Published in final edited form as:

Exp Hematol. 2011 January ; 39(1): 95–101. doi:10.1016/j.exphem.2010.09.008.

Disease Burden at the Progenitor Level is a feature of Primary Myelofibrosis: A multivariable analysis of 164 JAK2 V617F-positive Myeloproliferative Neoplasm patients

Brady L. Stein¹, Donna M. Williams², Ophelia Rogers², Mary Ann Isaacs², Jerry L. Spivak², and Alison R. Moliterno²

¹ Northwestern University Feinberg School of Medicine, Chicago, IL

² Johns Hopkins University School of Medicine, Baltimore, MD; Johns Hopkins University Medical Institutions, Baltimore, MD

Abstract

Objective—Suppression of normal hematopoiesis by the neoplastic clone (clonal dominance) is a feature of the myeloproliferative neoplasms, but the determinants that predict clonal dominance are unknown. The objective of this study was to identify clinical and laboratory variables that associate with the JAK2 V617F CD34⁺ progenitor allele burden and clonal dominance, which was defined by congruence of the JAK2 V617F CD34⁺ progenitor and neutrophil allele burdens.

Methods—A cross-sectional analysis was performed on 164 consecutive JAK2 V617F-positive patients: 30 with essential thrombocythosis (ET), 100 with polycythemia vera (PV), and 34 with myelofibrosis (MF), including 8 post-ETMF and 3 post-PVMF. The JAK2 V617F CD34⁺ progenitor and neutrophil allele burdens were measured using an allele-specific, quantitative real-time polymerase chain reaction (PCR) assay.

Results—After adjusting for genotype, sex, age at diagnosis and disease duration, disease type was the strongest predictor of clonal dominance, with the odds ratio being nearly 61.9 times higher for MF patients when compared to ET patients ($p < 0.001$), and 9.7 times higher when compared to PV patients ($p = 0.002$). Additionally, clonal dominance was associated with a clinical phenotype of an increased spleen size ($p = 0.006$), increased white blood cell count ($p = 0.009$), and lower hemoglobin ($p < 0.001$), even after adjusting for disease type and duration.

Conclusion—These data indicate that loss of wild type clones at the progenitor level is a feature of MF (PMF, post-ETMF, and post-PVMF), presumably due to expansion of the JAK2 V617F clone and that this characteristic is surprisingly independent of JAK2 V617F homozygosity, suggesting that additional genomic lesions may contribute to this unique molecular process that distinguishes MF from ET and PV.

Correspondence: Alison R. Moliterno, M.D., Division of Hematology, Johns Hopkins University School of Medicine, Ross Research Building 1025, 720 Rutland Avenue, Baltimore, MD 21205; amoliter@jhmi.edu.

Authorship and disclosures: BLS, JLS, and ARM designed the research and wrote the paper; DW, OR and MI designed and performed the research. The authors disclose no conflicts of interests.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Introduction

Essential thrombocytosis (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) share the same acquired genetic lesion, JAK2 V617F, but differ with respect to epidemiology, clinical phenotype and natural history. When measured in neutrophils, the ratio of mutant JAK2 to wild-type JAK2 (JAK2 V617F allele burden) has been shown to differ by disease type and correlate best with disease features including leukocytosis, splenomegaly, and thrombosis.[1] However, the myeloproliferative neoplasms (MPN) are stem cell disorders and suppression of normal hematopoiesis by the neoplastic clone is a feature of these diseases.[2–5] Therefore, correlations with the JAK2 V617F allele burden using CD34⁺ progenitor cells rather than neutrophils are of interest, since an analysis of CD34⁺ progenitors provides greater insight into the hematopoietic stem cell compartment. Following this approach, we have previously observed that the JAK2 V617F CD34⁺ allele burden better correlated with leukocytosis and splenomegaly than did the JAK2 V617F neutrophil allele burden.[6] Also, there was an additional discrepancy between the JAK2 V617F CD34⁺ and JAK2 V617F neutrophil allele burdens; the JAK2 V617F CD34⁺ was lower than the concomitant neutrophil allele burden in many MPN patients, and when this was the case, clonal assays showed that this discrepancy between the two measures was due to persistence of wild-type clones at the CD34⁺ level. This scenario was most common in ET, and less common in PMF. [6]

However, these associations between the JAK2 V617F CD34⁺ allele burden and the presence of clonal dominance with disease class and disease features were analyzed without adjusting for potentially confounding or mediating variables. Recently, we have shown that sex, age at diagnosis, disease class, and disease duration partially explain variability in the JAK2 V617F neutrophil allele burden, and whether the same factors may influence the JAK2 V617F CD34⁺ allele burden is not known.[7] Additionally, because clonal dominance at the progenitor level appeared to distinguish PMF from ET and PV, it was our objective to identify independent predictors of clonal dominance using a multivariable approach. We hypothesized that clonal dominance would be independently associated with JAK2 V617F homozygosity and disease duration. To test our hypothesis, we analyzed the association between these features and the presence of clonal dominance in 164 JAK2 V617F-positive MPN patients with ET, PV or myelofibrosis.

Methods

Study design and participants

This study was a cross-sectional analysis of 164 consecutive JAK2 V617F-positive MPN patients with ET, PV, or myelofibrosis (MF), (including primary myelofibrosis (PMF), post-ETMF, and post-PVMF) referred to the Johns Hopkins Center for Chronic Myeloproliferative Disorders between May 2005 and March 2010. The diagnosis of PV and ET were based on the Polycythemia Vera Study Group criteria [8,9]; the diagnosis of PMF was based on Italian Consensus criteria. [10,11] MF patients with an antecedent MPN were characterized accordingly as post-ETMF or post-PVMF [11]. The study was approved by an Institutional Review Board and all patients gave written consent.

JAK2 V617F assessment and cell preparation

The JAK2 V617F CD34⁺ progenitor and neutrophil allele burdens were expressed as continuous variables and measured using an allele-specific, quantitative real-time polymerase chain reaction (PCR) assay sensitive to a lower limit of detection of 5% of either the wild-type or mutant JAK2 allele; neutrophil DNA preparation has been previously described.[6] Peripheral blood CD34⁺ cells were isolated from Ficoll buffy coats using

immunomagnetic beads (Miltenyi, Auburn, CA, USA) as previously described. The peripheral blood CD34⁺ cells were 95% positive both for CD34 and CD38, were dimly positive for CD117, were CD41 negative, and were primarily in G0/G1 of the cell cycle, as previously described.[6] Of note, this analysis included CD34⁺ and neutrophil allele burden measurements in 96 previously studied patients (17 ET, 64 PV, and 15 MF), and 68 additional patients.

Clonal dominance

An absolute difference between the measured JAK2 V617F neutrophil and CD34⁺ allele burden of $\leq 10\%$ was considered an indicator of progenitor cell clonal dominance, which was expressed as a binary variable. This definition was based on prior observations of a markedly diminished presence of wild-type JAK2 CD34⁺ progenitors (compared to JAK2 V617F mutated progenitors) from BFU-e assays in samples from patients with congruent (difference $\leq 10\%$) JAK2 V617F CD34⁺ and neutrophil allele burdens.[6]

JAK2 V617F genotype

Genotype was also considered as a binary variable; a heterozygous genotype was determined based on a neutrophil allele burden of $< 55\%$ and a homozygous genotype was determined based on a neutrophil allele burden of $\geq 55\%$. The validity of this cut point as an indicator of genotype was supported by the identification of homozygous colonies in samples from patients with neutrophil allele burdens $\geq 55\%$ [6,1] and confirmation of uniparental disomy (UPD) at 9p via single nucleotide polymorphism (SNP) karyotype analysis in samples with allele burdens $\geq 55\%$ in 96% of cases (manuscript in preparation).

Clinical and laboratory parameters

Disease duration, blood cell counts, and treatment were assessed at the time of JAK2 V617F allele burden measurement. Most patients were not newly diagnosed as reflected in the median disease duration presented in the results. Age at diagnosis and thrombosis history was extracted from the medical records and splenomegaly was measured in centimeters below the costal margin by physical examination.

Statistical analysis

Continuous variables were assessed for normality using the Shapiro-Wilks test. The Kruskal-Wallis one way analysis of variance on ranks was used to assess for differences in group medians; Dunn's method was used when making multiple comparisons. Testing for differences in mean proportions was performed when appropriate with z tests. Multivariable linear regression was used to predict the expected mean JAK2 V617F CD34⁺ or neutrophil allele burden, adjusted for disease type, sex, age at diagnosis, disease duration and hydroxyurea therapy. Regression diagnostics suggested that robust regression be used due to data points with undue influence or leverage. Multivariable logistic regression was used to determine the association between the binary variable, clonal dominance, and disease subgroup, sex, genotype, age at diagnosis, disease duration; model fit was assessed with the Hosmer-Lemeshow test. Multivariable linear or logistic regression were used to determine the association between clonal dominance and clinical phenotype, adjusting for disease type, sex, JAK2 V617F clonal genotype, disease duration, age at diagnosis, white blood cell count and hydroxyurea therapy. Data were analyzed using the STATA SE 10.1 program (STATA Corp, College Station, TX) and SigmaStat 3.5 (Systat Software Inc, Richmond CA).

Results

Baseline characteristics of the cohort

The 164-patient cohort included 30 ET patients, 100 PV patients, 23 PMF patients, 8 post-ETMF patients, and 3 post-PVMF patients (Table 1). The majority of the cohort was women (59%), including 83% of ET patients and 62% of PV patients, though women comprised only 29% of all MF patients. ET patients were significantly younger than those with MF (difference in median 15 years, $p<0.05$), but there was no statistical difference in age between PV and ET patients or between PV and MF patients. Furthermore, there were no differences in disease duration at the time of allele burden analysis within any of the disease subgroups. Nearly one-quarter of the cohort were taking hydroxyurea at the time of JAK2 V617F assessment, but this proportion did not differ statistically between the disease subgroups. The clinical phenotypes of the cohort, with respect to splenomegaly, thrombosis, and laboratory parameters are also shown in Table 1.

Age at diagnosis and disease duration correlate with the JAK2 V617F CD34⁺ allele burden

From the multivariable regression model, the adjusted JAK2 V617F CD34⁺ allele burden was lower in females compared to males (4.3%), but this difference was not statistically significant (Table 2). Each one year increase in age at diagnosis or disease duration was expected to result in a rise of the JAK2 V617F CD34⁺ allele burden by 0.3% ($p=0.03$) and 1.3% ($p<0.001$) respectively, associations that were similar in magnitude when analyzing JAK2 V617F neutrophil allele burdens. The white blood cell count (<0.001) and spleen size (<0.001) were also correlated with the JAK2 V617F CD34⁺ allele burden, but hemoglobin and thrombosis history were not statistically associated with this measure (data not shown). Hydroxyurea therapy was not associated with the JAK2 V617F CD34⁺ allele burden in a univariate analysis, and was therefore not incorporated into the regression model.

The JAK2 V617F allele burden is similar in CD34⁺ progenitors and neutrophils in MF, but not ET or PV

When comparing the adjusted (disease duration, age at diagnosis) JAK2 V617F allele burden in CD34⁺ progenitors and neutrophils, there were differences by disease class. In ET and PV, the adjusted CD34⁺ allele burdens were at least 10% lower than the similarly adjusted neutrophil allele burdens (male ET: 23.7% vs. 42.5%; male PV: 56% vs. 71.1%; female ET 19.4% vs. 37.1%; female PV: 51.6% vs. 65.6%). In contrast, the adjusted JAK2 V617F CD34⁺ and neutrophil allele burdens were within 1% of one another in MF males (62.3% vs. 62.5%) and MF females (58.0% vs. 57.1%) (Table 2). We then compared the allele burdens in a subgroup of MF patients with antecedent ET or PV. Interestingly, MF patients with an antecedent history of either ET or PV had a similar relationship of the CD34⁺ to neutrophil JAK2 V617F allele burden as the de novo PMF patients ($n=11$; mean JAK2 V617F CD34⁺ allele burden vs. mean JAK2 V617F neutrophil allele burden: 66.8% vs. 68%, $p=0.9$).

Clonal dominance is primarily a feature of MF

Next, the association between disease class, sex, age at diagnosis, JAK2 V617F clonal genotype, disease duration, and the presence of clonal dominance were compared using logistic regression. In the univariate analysis, the odds of clonal dominance differed by disease, and was highest when comparing MF to ET (OR 49, 95% CI: 11.1, 214.6; $p<0.001$). Also, females, regardless of disease class, were less likely to be clonally dominant than men (OR 0.49, 95% CI: 0.26, 0.92; $p=0.03$). Each 1 year increase in age at diagnosis was associated with a 1.03 times higher odds of clonal dominance (95% CI 1.01, 1.05; $p=0.01$). A homozygous JAK2 V617F genotype appeared more likely among clonally dominant

patients compared to non-dominant patients, but this association was not significant (OR 1.76, 95% CI: 0.93, 3.3; $p=0.08$) (data not shown). Hydroxyurea therapy was not associated with clonal dominance in the univariate analysis ($p=0.5$).

A multivariable logistic regression model was then constructed to determine whether the association of clonal dominance with disease subgroup remained when adjusted for these potential confounding and mediating variables (Table 3). Due to sex and age differences between clonally dominant and non-dominant patients, these variables were included in the model; it was also hypothesized *a priori* that JAK2 V617F clonal genotype and disease duration would associate with clonal dominance, so these variables were also included in the model.

When using multivariable logistic regression, disease type had the most striking association with clonal dominance. The odds of clonal dominance was nearly 62 times higher for MF patients when compared to ET patients (95% CI: 11, 348, $p<0.001$), and 9.7 times higher when compared to PV patients (95% CI: 3.01, 31.2, $p<0.001$); clonal dominance was also 6.4 times as likely when comparing PV to ET patients (95% CI: 1.63, 25.1; $p=0.008$). These associations held when adjusted for sex, genotype, disease duration, and age at diagnosis. While disease duration ($p=0.01$) and age at diagnosis ($p=0.048$) were statistically associated with the presence of clonal dominance, the magnitudes of association were modest (Table 3). Interestingly, a homozygous JAK2 V617F genotype was not associated with the presence of clonal dominance ($p=0.49$).

Clonal dominance is associated with an increased white blood cell count, a lower hemoglobin, and increased spleen size

Finally, the association between the presence of clonal dominance and clinical phenotype was assessed, using either multivariable linear or logistic regression when appropriate (Table 4). In all regressions, adjustments were made for disease type, sex, JAK2 V617F genotype, disease duration, age at diagnosis, and hydroxyurea therapy. When analyzing continuous variables, those with clonal dominance had higher white blood cell counts (mean increase 2.8k/cu mm; $p=0.009$; 95% CI 0.7, 4.9 k/cu mm increase), lower hemoglobin values (mean decrease 1.1g/dl; $p<0.001$; 95% CI -0.5 , 1.7 g/dl decrease), and on average, an increase in spleen size by 2 cm ($p=0.006$; 95% CI: 0.6, 3.3cm), compared to non-clonally dominant patients. Binary variables were analyzed with logistic regression, and though the odds of past history of thrombosis were 2.1 times higher in clonally dominant patients (compared to non-dominant), this association was not statistically significant ($p=0.087$). However, when looking more specifically, the odds of arterial thrombosis history (compared to no thrombosis history) was 3.2 times higher in clonally dominant patients ($p=0.04$; 95% CI 1.03, 9.8). In this analysis of thrombosis, adjustments were also made for the white blood cell count, in addition to variables previously discussed.

Discussion

As the MPN are stem cell disorders, it is of interest to analyze the JAK2 V617F allele burden in CD34+ progenitors, in addition to neutrophils, to gain insight into disease pathogenesis. In this analysis of 164 MPN patients, we report interesting associations of the JAK2 V617F CD34+ allele burden with clinical features. For example, the JAK2 V617F CD34+ allele burden was influenced by age at diagnosis, and disease duration. Furthermore, the JAK2 V617F CD34+ allele burden was also influenced by disease type, and was lowest in ET compared to PV or MF; the JAK2 V617F CD34+ allele burden was similar in PV and MF patients. Previously, we have shown that the JAK2 V617F CD34+ allele burden better correlated with leukocytosis, reticulocytosis, and splenomegaly than did the neutrophil allele burden.[6] However, in isolation, the JAK2 V617F CD34+ allele burden provides less

insight into the character of the hematopoietic stem cell compartment; in fact, this measure becomes even more informative when comparisons to the JAK2 V617F neutrophil allele burden are made, because congruence of these measures suggests progenitor cell clonal dominance. For example, BFU-e assays in patient samples with congruent JAK2 V617F CD34⁺ and neutrophil allele burdens (difference $\leq 10\%$) revealed a near absence of wild-type JAK2 progenitors.[6]

Following this approach of comparing the JAK2 V617F CD34⁺ and neutrophil allele burdens, we found that the JAK2 V617F CD34⁺ allele burden was lower than the JAK2 V617F neutrophil allele burden in ET and PV patients, but the two measures did not differ in MF patients. The proportion of patients with clonal dominance also differed between MPN subgroups; though PV and MF could not be distinguished by the median CD34⁺ allele burden, the proportion with clonal dominance (concordant JAK2 V617F CD34⁺ and neutrophil allele burdens) was significantly greater among MF patients as compared to PV patients and ET patients.

With regard to predicting the odds of clonal dominance, the most striking and significant adjusted associations were with disease type. Even after adjusting for JAK2 V617F homozygosity and disease duration, clonal dominance at the progenitor level was still far more likely in MF patients compared to ET and PV patients. Progenitor cell clonal dominance was also a feature of post-ET or PV-MF, indicating that this is closely tied to MF biology. Finally, clonal dominance associated with a higher white blood cell count, lower hemoglobin and an increased spleen size, regardless of confounding variables, including disease type and treatment. These associations suggest that progenitor cell clonal dominance may contribute to a clinical phenotype of enhanced myeloproliferation (leukocytosis and splenomegaly). Interestingly, the association between the JAK2 V617F neutrophil allele burden and erythrocytosis[1] seems to be negated by progenitor cell clonal dominance, since lower hemoglobin's were statistically associated with clonal dominance. Though JAK2 V617F neutrophil allele burdens are similar in PV and PMF, it is possible that anemia in PMF may in part, be a consequence of the loss of wild-type JAK2 progenitors.

In this study, disease duration was also independently associated with clonal dominance, implying that, to an extent, clonal dominance may be a time-dependent process.[12] However, the effect of disease duration only had a modest impact on the odds of clonal dominance, especially when considering the magnitude of association of between disease type (e.g. MF) and clonal dominance. Hydroxyurea therapy did not influence results from this analysis, as its use did not differ between MPN subgroups, and it was not associated with clonal dominance in a univariate analysis. Moreover, hydroxyurea therapy does not modify the JAK2 V617F allele burden in neutrophils;[13] the same can only be inferred in the case of CD34⁺ cells.

Our findings contribute to the theory that heterogeneity in the progenitor cell compartment distinguishes the MPN subgroups, and may account for the discrepancy between genotype and phenotype in these disorders. In this model, in ET, the JAK2 V617F clone usually fails to achieve clonal dominance, and JAK2 wild-type clones continue to proliferate, possibly due to an aberrant negative feedback mechanism.[14] In PV, clonal heterogeneity exists still at the progenitor level, with JAK2 wild-type, JAK2 V617F heterozygous and V617F homozygous clones coexisting even in a single patient.[15][16] In contrast, in PMF and MF with antecedent ET or PV, dominance by the neoplastic clone, at the expense of wild-type precursors is present in the majority of cases, indicating a dominant biology of the disease MPN clone at the stem cell level.[17]

The lack of heterogeneity in the stem cell compartment in PMF complements other notable differences from ET and PV with respect to epidemiology, natural history and molecular pathogenesis[18]. For example, as opposed to ET and PV, PMF has a male sex predominance [7] anemia, and a poorer prognosis. Further, PMF has a greater magnitude of change in the JAK2 V617F neutrophil allele burden through the course of the disease than ET or PV.[7]

The mechanism by which the neoplastic clone achieves dominance in PMF is unknown, but may reflect an intrinsic genetic complexity, compared to ET and PV as recently suggested by single-nucleotide polymorphism karyotyping.[19] and is not only germane to disease pathogenesis of the MPN, but also to uncovering mechanisms of leukemic transformation. [20] In our study, JAK2 V617F homozygosity did not independently associate with odds of clonal dominance; this observation indicates that the biological advantage that JAK2V617F homozygous clones have over heterozygous clones may be most relevant to the differentiation of myeloid elements rather than to progenitors with self-renewing capacity. [21] In fact, the influence of JAK2 V617F on stem cell function remains unclear[22,23], but it may not influence gene expression in CD34+ progenitors within ET [24], and in PV, some gene expression changes appear independent from JAK2 V617F [25]. Further, in a JAK2 V617F knock-in model, mutant progenitors did not confer competitive advantage over wild-type hematopoietic stem cells in serial transplantation models [22][26]. Nevertheless, the presence of JAK2 V617F alone remains insufficient to explain the differences in the MPN progenitor compartment. Rather, additional acquired lesions (ASXL1, TET2, CBL, IDH, EZH2, and LNK) which can coexist with or without JAK2 V617F may provide an advantage over wild-type progenitor clones and account for progenitor compartment heterogeneity in the MPN.[27–29]. The segregation of these lesions to specific MPN phenotypes, and their specific biologic effect on hematopoietic stem cell function in the context of JAK2 V617F will be required to infer their pathophysiologic relationship to PMF.

Acknowledgments

This work was supported by grants from the National Institutes of Health (RO1HL082995), (K12HL087169-03), and the National Cancer Institute (PO1-CA108671).

References

1. 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]
2. Adamson JW, Fialkow PJ, Murphy S, Prchal JF, Steinmann L. Polycythemia vera: stem-cell and probable clonal origin of the disease. *N Engl J Med* 1976;295:913–916. [PubMed: 967201]
3. el Kassab N, Hetet G, Li Y, Briere J, Grandchamp B. Clonal analysis of haemopoietic cells in essential thrombocythaemia. *Br J Haematol* 1995;90:131–137. [PubMed: 7786775]
4. Ferraris AM, Mangerini R, Racchi O, et al. Heterogeneity of clonal development in chronic myeloproliferative disorders. *Am J Hematol* 1999;60:158–160. [PubMed: 9929111]
5. Catlin SN, Gutter P, Abkowitz JL. The kinetics of clonal dominance in myeloproliferative disorders. *Blood* 2005;106:2688–2692. [PubMed: 16002428]
6. Moliterno AR, Williams DM, Rogers O, Isaacs MA, Spivak JL. Phenotypic variability within the JAK2 V617F-positive MPD: roles of progenitor cell and neutrophil allele burdens. *Exp Hematol* 2008;36:1480–1486. [PubMed: 18723264]
7. Stein B, Williams D, Wang NY, et al. Sex differences in the JAK2V617F allele burden in the chronic myeloproliferative disorders. *Haematologica*. 2010
8. Berk PD, Goldberg JD, Donovan PB, Fruchtman SM, Berlin NI, Wasserman LR. Therapeutic recommendations in polycythemia vera based on Polycythemia Vera Study Group protocols. *Semin Hematol* 1986;23:132–143. [PubMed: 3704665]

9. Murphy S, Peterson P, Iland H, Laszlo J. Experience of the Polycythemia Vera Study Group with essential thrombocythemia: a final report on diagnostic criteria, survival, and leukemic transition by treatment. *Semin Hematol* 1997;34:29–39. [PubMed: 9025160]
10. Barosi G. Myelofibrosis with myeloid metaplasia: diagnostic definition and prognostic classification for clinical studies and treatment guidelines. *J Clin Oncol* 1999;17:2954–2970. [PubMed: 10561375]
11. Mesa RA, Verstovsek S, Cervantes F, et al. Primary myelofibrosis (PMF), post polycythemia vera myelofibrosis (post-PV MF), post essential thrombocythemia myelofibrosis (post-ET MF), blast phase PMF (PMF-BP): Consensus on terminology by the international working group for myelofibrosis research and treatment (IWG-MRT). *Leuk Res* 2007;31:737–740. [PubMed: 17210175]
12. Tefferi A, Lasho TL, Gilliland G, Kralovics R, Cazzola M, Skoda RC. JAK2 Mutations in Myeloproliferative Disorders. *N Engl J Med* 2005;353:1416–1417. [PubMed: 16192494]
13. Larsen TS, Pallisgaard N, de Stricker K, Moller MB, Hasselbalch HC. Limited efficacy of hydroxyurea in lowering of the JAK2 V617F allele burden. *Hematology* 2009;14:11–15. [PubMed: 19154659]
14. Lambert JR, Gale RE, Linch DC. The production of JAK2 wild-type platelets is not downregulated in patients with JAK2 V617F mutant-positive essential thrombocythaemia. *Br J Haematol* 2009;145:128–130. [PubMed: 19222478]
15. Dupont S, Masse A, James C, et al. The JAK2 617V>F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. *Blood* 2007;110:1013–1021. [PubMed: 17389763]
16. Li S, Kralovics R, De Libero G, Theocharides A, Gisslinger H, Skoda RC. Clonal heterogeneity in polycythemia vera patients with JAK2 exon12 and JAK2-V617F mutations. *Blood* 2008;111:3863–3866. [PubMed: 18195094]
17. James C, Mazurier F, Dupont S, et al. The hematopoietic stem cell compartment of JAK2V617F-positive myeloproliferative disorders is a reflection of disease heterogeneity. *Blood* 2008;112:2429–2438. [PubMed: 18612101]
18. Stein BL, Moliterno AR. Primary myelofibrosis and the myeloproliferative neoplasms: the role of individual variation. *JAMA* 2010;303:2513–2518. [PubMed: 20571018]
19. Stegelmann F, Bullinger L, Griesshammer M, et al. High-resolution single-nucleotide polymorphism array-profiling in myeloproliferative neoplasms identifies novel genomic aberrations. *Haematologica* 2010;95:666–669. [PubMed: 20015882]
20. Beer PA, Delhommeau F, LeCouedic J, et al. Two routes to leukemic transformation after a JAK2 mutation-positive myeloproliferative neoplasm. *Blood* 2010;115:2891–2900. [PubMed: 20008300]
21. Passamonti F, Rumi E, Pietra D, et al. Relation between JAK2 (V617F) mutation status, granulocyte activation, and constitutive mobilization of CD34+ cells into peripheral blood in myeloproliferative disorders. *Blood* 2006;107:3676–3682. [PubMed: 16373657]
22. Li J, Spensberger D, Ahn JS, et al. JAK2 V617F impairs hematopoietic stem cell function in a conditional knock-in mouse model of JAK2 V617F-positive essential thrombocythemia. *Blood* 2010;116:1528–1538. [PubMed: 20489053]
23. Skoda RC. JAK2 impairs stem cell function? *Blood* 2010;116:1392–1393. [PubMed: 20813904]
24. Catani L, Zini R, Sollazzo D, et al. Molecular profile of CD34+ stem/progenitor cells according to JAK2V617F mutation status in essential thrombocythemia. *Leukemia* 2009;23:997–1000. [PubMed: 19151790]
25. Berkofsky-Fessler W, Buzzai M, Kim MK, et al. Transcriptional profiling of polycythemia vera identifies gene expression patterns both dependent and independent from the action of JAK2V617F. *Clin Cancer Res* 2010;16:4339–4352. [PubMed: 20601445]
26. Mullally A, Lane SW, Ball B, et al. Physiological Jak2V617F expression causes a lethal myeloproliferative neoplasm with differential effects on hematopoietic stem and progenitor cells. *Cancer Cell* 2010;17:584–596. [PubMed: 20541703]
27. Carbuccioni N, Murati A, Trouplin V, et al. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia* 2009;23:2183–2186. [PubMed: 19609284]

28. Oh ST, Simonds EF, Jones C, et al. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood* 2010;116:988–992. [PubMed: 20404132]
29. 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. [PubMed: 20428194]

Table 1

Baseline characteristics of the Myeloproliferative Neoplasm Cohort

Number=	ET 30	PV 100	* MF 34	P value: ET vs. PV	P value: ET vs. MF	P value: PV vs. MF
Female (%)	25 (83)	62 (62)	10(29)	0.03	<0.001	<0.001
Age at diagnosis (IQR)	46 (30, 61)	54 (44, 66)	61 (54, 65)	NS	<0.05	NS
Disease duration (IQR)	5 (3, 10)	5 (2, 10)	3 (1, 10)	NS	NS	NS
**% Splenomegaly	0	43	91	<0.001	<0.001	<0.001
**% Thrombosis history (n)	23 (7)	29 (29)	13 (4)	NS	NS	NS
Thrombosis type, number						
Arterial only	5	17	0			
Venous only	2	9	3			
Both	0	3	1			
WBC (k/mm ³) (IQR)	7.5 (6.5, 9.0)	13.3 (9.1, 21.5)	15.1 (7.4, 27.4)	<0.05	<0.05	NS
Plt (k/mm ³) (IQR)	605 (489, 792)	489 (337, 789)	268 (164, 477)	NS	<0.05	<0.05
Hgb (g/dL) (IQR)	14.1 (13.3, 14.7)	12.1 (11.9, 14.0)	11.3 (9.2, 12.4)	ND	ND	ND
Retic (k/mm ³) (IQR)	54(45, 71)	89 (69, 103)	92(63, 132)	<0.05	<0.05	NS
**% Hydroxyurea	23	19	24	NS	NS	NS
%Neutrophil allele burden (IQR)	38 (33, 45)	66 (53, 81)	58 (48, 79)	<0.05	<0.05	NS
% CD34 allele burden (IQR)	15 (10, 28)	55 (37, 70)	62 (50, 77)	<0.05	<0.05	NS
**% Homozygous	0	74	65	<0.001	<0.001	NS
**% Clonal dominance (n)	13 (4)	42 (42)	88 (30)	0.004	<0.001	<0.001

Median values with the interquartile range are presented unless otherwise stated; hypothesis testing with the Kruskal-Wallis one way analysis of variance on ranks followed by Dunn's method for multiple comparison.

* Includes 23 patients with PMF; 8 patients with post-ET MF and 3 patients with post-PV MF

** Mean difference in proportions compared for hypothesis testing (z test)

NS=Not statistically significant

ND=Not done; hemoglobin altered by phlebotomy in PV and therefore not analyzed

IQR = interquartile range; WBC= white blood cell count; Plt=platelet; Hgb=Hemoglobin; Retic=reticulocyte count

Table 2

Determinants of JAK2 V617F variability: * Multivariable linear regression

	CD34+ Progenitors			Neutrophils		
	Adjusted JAK2 allele burden (Males)	Expected % change to JAK2 allele burden (95% CI)	P value	Adjusted JAK2 allele burden (Males)	Expected % change to JAK2 allele burden (95% CI)	P value
ET	23.7			42.5		
PV	56			71.1		
MF	62.3			62.5		
Female Sex		-4.3 (-11.8, 2.8)	0.23		-5.4 (-11.6, 0.71)	0.08
Age at diagnosis (1 year increase)		0.33 (0.09, 0.57)	0.008		0.19 (-0.02, 0.40)	0.08
Disease duration (1 year increase)		1.3 (0.72, 1.84)	<0.001		0.85 (0.36, 1.34)	0.001

* A single model was constructed using robust regression was used to downweight the influence of outliers identified during model checking procedures. Disease was considered a categorical variable, and interaction terms were explored, but none were found to be statistically significant.

Table 3

Multiple logistic regression: Predicting the odds of clonal dominance

	Odds Ratio	P value	95% Confidence interval
PV vs. ET	6.4	0.008	1.63, 25.1
MF vs. ET	61.9	<0.001	11, 348
MF vs. PV	9.7	0.002	3.01, 31.2
Female vs. Male sex	1.05	0.9	0.48, 2.31
Homozygous vs. Heterozygous genotype	0.69	0.42	0.28, 1.68
Disease duration (1 year increase)	1.1	0.01	1.02, 1.17
Age at diagnosis (1 year increase)	1.03	0.048	1.00, 1.06

Table 4

Phenotypic associations in those with clonal dominance compared to those without clonal dominance

	Measure of association	Magnitude	P value	95% Confidence interval
White blood cell count (k/cu mm)	MLR	+ 2.8	0.009	(0.7, 4.9)
Hemoglobin (g/dl)	MLR	−1.1	<0.001	(−1.7, 0.5)
Spleen size (cm)	MLR	+2	0.006	(0.6, 3.3)
Arterial thrombosis	MLogR	3.2	0.04	(1.03, 9.8)

MLR: Multivariable linear regression; magnitude of association reflects the mean increase in the parameter compared non-clonally dominant patients; adjustments made for disease type, sex, JAK2 V617F clonal genotype, age at diagnosis, disease duration and hydroxyurea therapy; MLogR; multivariable logistic regression; magnitude of association reflects the odds ratio comparing the parameter in clonally-dominant patients to that of non-dominant patients. Adjustments made for disease type, sex, JAK2 V617F clonal genotype, age at diagnosis, disease duration, hydroxyurea therapy, and white blood cell count.