n*=87)	NA
Arterial thrombosis at follow-up	0.25 (*n*=84)	NA
Venous thrombosis at diagnosis	0.43 (*n*=87)	NA
Venous thrombosis at follow-up	0.63 (*n*=84)	NA
Leukemic transformation	0.61	0.43
Fibrotic transformation	0.79	NA
Follow-up period	0.82	0.08
Survival	0.86	0.16

Abbreviation: NA, information either not available or not applicable.

Numbers in parenthesis denote the number of informative patients.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 5

Clinical features of 33 *TET2*-mutated or -unmutated patients with post-PV MF, post-ET MF or post-PV/ET/MF AML

	Post-PV MF	TET2 mutated	TET2 unmutated	Post-ET MF	TET2 mutated	TET2 unmutated	Post-PV/ ET/PMF AML	TET2 mutated	TET2 unmutated
N (%)	14 (100)	2 (14)	12 (86)	7 (100)	1 (14)	6 (86)	12 (100)	2 (17)	10 (83)
Males, *n* (%)	9 (64)	2 (100)	7 (58)	6 (86)	1 (100)	5 (83)	10 (83)	1 (50)	9 (90)
Age in years, median (range)	64.5 (43–74)	71.5 (69–74)	59.5 (43–72)	65 (53–73)	68	64 (53–73)	64 (53–81)	65 (62–68)	64 (53–81)
Post-PV AML, *n* (%)	NA	NA	NA	NA	NA	NA	4 (33)	1 (25)	3 (75)
Post-ET AML, *n* (%)	NA	NA	NA	NA	NA	NA	4 (33)	1 (25)	3 (75)
Post-PMF AML, *n* (%)	NA	NA	NA	NA	NA	NA	4 (33)	0	4 (100)
*JAK2*V617F, *n* (%)	14 (100)	2 (100)	12 (100)	4 (57)	1 (100)	3 (50)	7 (58)	2 (100)	5 (50)
Deaths, *n* (%)	9 (64)	2 (100)	7 (58)	6 (86)	1 (100)	5 (83)	12 (100)	2 (100)	10 (100)
Pre-transformation disease duration in months, median (range)	126 (25–294)	61 (30–92)	138 (25–294)	84 (54–168)	84	101.1 (54–168)	92.8 (13–217)	103.8 (62–145)	92.8 (13–217)
Post-transformation disease duration in months, median (range)	41 (4–249)	20 (4–35)	51 (20–249)	29 (20–156)	29	46 (20–156)	4 (1–11)	7 (6–9)	4 (1–11)

Abbreviations: AML, acute myeloid leukemia; ET, essential thrombocythemia; PMF, primary myelofibrosis; PV, polycythemia vera.