Published in final edited form as:

Clin Lymphoma Myeloma Leuk. 2016 August ; 16 Suppl: S105–S113. doi:10.1016/j.cml.2016.02.031.

Prognosis of primary myelofibrosis in the genomic era

Prithviraj Bose and

Department of Leukemia, University of Texas MD Anderson Cancer Center, Houston, TX, USA

Srdan Verstovsek

Abstract

Currently, prognostication in primary myelofibrosis (PMF) relies on the International Prognostic Scoring System (IPSS), dynamic IPSS (DIPSS) and DIPSS-plus, which incorporate age, blood counts, constitutional symptoms, circulating blasts, red cell transfusion need and karyotype. Although the *JAK2* V617F mutation was discovered a decade ago and *MPL* mutations shortly thereafter, it was the recent discovery of *CALR* mutations in the vast majority of *JAK2/MPL*-unmutated patients and recognition of the powerful impact of *CALR* mutations and “triple negative” (*JAK2/MPL/CALR*-negative) status on outcome that set the stage for revision of traditional prognostic models to include molecular information. Additionally, the advent of next-generation sequencing has identified a host of previously unrecognized somatic mutations across hematologic malignancies. Like in the myelodysplastic syndromes, the majority of common and prognostically informative mutations in PMF affect epigenetic regulation and mRNA splicing. Thus, a need has arisen to incorporate mutational information on genes such as *ASXL1* and *SRSF2* into risk stratification systems. Mutations in yet other genes appear to be important players in leukemic transformation, and new insights into disease pathogenesis are emerging. Finally, the number of prognostically detrimental mutations may affect both survival and response to ruxolitinib, which has significant implications for clinical decision making. In this review, we briefly summarize the prognostic models in use today and discuss in detail the somatic mutations commonly encountered in patients with PMF, along with their prognostic implications and role in leukemic transformation. Emerging prognostic models that incorporate new molecular information into existing systems or exclude clinical variables are also presented.

Keywords

prognosis; primary myelofibrosis; mutations; survival; leukemic transformation

Corresponding author: Prithviraj Bose, MD, Assistant Professor, Dept of Leukemia, 1400 Holcombe Blvd, FC4.3062, Unit 428, Houston, TX 77030, Phone: 713-792-7747, Fax: 713-792-7305, pbose@mdanderson.org.

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

Primary myelofibrosis (PMF), the most aggressive of the classic Philadelphia chromosome-negative (Ph⁻) myeloproliferative neoplasms (MPNs),¹ is a clonal, stem-cell disorder clinically characterized by anemia, splenomegaly, extramedullary hematopoiesis, a variety of constitutional symptoms, and relatively short survival.² In a Swedish population-based study of 9,384 individuals with Ph⁻ MPNs diagnosed from 1973 through 2008, survival was found to have improved significantly over time; however, the improvement was less pronounced after the year 2000 and was confined to patients with polycythemia vera (PV) and essential thrombocythemia (ET).³ Another European study examined survival trends amongst patients diagnosed with PMF between 1980 and 1995 (n=434) and between 1996 and 2007 (n=368), and found a significant improvement in median survival between the two eras (4.6 vs. 6.5 years); however, reduction in disease-specific mortality was restricted to the lower risk categories (discussed below), with no improvement in survival of intermediate-2 or high risk patients.⁴ Recently, the Janus kinase (JAK) 1/2 inhibitor, ruxolitinib, approved by the Food and Drug Administration (FDA) for the treatment of patients with intermediate or high risk patients with PMF, post-PV or post-ET MF, has demonstrated a survival benefit for these poor risk categories of patients in randomized, controlled clinical trials,^{5,6} in comparison to matched historical controls,^{7,8} as well as in a meta-analysis of pivotal, registration trials in the United States and Europe.⁹

Current prognostic classification systems in PMF

Although a number of prognostic scoring systems have been used over the years (reviewed in ref.¹⁰), robust prognostic modelling in PMF began with the publication of the International Prognostic Scoring System (IPSS) by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) in 2009.¹¹ This simple and widely used system uses 5 clinical variables - age >65 years, constitutional symptoms, hemoglobin <10 g/dl, leukocyte count >25 × 10⁹/l and circulating blasts 1% - each assigned one point, to delineate four prognostic categories: low, intermediate-1, intermediate-2 and high. The respective median survivals in these four categories were 135, 95, 48 and 27 months in the original cohort of 1054 consecutively diagnosed PMF patients.¹¹ In the IPSS dataset, patients without splenomegaly at diagnosis survived longer than those with splenomegaly, but the difference did not reach statistical significance, and including splenomegaly at diagnosis as a variable did not improve the prognostic model.¹¹

The IPSS risk factors were then validated at later time points, leading to the development of the Dynamic IPSS (DIPSS), which can be used at any time point in the patient's clinical course.¹² Anemia (hemoglobin <10 g/dl) was assigned 2 points in this model, with the other IPSS clinical variables receiving 1 point each. Median survivals were not reached, 14.2, 4 and 1.5 years for low, intermediate-1, intermediate-2 and high risk patients, respectively.¹² Importantly, the DIPSS has also been shown to predict progression to blast phase (BP) in PMF.¹³ Additionally, its usefulness in predicting outcomes after allogeneic hematopoietic cell transplantation (HCT) has been shown.¹⁴

Recognition that unfavorable cytogenetic abnormalities,¹⁵⁻¹⁸ red blood cell (RBC) transfusion dependence¹⁹ and thrombocytopenia^{15,17} impact prognosis in PMF led to the refinement of the DIPSS into the DIPSS-plus,²⁰ which adds these three adverse features to DIPSS risk. Thus, 1 point each is assigned to DIPSS intermediate-1 risk, unfavorable karyotype (defined as complex karyotype, or single or two abnormalities including +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3) or 11q23 rearrangement), platelets $<100 \times 10^9/l$, and RBC transfusion need.²⁰ DIPSS intermediate-2 and high risk are assigned 2 and 3 points, respectively. Low (0 points), intermediate-1 (1 point), intermediate-2 (2-3 points) and high (4-6 points) risk patients had median survivals of 180, 80, 35 and 16 months, respectively.²⁰ A model that assigns 2 points to “very high risk” cytogenetics (monosomal karyotype or inv(3)/i(17q)) and 1 point each to circulating blasts $\geq 2\%$ and platelets $<50 \times 10^9/l$ to classify patients as being at low (0 points), intermediate (1 point) or high (2 points) risk for leukemic transformation (LT) has been proposed by the IWG-MRT, with 3-year LT rates of 3%, 10% and 35%, respectively.²¹

Driver mutations in the Ph⁻ MPNs

A driver mutation is one that confers a selective advantage to a cell with self-renewal capacity, leading to the formation of a clone of mutated cells.²² Driver mutations can be founding (initiating) mutations, which give rise to the initial clone of a malignancy, or sub-clonal (cooperating) mutations, which occur in an already established clone and generate sub-clones carrying both the founding and the newly acquired mutation.²² Sub-clonal mutations are commonly associated with disease progression.²² In the case of Ph⁻ MPNs in particular, an important concept is that the founding “driver” mutations, while largely “driving” disease phenotype, are not necessarily the first somatic mutations leading to the development of these disorders.²² This is elucidated in the following paragraphs, as well as in the next section.

JAK2 mutations

The observation that acquired uniparental disomy (UPD) of chromosome 9p (loss of heterozygosity (LOH) due to mitotic recombination) was a frequent stem cell defect in PV²³ set the stage for the discovery in 2005 of the activating *JAK2* V617F mutation,²⁴⁻²⁷ found in approximately 95% of patients with PV and 50-60% of patients with ET and PMF. This mutation in the “pseudokinase” domain of JAK2 is unique to myeloid malignancies and removes its inhibitory influence on the catalytic domain, leading to constitutive activation of the kinase.²⁸ A specific constitutional *JAK2* haplotype, designated 46/1 (GGCC), confers MPN susceptibility by preferentially acquiring the V617F mutation,^{29,30} as does a germline *JAK2* single-nucleotide polymorphism (SNP), rs10974944.³¹ In addition to the well-known canonical actions of JAKs in transducing signals from membrane-bound cytokine and hematopoietic growth factor receptors, both wild type and mutant JAK2 translocate to the nucleus and phosphorylate histone H3 to regulate gene expression.³² Furthermore, mutant JAK2 phosphorylates the protein arginine methyltransferase PRMT5 with much greater affinity than wild type JAK2, leading to decreased methyltransferase activity and increased myeloproliferation.³³

Expression of *JAK2* V617F in mice induces a PV-like disease with secondary myelofibrosis,³⁴⁻³⁸ although experimental manipulation of the *JAK2* V617F allele burden can result in ET- or PMF-like phenotypes.^{39,40} *JAK2* V617F homozygous mice develop a severe hematopoietic stem cell defect, suggesting that additional lesions are needed to sustain clonal expansion.⁴¹ While homozygosity for *JAK2* V617F is most common in patients with PV, the mutant allele burden in patients with PMF is often equally high.⁴² In fact, the *JAK2* V617F allele burden is extremely low in hematopoietic stem cells (HSCs) from PV and ET patients at diagnosis, rising only at late stages of hematopoiesis,^{43,44} whereas it is much higher in HSCs from patients with PMF or post-PV/ET MF.^{45,46} The *JAK2* V617F mutation appears to provide only a minor advantage to HSCs, such that on its own, it would cause disease with a very long latency.^{47,48} Therefore, cooperation with other genetic events modifying HSC biology would greatly facilitate the development of the MPN phenotype.⁴⁹ It has been suggested that *JAK2* V617F-bearing HSCs remain harmless for a long time, until genetic or environmental changes such as hematopoietic stress or aging allow clonal dominance and MPN emergence.⁴⁹ Indeed, PMF has been considered an “accelerated phase” of the classic Ph⁻ MPNs.^{49,50} Finally, there is considerable evidence to support the acquisition of *JAK2* V617F as being a late event in at least some patients with Ph⁻ MPNs,⁵¹⁻⁵⁴ and nullizygosity for the *JAK2* 46/1 haplotype has been associated with shortened survival regardless of the presence or absence of the V617F mutation.⁵⁵ Taken together, these observations point to the underlying genomic complexity of Ph⁻ MPNs, particularly PMF, and suggest that other genetic lesions are also involved in disease pathogenesis, consistent with the two-hit theory of leukemogenesis.⁴⁹

A low, rather than high, *JAK2* V617F allele burden has been associated with inferior survival and LFS in PMF.^{56,57} While some studies have linked *JAK2* V617F positivity to poorer survival and a higher risk of LT in PMF,^{58,59} others have not,⁶⁰ and the mutation is often lost upon progression to BP.^{61,62}

MPL mutations

Approximately 5-10% of patients with PMF bear activating mutations in *MPL*, the gene encoding the thrombopoietin receptor, most commonly W515L/K affecting exon 10.⁶³⁻⁶⁵ *MPL* mutations in PMF also arise at the stem cell level⁶⁶ and are generally mutually exclusive of *JAK2* mutations. As in the case of *JAK2* V617F, acquired UPD (of chromosome 1p) has been linked to homozygosity for *MPL* W515L,^{67,68} and the 46/1 *JAK2* haplotype also predisposes to *MPL* exon 10 mutations.⁶⁹

CALR mutations

Recently, activating mutations in the endoplasmic reticulum (ER) chaperone protein, calreticulin (CALR) were discovered in the vast majority of patients with *JAK2*/*MPL*-unmutated ET (67-71%) and PMF (56-88%).^{70,71} *CALR* mutations were not described in PV, of which nearly all cases bear activating mutations in *JAK2*, either in exon 14 (V617F) or 12.⁷²⁻⁷⁴ All *CALR* mutations described to date (predominantly “type 1” 52-base pair deletions in PMF and less frequently “type 2” 5-base pair insertions) appear to be mutually exclusive of *JAK2* and *MPL* mutations, affect exon 9 and result in the generation of a protein

with an abnormal carboxyl terminal with loss of the ER retention motif and impaired calcium binding.⁷⁰

Patients who lack any of the afore-mentioned founding driver mutations are said to have “triple negative” disease (up to 10%).^{75,76} A key concept is the uniform activation of the JAK-STAT (signal transducer and activator of transcription) pathway in Ph⁺ MPNs and hence, susceptibility to therapeutic JAK inhibition.⁷⁷ A transcriptional signature consistent with activated JAK2 signaling is seen in all Ph⁺ MPN patients regardless of clinical phenotype or mutational status.⁷⁸ Indeed, the clinical benefit of ruxolitinib in patients with PMF or post-PV/ET MF in the pivotal COMFORT trials was observed regardless of *JAK2* mutation status,^{79,80} and the drug has specifically been shown to be an effective treatment for patients with *CALR*-mutated PMF.⁸¹

Two large studies have examined the clinical features and outcomes of molecularly annotated patients with PMF.^{75,76} In the Italian study in 617 subjects, median survival was 17.7 years in *CALR*-mutant, 9.2 years in *JAK2*-mutant, 9.1 years in *MPL*-mutant, and 3.2 years in triple-negative patients.⁷⁵ Patients with *CALR* mutation were younger, had higher platelet counts, and a lower risk of developing anemia, thrombocytopenia, and marked leukocytosis compared with other subtypes.^{75,76} Both studies demonstrated a favorable effect of *CALR* mutations on survival and LFS and documented the poor survival and LFS of “triple negative” patients. Furthermore, the impact of genetic lesions on survival was independent of the current prognostic scoring systems.^{75,76} However, in contrast to the Mayo Clinic experience,⁸² the Italian investigators did not find major differences in phenotype or prognostic impact between type 1 and type 2 *CALR* mutations.⁷⁵ The favorable effect of *CALR* mutations on survival and the poor survival of triple negative patients as compared to *JAK2/MPL*-mutated patients has also been shown in the post-allogeneic HCT setting in a German study (n=133, 97 PMF, 36 post-PV/ET MF) on univariate analysis.⁸³ However, on multivariate analysis, only older age and female donor sex remained independent predictors of shorter survival, with *CALR* mutation status showing a trend for better survival.⁸³

The Italian investigators also developed as a proof-of-concept a “clinical molecular prognostic model” to see if information on *JAK2*, *MPL* and *CALR* mutation status improves IPSS-based risk stratification.⁷⁵ They assigned 1 point each for presence of constitutional symptoms, circulating blasts ≥ 1%, hemoglobin <10 g/dl and presence of a *JAK2* mutation, and 2 points each for presence of a *MPL* mutation, triple negativity, leukocytes >25 × 10⁹/l, and age >65 years.⁷⁵ The resulting risk categories were very low (score 0), low (score 1), intermediate (score 2-3), high (score 4-5), and very high (score ≥ 6). The survival curves generated in this manner were significantly different, and the model performed better than the IPSS using established statistical criteria.⁷⁵

Mutations in negative regulators of JAK2

A small proportion of patients with Ph⁺ MPNs bear mutations in *CBL* and *LNK*, encoding two negative regulators of JAK2 signaling. Loss-of-function mutations in the inhibitory adaptor protein LNK were first described in two patients with *JAK2* V617F-negative MPNs as a novel mechanism of JAK-STAT activation.⁸⁴ *LNK* mutations are not mutually

exclusive, however, of *JAK2* V617F and may be more common in BP PMF.⁸⁵ Gain-of-function mutations in *CBL* are strongly associated with acquired UPD of chromosome 11q, ultimately leading to loss of its tumor suppressor function through loss of the wild type alleles.^{86,87} *CBL* mutations have been reported to occur in 6% of PMF patients,⁸⁸ and may play a larger role during LT.^{49,89}

Mutations in epigenetic regulators, splicing genes and genes involved in leukemic transformation

Mutations in a host of other genes are commonly encountered in patients with Ph⁻ MPNs, particularly PMF. Most of these affect epigenetic regulators and spliceosome components, as in the myelodysplastic syndromes (MDS).⁹⁰ The major ones, especially those that have been shown to impact prognosis in PMF, are discussed below. Less frequently occurring mutations (3-8% of patients) in other genes (*SCRIB*, *MIR662*, *BARD1*, *TCF12*, *FAT4*, *DAP3*, *POLG*, *NRAS*) have also been described.⁹¹

TET2 mutations

The Ten-Eleven-Translocation (TET) enzymes hydroxylate 5-methylcytosine (mC) to produce 5-hydroxymethylcytosine (5hmC).⁹²⁻⁹⁴ Although named after *TET1*, located on chromosome 10, *TET2* is located on chromosome 4q24, and UPD and LOH of 4q24 have been associated with *TET2* mutations in MDS⁹⁵ and MDS/MPN overlap syndromes.⁹⁶ In animal models, loss of *TET2* leads to progressive enlargement of the HSC compartment and eventual myeloproliferation, including splenomegaly, monocytosis, and extramedullary hematopoiesis.⁹⁷ 5hmC is believed to represent a step towards cytosine demethylation,⁴⁹ which is consistent with the observed clinical benefit from hypomethylating agents in *TET2*-mutated MDS.⁹⁸ *TET2* mutations occur across the spectrum of myeloid malignancies, and in the original report, were found in 12% of patients with Ph⁻ MPNs.⁹⁹ Importantly, *TET2* mutations can precede the acquisition of *JAK2* V617F in Ph⁻ MPNs,⁹⁹ or occur as a late event during progression.¹⁰⁰ It was recently demonstrated that the order in which *TET2* mutations and *JAK2* V617F are acquired influences clinical characteristics, response to ruxolitinib *in vitro*, stem and progenitor cell biology and clonal evolution in the Ph⁻ MPNs.¹⁰¹ *TET2* mutations increase in frequency with age, even in healthy individuals, and are associated with clonal hematopoiesis and an increased risk of future hematologic cancers.¹⁰²⁻¹⁰⁴ In one study, no effect on survival or LT was found among 60 PMF patients, 17% of whom had *TET2* mutations,¹⁰⁵ but other investigators have found *TET2* mutations to be associated with LT^{89,106} and poor outcomes.¹⁰⁷

IDH1/2 mutations

Mutations in the genes encoding the isocitrate dehydrogenase 1 and 2 (IDH1/2) enzymes of the Krebs' cycle result in the production of the abnormal metabolite 2-hydroxyglutarate (2HG) instead of the normal α -ketoglutarate (α KG) from isocitrate.^{49,108} In *IDH1/2*-mutant acute myeloid leukemia (AML), 2HG inhibits the function of TET2, an α KG-dependent enzyme, leading to a hypermethylation phenotype and impaired hematopoietic differentiation.¹⁰⁹ Thus, *TET2* loss-of-function mutations are associated with similar epigenetic defects as *IDH1/2* mutants, and the two are mutually exclusive in AML.¹⁰⁹

IDH1/2 mutations have been implicated in the progression of chronic phase (CP) Ph⁺ MPNs to AML.^{106,110,111} In an analysis of *IDH1/2* mutations among 200 patients with CP (n=166) or BP (n=34) MPNs, mutational frequencies were approximately 21% (7 of 34) for BP MPNs and approximately 4% (3 of 77) for PMF. Only 1 of 12 patients with paired samples available had *IDH* mutations both in CP and BP.¹¹² In a much larger, multi-institutional study of 1473 patients with Ph⁺ MPNs, the *IDH* mutation frequencies were virtually identical: 4.2% in PMF and 21.6% in BP MPNs.¹¹³ *IDH*-mutated patients were more likely to be nullizygous for the *JAK2* 46/1 haplotype, especially in PMF. In CP PMF, *JAK2* 46/1 haplotype nullizygosity, but not *IDH* mutational status had an adverse effect on survival.¹¹³ In contrast, in both BP PMF and BP MPN, the presence of an *IDH* mutation predicted worse survival.¹¹³ However, in a follow-up study in 301 consecutive patients with CP PMF, *IDH* mutations predicted for worse survival and LFS, particularly in *JAK2* V617F-positive patients.¹¹¹

EZH2 mutations

Enhancer of Zeste homolog 2 (*EZH2*) is a Polycomb group protein that interacts with DNA methyltransferases (DNMTs) within the context of Polycomb repressive complexes 2 and 3 (PRC2/3) and controls the binding of DNMTs to the promoters of several genes repressed by methylation, serving as a recruitment platform for DNMTs.¹¹⁴ *EZH2* encodes the catalytic subunit of PRC2, a highly conserved histone H3 lysine 27 (H3K27) methyltransferase that mediates repression of gene transcription.¹¹⁵ *EZH2* acts as a tumor suppressor gene in myeloid malignancies,¹¹⁶ and homozygous inactivating mutations in *EZH2* were first described in individuals with acquired UPD of chromosome 7q, abnormalities of which are common in myeloid neoplasms.¹¹⁷ In the original report, *EZH2* mutations were found in 13% of patients with myelofibrosis.¹¹⁷ In a subsequent study of 370 subjects with PMF and 148 with post-PV/ET MF, *EZH2* mutations were detected in 5.9% of PMF, 1.2% of post-PV MF, and 9.4% of post-ET MF patients.¹¹⁸ *EZH2*-mutated patients had significantly poorer survival and LFS; on multivariate analysis, inferior survival was predicted only by IPSS high risk, low *JAK2* V617F allele burden and mutated *EZH2*.¹¹⁸ In another study, *EZH2* mutations were detected in 3 of 46 (7%) cases of PMF from one institution and none of 25 from another, as well as in no cases of post-PV/ET MF (n=22) or BP MPN (n=11); no association with clinical outcome was observed in this study.¹¹⁹

ASXL1 mutations

Additional sex combs like 1 (*ASXL1*) is a PRC gene required for long-term repression of homeobox genes.¹²⁰ It is part of a novel complex (Polycomb repressive deubiquitinase, PR-DUB) that removes monoubiquitin from histone H2A in nucleosomes, an action opposite to that of PRC1 and the related dRing-associated factors (dRAF) complex, thus helping maintain a dynamic balance between H2A ubiquitination and deubiquitination.¹²¹ In a small study (n=64), heterozygous *ASXL1* mutations were found in one out of 35 cases of ET, 3 out of 10 cases of PMF (one in accelerated phase) and one out of 5 cases of BP MPNs (a case of post-ET AML).¹²² In a study of 63 patients with AML arising from a pre-existing Ph⁺ MPN, *ASXL1* mutations were found in 19.3%, and were almost always detected in paired samples from individual patients, suggesting that they were not acquired at LT.¹⁰⁶ Like *TET2* mutations, *ASXL1* mutations can precede the acquisition of *JAK2* V617F.¹⁰⁶ In

a larger, multi-institutional cohort, the frequency of *ASXL1* mutations was found to be 12-13% in PMF and 23% in post-PV/ET MF.¹¹⁹ While an adverse impact on survival was suggested on univariate analysis, this was lost upon multivariate analysis that included DIPSS risk.¹¹⁹ However, a *CALR/ASXL1* mutations-based prognostic model was found to be DIPSS-plus independent in a large international study (n=570) of PMF patients.¹²³ Survival was the longest in *CALR*⁺*ASXL1*⁻ (median 10.4 years) and shortest in *CALR*⁻*ASXL1*⁺ patients (median 2.3 years). *CALR*⁺*ASXL1*⁺ and *CALR*⁻*ASXL1*⁻ patients had similar survival and were grouped together in an intermediate-risk category (median survival 5.8 years).¹²³

Spliceosome mutations

Somatic mutations in spliceosomal genes, e.g., *SF3B1*, *U2AF1*, *SRSF2*, *ZRSR2*, etc., resulting in altered pre-mRNA splicing patterns, are ubiquitous across myeloid and lymphoid leukemias, and represent a recently discovered leukemogenic pathway.¹²⁴ In the original publication, spliceosome pathway mutations were reported to occur in 9.4% of patients with Ph⁻ MPNs.¹²⁵ In a subsequent study in PMF, monoallelic mutations in *SRSF2* were found in 32 of 187 (17%) patients, and correlated with shortened survival and LFS.¹²⁶ There was significant clustering with *IDH* mutations, and the adverse impact on survival was independent of both DIPSS-plus and *IDH* mutations.¹²⁶ In contrast, *SF3B1* mutations, common in MDS with ringed sideroblasts,¹²⁷ MDS/MPN overlap syndromes¹²⁸ and chronic lymphocytic leukemia,^{129,130} were found in only 10 of 155 (6.5%) of patients with PMF and did not influence survival.¹³¹

TP53 mutations

Mutations in *TP53* are strongly associated with progression of Ph⁻ MPNs to AML.^{89,107,132-134} In one study,¹³² somatic mutations in *TP53* were found in 27.3% of Ph⁻ MPN patients in whom LT had occurred, and 18.18% of patients had amplifications of chromosome 1q, which harbors *MDM4*, encoding a negative regulator of p53 frequently overexpressed in cancer.¹³⁵ *TP53* mutations can be present in the heterozygous state for prolonged periods during the chronic phase of MPNs without clonal expansion; however, loss of the wild type allele by chromosomal deletion or UPD leads to rapid expansion of the hemi- or homozygous mutant *TP53* clone, ultimately causing LT.¹⁰⁷ The low allele burden of mutant *TP53* in the chronic phase of MPNs suggests the need for sensitive methods such as next-generation sequencing (NGS) for reliable detection, as this is clearly a high-risk group of patients.¹⁰⁷

DNMT3A mutations

Loss-of-function mutations in DNA methyltransferase 3A (*DNMT3A*) are common in AML¹³⁶ and are associated with poor outcomes in both AML and MDS.^{136,137} In a study of 80 patients with CP MPNs (30 PV, 30 ET and 20 MF, including PMF and post-PV/ET MF) and 35 with BP MPNs, 12 *DNMT3A* sequence variants were identified (10% overall; BP MPNs, 17%; MF, 15%; PV, 7%; ET, 3%), of which five could be proved to be of somatic origin.¹³⁸ In a cohort of 46 patients with PMF, 22 with post-PV/ET MF, 11 with BP MPNs and 15 with chronic myelomonocytic leukemia (CMML), *DNMT3A* mutations were

uncovered in only 3 (7%) PMF patients and in none of the others.¹³⁹ No correlation with outcomes or presenting features was identified.¹³⁹

RUNX1 mutations

Runt-related transcription factor 1 (*RUNX1*) encodes a heteromeric transcription factor with a major role in hematopoiesis⁴⁹ that is frequently mutated in AML¹⁴⁰ and MDS,¹⁴¹ where it has been associated with adverse outcomes. *RUNX1* has been implicated in LT of Ph⁻ MPNs.^{89,133,142} Inactivating mutations in *RUNX1* cause differentiation arrest and both point mutations and chromosomal aberrations at the *RUNX1* locus are found in post-MPN AML samples.¹³³

IKZF1 deletions

The Ikaros family zinc finger 1 (*IKZF1*) gene located on chromosome 7p encodes for the Ikaros transcription factors, which are important regulators of lymphoid differentiation.¹⁴³ *IKZF1* gene deletions are implicated in the pathogenesis of the lymphoid BP of chronic myeloid leukemia and Philadelphia chromosome-positive acute lymphoblastic leukemia.¹⁴⁴ *IKZF1* deletions have been reported to be strongly associated with the development of post-MPN AML, deletion 7p and a complex karyotype.¹⁴⁵ Hemizygous loss of *IKZF1* (including monosomy 7) was detected in 21% of post-MPN AML and 0.2% of non-leukemic MPN patients.¹⁴⁵

Mutations and leukemic transformation in the Ph⁻ MPNs

Given that a *JAK2* V617F-positive MPN can evolve either into a *JAK2* V617F-positive or wild type AML, two routes for LT have been proposed.⁸⁹ In the former situation, evolution to *JAK2*-mutant AML is preceded by the acquisition of genetic changes that give rise to the phenotype of accelerated-phase disease, manifested clinically as PMF^{49,50} or myelofibrotic transformation of ET/PV.⁸⁹ Mutant *JAK2*-induced inhibition of Bcl-xL deamidation,¹⁴⁶ reactive oxygen species generation¹⁴⁷ and stimulation of homologous recombination¹⁴⁸ could contribute to genetic instability and the aberrant survival of DNA-damaged cells in this setting. However, the observation that CP MPN patients do not accumulate chromosomal aberrations¹³³ and somatic mutations¹⁰⁷ over time argues against the concept that *JAK2* V617F induces genomic instability. In the latter situation, the chronic and leukemic phases of the disease could either be clonally related, having arisen from a shared pre-*JAK2* V617F clone, or be clonally unrelated, reflecting transformation of independent stem cells.⁸⁹ As discussed above, *TET2* and *ASXL1* mutations can be acquired pre-*JAK2* V617F,^{99,106} as can deletion 20q, which is likely not a predisposing factor for acquisition of *JAK2* V617F.¹⁴⁹

Identification of a high molecular risk signature in PMF, importance of number of mutations and development of new prognostic models

A “high molecular risk” (HMR) signature was recently developed for patients with PMF in a multi-national study of 879 patients.¹⁵⁰ Genes analyzed included *ASXL1*, *SRSF2*, *EZH2*, *TET2*, *DNMT3A*, *CBL*, *IDH1*, *IDH2*, *MPL* and *JAK2*. In both the learning (n=483) and

validation (n=396) cohorts, mutations in *ASXL1*, *SRSF2* and *EZH2* predicted shortened survival, but only *ASXL1* mutations remained significant in the context of IPSS and DIPSS-plus, respectively.¹⁵⁰ LFS was adversely affected by mutations in *IDH1* and *SRSF2* in both cohorts and, additionally, by *IDH2* and *ASXL1* mutations in the learning cohort.¹⁵⁰ Based on these findings, PMF patients with mutations in any of these five genes were identified as belonging to a “HMR” category.

Conventional karyotyping using classic metaphase cytogenetics identifies chromosomal abnormalities in around 30% of patients with Ph⁻ MPNs, and SNP arrays do so in over half of patients.¹³³ In contrast, at least one somatic mutation can be found in 90% of MPN patients.¹⁰⁷ It was recently shown that one of the strongest predictors of outcome is the number of somatic mutations in addition to *JAK2*, *CALR* or *MPL*.¹⁰⁷ Interestingly, in this study, the group of patients carrying either no detectable somatic mutation or a mutation in *JAK2*, *CALR* or *MPL* only had a particularly favorable prognosis, with no cases of LT.¹⁰⁷ While this may appear contradictory to the known poor LFS of triple negative patients, the discrepancy is likely explained by the fact that the mutation profiles of 105 genes were analyzed in the above study using NGS and allele-specific polymerase chain reaction,¹⁰⁷ unlike in studies that examined the clinical correlates of mutations in *JAK2*, *MPL*, or *CALR* versus triple negative status.^{75,76,151} Indeed, an international study of 797 patients with PMF has shown that the number of mutated genes (from amongst the five comprising the HMR category) is predictive of both survival and LFS.¹⁵² Median survival was 2.6 years for patients with 2 mutations, 7 years for patients with one mutation, and 12.3 years for those with no mutations. The prognostic significance of number of mutations was independent of both IPSS and DIPSS-plus.¹⁵² 2 mutations were also associated with shortened LFS. However, *CALR* mutations favorably impacted survival, irrespective of both number of prognostically detrimental mutations and DIPSS-plus.¹⁵² More recently, targeted NGS of a panel of 28 genes recurrently mutated in myeloid malignancies was performed on samples obtained at study entry from 95 patients with MF who received ruxolitinib on a phase 1/2 study.¹⁵³ Mutations in 15 of these genes were found in all but 2 patients. Patients with 3 mutations of any type had significantly lower odds of a spleen response (nine-fold lower odds than those with 2 mutations) and a shorter time to treatment discontinuation (TTD).¹⁵³ In multivariate analysis, only the presence of 2 mutations and spleen response were associated with shorter TTD. Patients with 1 mutation(s) in *ASXL1*, *EZH2*, *IDH1* or *IDH2* (*SRSF2* mutations were not tested for in this study) had shorter survival and TTD, and were significantly less likely to have a spleen response to ruxolitinib.¹⁵³ Of note, spleen response in this group of patients had previously been shown to correlate with improved survival.⁷ Reductions in spleen size with ruxolitinib treatment also correlated with longer survival in a pooled analysis of survival data from the pivotal COMFORT-I and -II trials.⁹ Collectively, these observations suggest that multi-gene profiling to identify prognostically detrimental mutations should be incorporated into the routine diagnostic workup of patients with MF and integrated into prognostic models for both outcome prediction and treatment planning.

Accordingly, a new mutation-enhanced IPSS (MIPSS) for PMF was recently presented.¹⁵⁴ Upon multivariable analysis of the learning cohort (n=588), the following factors remained significant for survival: age >60 years, constitutional symptoms, hemoglobin <10 g/dl,

platelets $<200 \times 10^9/l$, triple negativity, *JAK2/MPL* mutation, *ASXL1* mutation and *SRSF2* mutation.¹⁵⁴ These adverse factors were weighted 1.5, 0.5, 0.5, 1.0, 1.5, 0.5, 0.5 and 0.5, respectively. Four risk groups with non-overlapping survival curves could thus be delineated: low (score 0-0.5), intermediate-1 (score 1-1.5), intermediate-2 (score 2-3.5) and high (score 4). MIPSS performed better than IPSS in predicting survival, and its performance was validated in an independent cohort of 398 patients with PMF.¹⁵⁴ High and intermediate-1/2 risk MIPSS also predicted LFS in both cohorts.¹⁵⁴ However, karyotype retained MIPSS-independent prognostic value for both survival and LFS in the validation cohort.¹⁵⁴ Because of the latter observation, a prognostic model based only on age and genomic information, the Genetics-based Prognostic Scoring System (GPSS) was developed.¹⁵⁵ Four cytogenetic risk categories were defined: very high (monosomal karyotype,¹⁵⁶ *inv*(3), *i*(17q), -7/7q-, 11q or 12p abnormalities), high (complex non-monosomal, two abnormalities not included in very high risk category, 5q-, +8, other autosomal trisomies except +9, and other sole abnormalities not included in other risk categories), intermediate (sole abnormalities of 20q-, 1q+ or any other sole translocation, and -Y or other sex chromosome abnormality) and low (normal or sole abnormalities of 13q- or +9).¹⁵⁵ Age-adjusted multivariable analysis identified the following factors as independent predictors of shortened survival in the training set of 964 patients with PMF: very high risk karyotype (3 points), high risk karyotype (1 point), triple-negative (2 points), *JAK2* mutation (2 points), *MPL* mutation (2 points), type 2/type 2-like *CALR* mutation (2 points), *ASXL1* mutation (1 point) and *SRSF2* mutation (1 point).¹⁵⁵ Age >60 years received 2 points. Four risk categories could be demarcated in this way: low (0 points), intermediate-1 (1-2 points), intermediate-2 (3-4 points) and high (5 points), with corresponding median survival times of >17, 9, 5 and 2.2 years, respectively.¹⁵⁵ High-risk patients also had a higher risk of LT. The ability of the GPSS to effectively discriminate between higher (high/intermediate-2) and lower (low/intermediate-1) risk categories in terms of both survival and LFS was validated in an independent cohort of 183 patients with PMF.¹⁵⁵

Conclusion

Prognostication of PMF has traditionally relied on clinical variables, and there is no doubt that the IPSS¹¹ and DIPSS¹² are useful in classifying patients into different risk categories at diagnosis and at later time points, respectively. Although more cumbersome to use, the DIPSS-plus²⁰ added significant value to the DIPSS by incorporating cytogenetic risk. Indeed, certain karyotypic features, e.g., monosomal¹⁵⁶ or complex karyotype,¹⁶ chromosome 17 aberrations,^{16,17} particularly *i*(17q) and *inv*(3)¹⁸ confer an extremely poor prognosis. However, karyotypic abnormalities are not detected in the majority of patients. The explosion of knowledge in recent years of the molecular underpinnings of the disease as a result of the wide availability of techniques like NGS has uncovered a wealth of prognostically useful information, the incorporation of which into prognostic models for routine use in the clinic is just beginning. One potentially problematic issue is that all major studies of prognostic modelling in PMF to date have excluded patients with early/prefibrotic PMF, and indolent and difficult-to-diagnose entity^{157,158} which, nevertheless, is included under PMF in the World Health Organization classification.¹⁵⁹ Another point worth noting is that there may be important biologic differences between PMF and post-PV/ET MF, such

that the current practice of using the risk stratification systems developed for PMF for these patients may not be optimal.¹⁶⁰ As prognostic models in PMF continue to evolve in the genomic era, hopefully, large studies will be conducted to also address these issues.

References

- Dameshek W. Some speculations on the myeloproliferative syndromes. *Blood*. 1951; 6:372–5. [PubMed: 14820991]
- Tefferi A. Myelofibrosis with myeloid metaplasia. *N Engl J Med*. 2000; 342:1255–65. [PubMed: 10781623]
- Hultcrantz M, Kristinsson SY, Andersson TM, Landgren O, Eloranta S, Derolf AR, Dickman PW, Björkholm M. Patterns of survival among patients with myeloproliferative neoplasms diagnosed in Sweden from 1973 to 2008: a population-based study. *J Clin Oncol*. 2012; 30:2995–3001. [PubMed: 22802311]
- Cervantes F, Dupriez B, Passamonti F, Vannucchi AM, Morra E, Reilly JT, Demory JL, Rumi E, Guglielmelli P, Roncoroni E, Tefferi A, Pereira A. Improving survival trends in primary myelofibrosis: an international study. *J Clin Oncol*. 2012; 30:2981–7. [PubMed: 22826273]
- Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, Catalano JV, Deininger M, Miller C, Silver RT, Talpaz M, Winton EF, Harvey JH Jr, Arcasoy MO, Hexner E, Lyons RM, Paquette R, Raza A, Vaddi K, Erickson-Viitanen S, Koumenis IL, Sun W, Sandor V, Kantarjian HM. A double-blind, placebo-controlled trial of ruxolitinib for myelofibrosis. *N Engl J Med*. 2012; 366:799–807. [PubMed: 22375971]
- Cervantes F, Vannucchi AM, Kiladjian JJ, Al-Ali HK, Sirulnik A, Stalbovskaya V, McQuitty M, Hunter DS, Levy RS, Passamonti F, Barbui T, Barosi G, Harrison CN, Knoop L, Gisslinger H. COMFORT-II investigators. Three-year efficacy, safety, and survival findings from COMFORT-II, a phase 3 study comparing ruxolitinib with best available therapy for myelofibrosis. *Blood*. 2013; 122:4047–53. [PubMed: 24174625]
- Verstovsek S, Kantarjian HM, Estrov Z, Cortes JE, Thomas DA, Kadia T, Pierce S, Jabbour E, Borthakur G, Rumi E, Pungolino E, Morra E, Caramazza D, Cazzola M, Passamonti F. Long-term outcomes of 107 patients with myelofibrosis receiving JAK1/JAK2 inhibitor ruxolitinib: survival advantage in comparison to matched historical controls. *Blood*. 2012; 120:1202–9. [PubMed: 22718840]
- Passamonti F, Maffioli M, Cervantes F, Vannucchi AM, Morra E, Barbui T, Caramazza D, Pieri L, Rumi E, Gisslinger H, Knoop L, Kiladjian JJ, Mora B, Hollaender N, Pascutto C, Harrison C, Cazzola M. Impact of ruxolitinib on the natural history of primary myelofibrosis: a comparison of the DIPSS and the COMFORT-2 cohorts. *Blood*. 2014; 123:1833–5. [PubMed: 24443442]
- Vannucchi AM, Kantarjian HM, Kiladjian JJ, Gotlib J, Cervantes F, Mesa RA, Sarlis NJ, Peng W, Sandor V, Gopalakrishna P, Hmissi A, Stalbovskaya V, Gupta V, Harrison C, Verstovsek S. A pooled analysis of overall survival in COMFORT-I and COMFORT-II, 2 randomized phase 3 trials of ruxolitinib for the treatment of myelofibrosis. *Haematologica*. 2015
- Cervantes F, Passamonti F, Barosi G. Life expectancy and prognostic factors in the classic BCR/ABL-negative myeloproliferative disorders. *Leukemia*. 2008; 22:905–14. [PubMed: 18385755]
- Cervantes F, Dupriez B, Pereira A, Passamonti F, Reilly JT, Morra E, Vannucchi AM, Mesa RA, Demory JL, Barosi G, Rumi E, Tefferi A. New prognostic scoring system for primary myelofibrosis based on a study of the International Working Group for Myelofibrosis Research and Treatment. *Blood*. 2009; 113:2895–901. [PubMed: 18988864]
- Passamonti F, Cervantes F, Vannucchi AM, Morra E, Rumi E, Pereira A, Guglielmelli P, Pungolino E, Caramella M, Maffioli M, Pascutto C, Lazzarino M, Cazzola M, Tefferi A. A dynamic prognostic model to predict survival in primary myelofibrosis: a study by the IWG-MRT (International Working Group for Myeloproliferative Neoplasms Research and Treatment). *Blood*. 2010; 115:1703–8. [PubMed: 20008785]
- Passamonti F, Cervantes F, Vannucchi AM, Morra E, Rumi E, Cazzola M, Tefferi A. Dynamic International Prognostic Scoring System (DIPSS) predicts progression to acute myeloid leukemia in primary myelofibrosis. *Blood*. 2010; 116:2857–8. [PubMed: 20947690]

14. Scott BL, Gooley TA, Sorrow ML, Rezvani AR, Linenberger ML, Grim J, Sandmaier BM, Myerson D, Chauncey TR, Storb R, Buxhofer-Ausch V, Radich JP, Appelbaum FR, Deeg HJ. The Dynamic International Prognostic Scoring System for myelofibrosis predicts outcomes after hematopoietic cell transplantation. *Blood*. 2012; 119:2657–64. [PubMed: 22234678]
15. Caramazza D, Begna KH, Gangat N, Vaidya R, Siragusa S, Van Dyke DL, Hanson C, Pardanani A, Tefferi A. Refined cytogenetic-risk categorization for overall and leukemia-free survival in primary myelofibrosis: a single center study of 433 patients. *Leukemia*. 2011; 25:82–8. [PubMed: 20944670]
16. Tam CS, Abruzzo LV, Lin KI, Cortes J, Lynn A, Keating MJ, Thomas DA, Pierce S, Kantarjian H, Verstovsek S. The role of cytogenetic abnormalities as a prognostic marker in primary myelofibrosis: applicability at the time of diagnosis and later during disease course. *Blood*. 2009; 113:4171–8. [PubMed: 19131547]
17. Tam CS, Kantarjian H, Cortes J, Lynn A, Pierce S, Zhou L, Keating MJ, Thomas DA, Verstovsek S. Dynamic model for predicting death within 12 months in patients with primary or post-polycythemia vera/essential thrombocythemia myelofibrosis. *J Clin Oncol*. 2009; 27:5587–93. [PubMed: 19786661]
18. Tefferi A, Jimma T, Gangat N, Vaidya R, Begna KH, Hanson CA, Van Dyke DL, Caramazza D, Pardanani A. Predictors of greater than 80% 2-year mortality in primary myelofibrosis: a Mayo Clinic study of 884 karyotypically annotated patients. *Blood*. 2011; 118:4595–8. [PubMed: 21881047]
19. Tefferi A, Mesa RA, Pardanani A, Hussein K, Schwager S, Hanson CA, Steensma DP. Red blood cell transfusion need at diagnosis adversely affects survival in primary myelofibrosis-increased serum ferritin or transfusion load does not. *Am J Hematol*. 2009; 84:265–7. [PubMed: 19396855]
20. Gangat N, Caramazza D, Vaidya R, George G, Begna K, Schwager S, Van Dyke D, Hanson C, Wu W, Pardanani A, Cervantes F, Passamonti F, Tefferi A. DIPSS plus: a refined Dynamic International Prognostic Scoring System for primary myelofibrosis that incorporates prognostic information from karyotype, platelet count, and transfusion status. *J Clin Oncol*. 2011; 29:392–7. [PubMed: 21149668]
21. Tefferi A, Pardanani A, Gangat N, Begna KH, Hanson CA, Van Dyke DL, Caramazza D, Vannucchi AM, Morra E, Cazzola M, Pereira A, Cervantes F, Passamonti F. Leukemia risk models in primary myelofibrosis: an International Working Group study. *Leukemia*. 2012; 26:1439–41. [PubMed: 22289985]
22. Cazzola M, Kralovics R. From Janus kinase 2 to calreticulin: the clinically relevant genomic landscape of myeloproliferative neoplasms. *Blood*. 2014; 123:3714–9. [PubMed: 24786775]
23. Kralovics R, Guan Y, Prchal JT. Acquired uniparental disomy of chromosome 9p is a frequent stem cell defect in polycythemia vera. *Exp Hematol*. 2002; 30:229–36. [PubMed: 11882360]
24. Baxter EJ, Scott LM, Campbell PJ, East C, Fourouclas N, Swanton S, Vassiliou GS, Bench AJ, Boyd EM, Curtin N, Scott MA, Erber WN, Green AR. Cancer Genome Project. Acquired mutation of the tyrosine kinase JAK2 in human myeloproliferative disorders. *Lancet*. 2005; 365:1054–61. [PubMed: 15781101]
25. James C, Ugo V, Le Couedic JP, Staerk J, Delhommeau F, Lacout C, Garcon L, Raslova H, Berger R, Bennaceur-Griscelli A, Villeval JL, Constantinescu SN, Casadevall N, Vainchenker W. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. *Nature*. 2005; 434:1144–8. [PubMed: 15793561]
26. Kralovics R, Passamonti F, Buser AS, Teo SS, Tiedt R, Passweg JR, Tichelli A, Cazzola M, Skoda RC. A gain-of-function mutation of JAK2 in myeloproliferative disorders. *N Engl J Med*. 2005; 352:1779–90. [PubMed: 15858187]
27. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, Boggon TJ, Wlodarska I, Clark JJ, Moore S, Adelsperger J, Koo S, Lee JC, Gabriel S, Mercher T, D'Andrea A, Frohling S, Dohner K, Marynen P, Vandenberghe P, Mesa RA, Tefferi A, Griffin JD, Eck MJ, Sellers WR, Meyerson M, Golub TR, Lee SJ, Gilliland DG. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005; 7:387–97. [PubMed: 15837627]
28. Levine RL, Pardanani A, Tefferi A, Gilliland DG. Role of JAK2 in the pathogenesis and therapy of myeloproliferative disorders. *Nat Rev Cancer*. 2007; 7:673–83. [PubMed: 17721432]

29. Jones AV, Chase A, Silver RT, Oscier D, Zoi K, Wang YL, Cario H, Pahl HL, Collins A, Reiter A, Grand F, Cross NC. JAK2 haplotype is a major risk factor for the development of myeloproliferative neoplasms. *Nat Genet.* 2009; 41:446–9. [PubMed: 19287382]
30. Olcaydu D, Harutyunyan A, Jager R, Berg T, Gisslinger B, Pabinger I, Gisslinger H, Kralovics R. A common JAK2 haplotype confers susceptibility to myeloproliferative neoplasms. *Nat Genet.* 2009; 41:450–4. [PubMed: 19287385]
31. Kilpivaara O, Mukherjee S, Schram AM, Wadleigh M, Mullally A, Ebert BL, Bass A, Marubayashi S, Heguy A, Garcia-Manero G, Kantarjian H, Offit K, Stone RM, Gilliland DG, Klein RJ, Levine RL. A germline JAK2 SNP is associated with predisposition to the development of JAK2(V617F)-positive myeloproliferative neoplasms. *Nat Genet.* 2009; 41:455–9. [PubMed: 19287384]
32. Dawson MA, Bannister AJ, Gottgens B, Foster SD, Bartke T, Green AR, Kouzarides T. JAK2 phosphorylates histone H3Y41 and excludes HP1alpha from chromatin. *Nature.* 2009; 461:819–22. [PubMed: 19783980]
33. Liu F, Zhao X, Perna F, Wang L, Koppikar P, Abdel-Wahab O, Harr MW, Levine RL, Xu H, Tefferi A, Deblasio A, Hatlen M, Menendez S, Nimer SD. JAK2V617F-mediated phosphorylation of PRMT5 downregulates its methyltransferase activity and promotes myeloproliferation. *Cancer Cell.* 2011; 19:283–94. [PubMed: 21316606]
34. 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; 108:1652–60. [PubMed: 16670266]
35. 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; 107:4274–81. [PubMed: 16478879]
36. Xing S, Wanting TH, Zhao W, Ma J, Wang S, Xu X, Li Q, Fu X, Xu M, Zhao ZJ. Transgenic expression of JAK2V617F causes myeloproliferative disorders in mice. *Blood.* 2008; 111:5109–17. [PubMed: 18334677]
37. Marty C, Lacout C, Martin A, Hasan S, Jacquot S, Birling MC, Vainchenker W, Villeval JL. Myeloproliferative neoplasm induced by constitutive expression of JAK2V617F in knock-in mice. *Blood.* 2010; 116:783–7. [PubMed: 20472827]
38. Akada H, Yan D, Zou H, Fiering S, Hutchison RE, Mohi MG. Conditional expression of heterozygous or homozygous Jak2V617F from its endogenous promoter induces a polycythemia vera-like disease. *Blood.* 2010; 115:3589–97. [PubMed: 20197548]
39. Tiedt R, Hao-Shen H, Sobas MA, Looser R, Dirnhofer S, Schwaller J, Skoda RC. Ratio of mutant JAK2-V617F to wild-type Jak2 determines the MPD phenotypes in transgenic mice. *Blood.* 2008; 111:3931–40. [PubMed: 18160670]
40. Shide K, Shimoda HK, Kumano T, Karube K, Kameda T, Takenaka K, Oku S, Abe H, Katayose KS, Kubuki Y, Kusumoto K, Hasuike S, Tahara Y, Nagata K, Matsuda T, Ohshima K, Harada M, Shimoda K. Development of ET, primary myelofibrosis and PV in mice expressing JAK2 V617F. *Leukemia.* 2008; 22:87–95. [PubMed: 18033315]
41. Li J, Kent DG, Godfrey AL, Manning H, Nangalia J, Aziz A, Chen E, Saeb-Parsy K, Fink J, Sneade R, Hamilton TL, Pask DC, Silber Y, Zhao X, Ghevaert C, Liu P, Green AR. JAK2V617F homozygosity drives a phenotypic switch in myeloproliferative neoplasms, but is insufficient to sustain disease. *Blood.* 2014; 123:3139–51. [PubMed: 24692758]
42. 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; 22:1299–307. [PubMed: 18496562]
43. Dupont S, Masse A, James C, Teyssandier I, Lecluse Y, Larbret F, Ugo V, Saulnier P, Koscielny S, Le Couedic JP, Casadevall N, Vainchenker W, Delhommeau F. The JAK2 617V>F mutation triggers erythropoietin hypersensitivity and terminal erythroid amplification in primary cells from patients with polycythemia vera. *Blood.* 2007; 110:1013–21. [PubMed: 17389763]
44. 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; 36:1480–6. [PubMed: 18723264]

45. James C, Mazurier F, Dupont S, Chaligne R, Lamrissi-Garcia I, Tulliez M, Lippert E, Mahon FX, Pasquet JM, Etienne G, Delhommeau F, Giraudier S, Vainchenker W, de Verneuil H. The hematopoietic stem cell compartment of JAK2V617F-positive myeloproliferative disorders is a reflection of disease heterogeneity. *Blood*. 2008; 112:2429–38. [PubMed: 18612101]
46. Stein BL, Williams DM, Rogers O, Isaacs MA, Spivak JL, Moliterno AR. Disease burden at the progenitor level is a feature of primary myelofibrosis: a multivariable analysis of 164 JAK2 V617F-positive myeloproliferative neoplasm patients. *Exp Hematol*. 2011; 39:95–101. [PubMed: 20888389]
47. Catlin SN, Gutter P, Abkowitz JL. The kinetics of clonal dominance in myeloproliferative disorders. *Blood*. 2005; 106:2688–92. [PubMed: 16002428]
48. Vickers MA. JAK2 617V>F positive polycythemia rubra vera maintained by approximately 18 stochastic stem-cell divisions per year, explaining age of onset by a single rate-limiting mutation. *Blood*. 2007; 110:1675–80. [PubMed: 17452518]
49. Vainchenker W, Delhommeau F, Constantinescu SN, Bernard OA. New mutations and pathogenesis of myeloproliferative neoplasms. *Blood*. 2011; 118:1723–35. [PubMed: 21653328]
50. Campbell PJ, Green AR. The myeloproliferative disorders. *N Engl J Med*. 2006; 355:2452–66. [PubMed: 17151367]
51. Kralovics R, Teo SS, Li S, Theodorides A, Buser AS, Tichelli A, Skoda RC. Acquisition of the V617F mutation of JAK2 is a late genetic event in a subset of patients with myeloproliferative disorders. *Blood*. 2006; 108:1377–80. [PubMed: 16675710]
52. Levine RL, Belisle C, Wadleigh M, Zahrieh D, Lee S, Chagnon P, Gilliland DG, Busque L. 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; 107:4139–41. [PubMed: 16434490]
53. Nussenzweig RH, Swierczek SI, Jelinek J, Gaikwad A, Liu E, Verstovsek S, Prchal JF, Prchal JT. Polycythemia vera is not initiated by JAK2V617F mutation. *Exp Hematol*. 2007; 35:32–8. [PubMed: 17198871]
54. Jamal R, Belisle C, Lessard MC, Hebert J, Roy DC, Levine R, Busque L. Evidence suggesting the presence of a stem cell clone anteceding the acquisition of the JAK2-V617F mutation. *Leukemia*. 2008; 22:1472–4. [PubMed: 18239619]
55. Tefferi A, Lasho TL, Patnaik MM, Finke CM, Hussein K, Hogan WJ, Elliott MA, Litzow MR, Hanson CA, Pardanani A. JAK2 germline genetic variation affects disease susceptibility in primary myelofibrosis regardless of V617F mutational status: nullizygosity for the JAK2 46/1 haplotype is associated with inferior survival. *Leukemia*. 2010; 24:105–9. [PubMed: 19847199]
56. Tefferi A, Lasho TL, Huang J, Finke C, Mesa RA, Li CY, Wu W, Hanson CA, Pardanani A. 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; 22:756–61. [PubMed: 18216871]
57. Guglielmelli P, Barosi G, Specchia G, Rambaldi A, Lo Coco F, Antonioli E, Pieri L, Pancrazzi A, Ponziani V, Delaini F, Longo G, Ammatuna E, Liso V, Bosi A, Barbui T, Vannucchi AM. Identification of patients with poorer survival in primary myelofibrosis based on the burden of JAK2V617F mutated allele. *Blood*. 2009; 114:1477–83. [PubMed: 19549988]
58. Barosi G, Bergamaschi G, Marchetti M, Vannucchi AM, Guglielmelli P, Antonioli E, Massa M, Rosti V, Campanelli R, Villani L, Viarengo G, Gattoni E, Gerli G, Specchia G, Tinelli C, Rambaldi A, Barbui T. Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Italian Registry of Myelofibrosis. JAK2 V617F mutational status predicts progression to large splenomegaly and leukemic transformation in primary myelofibrosis. *Blood*. 2007; 110:4030–6. [PubMed: 17712047]
59. Campbell PJ, Griesshammer M, Dohner K, Dohner H, Kusec R, Hasselbalch HC, Larsen TS, Pallisgaard N, Giraudier S, Le Bousse-Kerdiles MC, Desterke C, Guerton B, Dupriez B, Bordessoule D, Fenaux P, Kiladjian JJ, Viallard JF, Briere J, Harrison CN, Green AR, Reilly JT. V617F mutation in JAK2 is associated with poorer survival in idiopathic myelofibrosis. *Blood*. 2006; 107:2098–100. [PubMed: 16293597]

60. Tefferi A, Lasho TL, Schwager SM, Steensma DP, Mesa RA, Li CY, Wadleigh M, Gary Gilliland D. The JAK2(V617F) tyrosine kinase mutation in myelofibrosis with myeloid metaplasia: lineage specificity and clinical correlates. *Br J Haematol.* 2005; 131:320–8. [PubMed: 16225651]
61. Campbell PJ, Baxter EJ, Beer PA, Scott LM, Bench AJ, Huntly BJ, Erber WN, Kusec R, Larsen TS, Giraudier S, Le Bousse-Kerdiles MC, Griesshammer M, Reilly JT, Cheung BY, Harrison CN, Green AR. Mutation of JAK2 in the myeloproliferative disorders: timing, clonality studies, cytogenetic associations, and role in leukemic transformation. *Blood.* 2006; 108:3548–55. [PubMed: 16873677]
62. Theoharides A, Boissinot M, Girodon F, Garand R, Teo SS, Lippert E, Talmant P, Tichelli A, Hermouet S, Skoda RC. Leukemic blasts in transformed JAK2-V617F-positive myeloproliferative disorders are frequently negative for the JAK2-V617F mutation. *Blood.* 2007; 110:375–9. [PubMed: 17363731]
63. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, Cuker A, Wernig G, Moore S, Galinsky I, DeAngelo DJ, Clark JJ, Lee SJ, Golub TR, Wadleigh M, Gilliland DG, Levine RL. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. *PLoS Med.* 2006; 3:e270. [PubMed: 16834459]
64. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, Steensma DP, Elliott MA, Wolanskyj AP, Hogan WJ, McClure RF, Litzow MR, Gilliland DG, Tefferi A. MPLS15 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood.* 2006; 108:3472–6. [PubMed: 16868251]
65. Pardanani A, Guglielmelli P, Lasho TL, Pancrazzi A, Finke CM, Vannucchi AM, Tefferi A. Primary myelofibrosis with or without mutant MPL: comparison of survival and clinical features involving 603 patients. *Leukemia.* 2011; 25:1834–9. [PubMed: 21691276]
66. Chaligne R, James C, Tonetti C, Besancenot R, Le Couedic JP, Fava F, Mazurier F, Godin I, Maloum K, Larbret F, Lecluse Y, Vainchenker W, Giraudier S. Evidence for MPL W515L/K mutations in hematopoietic stem cells in primitive myelofibrosis. *Blood.* 2007; 110:3735–43. [PubMed: 17709604]
67. Rumi E, Pietra D, Guglielmelli P, Bordoni R, Casetti I, Milanesi C, Sant'Antonio E, Ferretti V, Pancrazzi A, Rotunno G, Severgnini M, Pietrelli A, Astori C, Fugazza E, Pascutto C, Boveri E, Passamonti F, De Bellis G, Vannucchi A, Cazzola M. Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative. Acquired copy-neutral loss of heterozygosity of chromosome 1p as a molecular event associated with marrow fibrosis in MPL-mutated myeloproliferative neoplasms. *Blood.* 2013; 121:4388–95. [PubMed: 23575445]
68. Szpurka H, Gondek LP, Mohan SR, Hsi ED, Theil KS, Maciejewski JP. UPD1p indicates the presence of MPL W515L mutation in RARS-T, a mechanism analogous to UPD9p and JAK2 V617F mutation. *Leukemia.* 2009; 23:610–4. [PubMed: 18818701]
69. Jones AV, Campbell PJ, Beer PA, Schnittger S, Vannucchi AM, Zoi K, Percy MJ, McMullin MF, Scott LM, Tapper W, Silver RT, Oscier D, Harrison CN, Grallert H, Kisialiou A, Strike P, Chase AJ, Green AR, Cross NC. The JAK2 46/1 haplotype predisposes to MPL-mutated myeloproliferative neoplasms. *Blood.* 2010; 115:4517–23. [PubMed: 20304805]
70. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, Them NC, Berg T, Gisslinger B, Pietra D, Chen D, Vladimer GI, Bagienski K, Milanesi C, Casetti IC, Sant'Antonio E, Ferretti V, Elena C, Schischlik F, Cleary C, Six M, Schalling M, Schonegger A, Bock C, Malcovati L, Pascutto C, Superti-Furga G, Cazzola M, Kralovics R. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med.* 2013; 369:2379–90. [PubMed: 24325356]
71. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, Avezov E, Li J, Kollmann K, Kent DG, Aziz A, Godfrey AL, Hinton J, Martincorena I, Van Loo P, Jones AV, Guglielmelli P, Tarpey P, Harding HP, Fitzpatrick JD, Goudie CT, Ortmann CA, Loughran SJ, Raine K, Jones DR, Butler AP, Teague JW, O'Meara S, McLaren S, Bianchi M, Silber Y, Dimitropoulou D, Bloxham D, Mudie L, Maddison M, Robinson B, Keohane C, Maclean C, Hill K, Orchard K, Tauro S, Du MQ, Greaves M, Bowen D, Huntly BJ, Harrison CN, Cross NC, Ron D, Vannucchi AM, Papaemmanuil E, Campbell PJ, Green AR. Somatic CALR mutations in myeloproliferative neoplasms with nonmutated JAK2. *N Engl J Med.* 2013; 369:2391–405. [PubMed: 24325359]

72. Scott LM, Tong W, Levine RL, Scott MA, Beer PA, Stratton MR, Futreal PA, Erber WN, McMullin MF, Harrison CN, Warren AJ, Gilliland DG, Lodish HF, Green AR. JAK2 exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. *N Engl J Med*. 2007; 356:459–68. [PubMed: 17267906]
73. Pietra D, Li S, Brisci A, Passamonti F, Rumi E, Theocharides A, Ferrari M, Gisslinger H, Kralovics R, Cremonesi L, Skoda R, Cazzola M. Somatic mutations of JAK2 exon 12 in patients with JAK2 (V617F)-negative myeloproliferative disorders. *Blood*. 2008; 111:1686–9. [PubMed: 17984312]
74. Passamonti F, Elena C, Schnittger S, Skoda RC, Green AR, Girodon F, Kiladjian JJ, McMullin MF, Ruggeri M, Besses C, Vannucchi AM, Lippert E, Gisslinger H, Rumi E, Lehmann T, Ortmann CA, Pietra D, Pascutto C, Haferlach T, Cazzola M. Molecular and clinical features of the myeloproliferative neoplasm associated with JAK2 exon 12 mutations. *Blood*. 2011; 117:2813–6. [PubMed: 21224469]
75. Rumi E, Pietra D, Pascutto C, Guglielmelli P, Martinez-Trillos A, Casetti I, Colomer D, Pieri L, Praticorona M, Rotunno G, Sant'Antonio E, Bellini M, Cavalloni C, Mannarelli C, Milanesi C, Boveri E, Ferretti V, Astori C, Rosti V, Cervantes F, Barosi G, Vannucchi AM, Cazzola M. Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative Investigators. Clinical effect of driver mutations of JAK2, CALR, or MPL in primary myelofibrosis. *Blood*. 2014; 124:1062–9. [PubMed: 24986690]
76. Tefferi A, Lasho TL, Finke CM, Knudson RA, Ketterling R, Hanson CH, Maffioli M, Caramazza D, Passamonti F, Pardanani A. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. *Leukemia*. 2014; 28:1472–7. [PubMed: 24402162]
77. Anand S, Stedham F, Gudgin E, Campbell P, Beer P, Green AR, Huntly BJ. Increased basal intracellular signaling patterns do not correlate with JAK2 genotype in human myeloproliferative neoplasms. *Blood*. 2011; 118:1610–21. [PubMed: 21653937]
78. Rampal R, Al-Shahrour F, Abdel-Wahab O, Patel JP, Brunel JP, Mermel CH, Bass AJ, Pretz J, Ahn J, Hricik T, Kilpivaara O, Wadleigh M, Busque L, Gilliland DG, Golub TR, Ebert BL, Levine RL. Integrated genomic analysis illustrates the central role of JAK-STAT pathway activation in myeloproliferative neoplasm pathogenesis. *Blood*. 2014; 123:e123–33. [PubMed: 24740812]
79. Verstovsek S, Mesa RA, Gotlib J, Levy RS, Gupta V, DiPersio JF, Catalano JV, Deininger M, Miller C, Silver RT, Talpaz M, Winton EF, Harvey JH Jr, Arcasoy MO, Hexner E, Lyons RM, Paquette R, Raza A, Vaddi K, Erickson-Viitanen S, Sun W, Sandor V, Kantarjian HM. The clinical benefit of ruxolitinib across patient subgroups: analysis of a placebo-controlled, Phase III study in patients with myelofibrosis. *Br J Haematol*. 2013; 161:508–16. [PubMed: 23480528]
80. Guglielmelli P, Biamonte F, Rotunno G, Artusi V, Artuso L, Bernardis I, Tenedini E, Pieri L, Paoli C, Mannarelli C, Fjerza R, Rumi E, Stalbovskaya V, Squires M, Cazzola M, Manfredini R, Harrison C, Tagliafico E, Vannucchi AM. COMFORT-II Investigators, Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative (AGIMM) Investigators. Impact of mutational status on outcomes in myelofibrosis patients treated with ruxolitinib in the COMFORT-II study. *Blood*. 2014; 123:2157–60. [PubMed: 24458439]
81. Guglielmelli P, Rotunno G, Bogani C, Mannarelli C, Giunti L, Provenzano A, Giglio S, Squires M, Stalbovskaya V, Gopalakrishna P, Vannucchi AM. COMFORT-II Investigators. Ruxolitinib is an effective treatment for CALR-positive patients with myelofibrosis. *Br J Haematol*. 2015
82. Tefferi A, Lasho TL, Finke C, Belachew AA, Wassie EA, Ketterling RP, Hanson CA, Pardanani A. Type 1 vs type 2 calreticulin mutations in primary myelofibrosis: differences in phenotype and prognostic impact. *Leukemia*. 2014; 28:1568–70. [PubMed: 24569778]
83. Panagiota V, Thol F, Markus B, Fehse B, Alchalby H, Badbaran A, Lehmann U, Koenecke C, Shahswar R, Chaturvedi A, Stadler M, Eder M, Gohring G, Koenigsman M, Kloos A, Trummer A, Schroeder T, Kobbe G, Thiede C, Platzbecker U, Schlegelberger B, Kreipe HH, Ganser A, Kroger N, Heuser M. Prognostic effect of calreticulin mutations in patients with myelofibrosis after allogeneic hematopoietic stem cell transplantation. *Leukemia*. 2014; 28:1552–5. [PubMed: 24504025]

84. Oh ST, Simonds EF, Jones C, Hale MB, Goltsev Y, Gibbs KD Jr, Merker JD, Zehnder JL, Nolan GP, Gotlib J. Novel mutations in the inhibitory adaptor protein LNK drive JAK-STAT signaling in patients with myeloproliferative neoplasms. *Blood*. 2010; 116:988–92. [PubMed: 20404132]
85. Pardanani A, Lasho T, Finke C, Oh ST, Gotlib J, Tefferi A. LNK mutation studies in blast-phase myeloproliferative neoplasms, and in chronic-phase disease with TET2, IDH, JAK2 or MPL mutations. *Leukemia*. 2010; 24:1713–8. [PubMed: 20724988]
86. Dunbar AJ, Gondek LP, O'Keefe CL, Makishima H, Rataul MS, Szpurka H, Sekeres MA, Wang XF, McDevitt MA, Maciejewski JP. 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. *Cancer Res*. 2008; 68:10349–57. [PubMed: 19074904]
87. Sanada M, Suzuki T, Shih LY, Otsu M, Kato M, Yamazaki S, Tamura A, Honda H, Sakata-Yanagimoto M, Kumano K, Oda H, Yamagata T, Takita J, Gotoh N, Nakazaki K, Kawamata N, Onodera M, Nobuyoshi M, Hayashi Y, Harada H, Kurokawa M, Chiba S, Mori H, Ozawa K, Omine M, Hirai H, Nakauchi H, Koeffler HP, Ogawa S. Gain-of-function of mutated C-CBL tumour suppressor in myeloid neoplasms. *Nature*. 2009; 460:904–8. [PubMed: 19620960]
88. Grand FH, Hidalgo-Curtis CE, Ernst T, Zoi K, Zoi C, McGuire C, Kreil S, Jones A, Score J, Metzgeroth G, Oscier D, Hall A, Brandts C, Serve H, Reiter A, Chase AJ, Cross NC. Frequent CBL mutations associated with 11q acquired uniparental disomy in myeloproliferative neoplasms. *Blood*. 2009; 113:6182–92. [PubMed: 19387008]
89. Beer PA, Delhommeau F, LeCouedic JP, Dawson MA, Chen E, Bareford D, Kusec R, McMullin MF, Harrison CN, Vannucchi AM, Vainchenker W, Green AR. Two routes to leukemic transformation after a JAK2 mutation-positive myeloproliferative neoplasm. *Blood*. 2010; 115:2891–900. [PubMed: 20008300]
90. Bejar R, Steensma DP. Recent developments in myelodysplastic syndromes. *Blood*. 2014; 124:2793–803. [PubMed: 25237199]
91. Tenedini E, Bernardis I, Artusi V, Artuso L, Roncaglia E, Guglielmelli P, Pieri L, Bogani C, Biamonte F, Rotunno G, Mannarelli C, Bianchi E, Pancrazzi A, Fanelli T, Malagoli Tagliazucchi G, Ferrari S, Manfredini R, Vannucchi AM, Tagliafico E. AGIMM investigators. Targeted cancer exome sequencing reveals recurrent mutations in myeloproliferative neoplasms. *Leukemia*. 2014; 28:1052–9. [PubMed: 24150215]
92. Tahiliani M, Koh KP, Shen Y, Pastor WA, Bandukwala H, Brudno Y, Agarwal S, Iyer LM, Liu DR, Aravind L, Rao A. Conversion of 5-methylcytosine to 5-hydroxymethylcytosine in mammalian DNA by MLL partner TET1. *Science*. 2009; 324:930–5. [PubMed: 19372391]
93. Ko M, Huang Y, Jankowska AM, Pape UJ, Tahiliani M, Bandukwala HS, An J, Lamperti ED, Koh KP, Ganetzky R, Liu XS, Aravind L, Agarwal S, Maciejewski JP, Rao A. Impaired hydroxylation of 5-methylcytosine in myeloid cancers with mutant TET2. *Nature*. 2010; 468:839–43. [PubMed: 21057493]
94. Ito S, D'Alessio AC, Taranova OV, Hong K, Sowers LC, Zhang Y. Role of Tet proteins in 5mC to 5hmC conversion, ES-cell self-renewal and inner cell mass specification. *Nature*. 2010; 466:1129–33. [PubMed: 20639862]
95. Mohamedali AM, Smith AE, Gaken J, Lea NC, Mian SA, Westwood NB, Strupp C, Gattermann N, Germing U, Mufti GJ. Novel TET2 mutations associated with UPD4q24 in myelodysplastic syndrome. *J Clin Oncol*. 2009; 27:4002–6. [PubMed: 19528370]
96. Jankowska AM, Szpurka H, Tiu RV, Makishima H, Afable M, Huh J, O'Keefe CL, Ganetzky R, McDevitt MA, Maciejewski JP. Loss of heterozygosity 4q24 and TET2 mutations associated with myelodysplastic/myeloproliferative neoplasms. *Blood*. 2009; 113:6403–10. [PubMed: 19372255]
97. Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, Figueroa ME, Vasanthakumar A, Patel J, Zhao X, Perna F, Pandey S, Madzo J, Song C, Dai Q, He C, Ibrahim S, Beran M, Zavadil J, Nimer SD, Melnick A, Godley LA, Aifantis I, Levine RL. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell*. 2011; 20:11–24. [PubMed: 21723200]
98. Bejar R, Lord A, Stevenson K, Bar-Natan M, Perez-Ladaga A, Zaneveld J, Wang H, Caughey B, Stojanov P, Getz G, Garcia-Manero G, Kantarjian H, Chen R, Stone RM, Neuberg D, Steensma

- DP, Ebert BL. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood*. 2014; 124:2705–12. [PubMed: 25224413]
99. Delhommeau F, Dupont S, Della Valle V, James C, Trannoy S, Masse A, Kosmider O, Le Couedic JP, Robert F, Alberdi A, Lecluse Y, Plo I, Dreyfus FJ, Marzac C, Casadevall N, Lacombe C, Romana SP, Dessen P, Soulier J, Viguie F, Fontenay M, Vainchenker W, Bernard OA. Mutation in TET2 in myeloid cancers. *N Engl J Med*. 2009; 360:2289–301. [PubMed: 19474426]
 100. Schaub FX, Looser R, Li S, Hao-Shen H, Lehmann T, Tichelli A, Skoda RC. Clonal analysis of TET2 and JAK2 mutations suggests that TET2 can be a late event in the progression of myeloproliferative neoplasms. *Blood*. 2010; 115:2003–7. [PubMed: 20061559]
 101. Ortmann CA, Kent DG, Nangalia J, Silber Y, Wedge DC, Grinfeld J, Baxter EJ, Massie CE, Papaemmanuil E, Menon S, Godfrey AL, Dimitropoulou D, Guglielmelli P, Bellosillo B, Besses C, Dohner K, Harrison CN, Vassiliou GS, Vannucchi A, Campbell PJ, Green AR. Effect of mutation order on myeloproliferative neoplasms. *N Engl J Med*. 2015; 372:601–12. [PubMed: 25671252]
 102. Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z, Mollica L, Li J, Viale A, Heguy A, Hassimi M, Socci N, Bhatt PK, Gonen M, Mason CE, Melnick A, Godley LA, Brennan CW, Abdel-Wahab O, Levine RL. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet*. 2012; 44:1179–81. [PubMed: 23001125]
 103. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Chambert K, Mick E, Neale BM, Fromer M, Purcell SM, Svantesson O, Landen M, Hoglund M, Lehmann S, Gabriel SB, Moran JL, Lander ES, Sullivan PF, Sklar P, Gronberg H, Hultman CM, McCarroll SA. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014; 371:2477–87. [PubMed: 25426838]
 104. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, Lindsley RC, Mermel CH, Burt N, Chavez A, Higgins JM, Moltchanov V, Kuo FC, Kluk MJ, Henderson B, Kinnunen L, Koistinen HA, Ladenvall C, Getz G, Correa A, Banahan BF, Gabriel S, Kathiresan S, Stringham HM, McCarthy MI, Boehnke M, Tuomilehto J, Haiman C, Groop L, Atzmon G, Wilson JG, Neuberg D, Altshuler D, Ebert BL. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014; 371:2488–98. [PubMed: 25426837]
 105. Tefferi A, Pardanani A, Lim KH, Abdel-Wahab O, Lasho TL, Patel J, Gangat N, Finke CM, Schwager S, Mullally A, Li CY, Hanson CA, Mesa R, Bernard O, Delhommeau F, Vainchenker W, Gilliland DG, Levine RL. TET2 mutations and their clinical correlates in polycythemia vera, essential thrombocythemia and myelofibrosis. *Leukemia*. 2009; 23:905–11. [PubMed: 19262601]
 106. Abdel-Wahab O, Manshouri T, Patel J, Harris K, Yao J, Hedvat C, Heguy A, Bueso-Ramos C, Kantarjian H, Levine RL, Verstovsek S. Genetic analysis of transforming events that convert chronic myeloproliferative neoplasms to leukemias. *Cancer Res*. 2010; 70:447–52. [PubMed: 20068184]
 107. Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, Girsberger S, Lehmann T, Passweg J, Stern M, Beisel C, Kralovics R, Skoda RC. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood*. 2014; 123:2220–8. [PubMed: 24478400]
 108. Tefferi A. Novel mutations and their functional and clinical relevance in myeloproliferative neoplasms: JAK2, MPL, TET2, ASXL1, CBL, IDH and IKZF1. *Leukemia*. 2010; 24:1128–38. [PubMed: 20428194]
 109. Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A, Li Y, Bhagwat N, Vasanthakumar A, Fernandez HF, Tallman MS, Sun Z, Wolniak K, Peeters JK, Liu W, Choe SE, Fantin VR, Paietta E, Lowenberg B, Licht JD, Godley LA, Delwel R, Valk PJ, Thompson CB, Levine RL, Melnick A. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. *Cancer Cell*. 2010; 18:553–67. [PubMed: 21130701]
 110. Green A, Beer P. Somatic mutations of IDH1 and IDH2 in the leukemic transformation of myeloproliferative neoplasms. *N Engl J Med*. 2010; 362:369–70. [PubMed: 20107228]
 111. Tefferi A, Jimma T, Sulai NH, Lasho TL, Finke CM, Knudson RA, McClure RF, Pardanani A. IDH mutations in primary myelofibrosis predict leukemic transformation and shortened survival:

- clinical evidence for leukemogenic collaboration with JAK2V617F. *Leukemia*. 2012; 26:475–80. [PubMed: 21912393]
112. Pardanani A, Lasho TL, Finke CM, Mai M, McClure RF, Tefferi A. IDH1 and IDH2 mutation analysis in chronic- and blast-phase myeloproliferative neoplasms. *Leukemia*. 2010; 24:1146–51. [PubMed: 20410924]
 113. Tefferi A, Lasho TL, Abdel-Wahab O, Guglielmelli P, Patel J, Caramazza D, Pieri L, Finke CM, Kilpivaara O, Wadleigh M, Mai M, McClure RF, Gilliland DG, Levine RL, Pardanani A, Vannucchi AM. IDH1 and IDH2 mutation studies in 1473 patients with chronic-, fibrotic- or blast-phase essential thrombocythemia, polycythemia vera or myelofibrosis. *Leukemia*. 2010; 24:1302–9. [PubMed: 20508616]
 114. Vire E, Brenner C, Deplus R, Blanchon L, Fraga M, Didelot C, Morey L, Van Eynde A, Bernard D, Vanderwinden JM, Bollen M, Esteller M, Di Croce L, de Launoit Y, Fuks F. The Polycomb group protein EZH2 directly controls DNA methylation. *Nature*. 2006; 439:871–4. [PubMed: 16357870]
 115. Martinez-Garcia E, Licht JD. Deregulation of H3K27 methylation in cancer. *Nat Genet*. 2010; 42:100–1. [PubMed: 20104248]
 116. Nikoloski G, Langemeijer SM, Kuiper RP, Knops R, Massop M, Tonnissen ER, van der Heijden A, Scheele TN, Vandenbergh P, de Witte T, van der Reijden BA, Jansen JH. Somatic mutations of the histone methyltransferase gene EZH2 in myelodysplastic syndromes. *Nat Genet*. 2010; 42:665–7. [PubMed: 20601954]
 117. Ernst T, Chase AJ, Score J, Hidalgo-Curtis CE, Bryant C, Jones AV, Waghorn K, Zoi K, Ross FM, Reiter A, Hochhaus A, Drexler HG, Duncombe A, Cervantes F, Oscier D, Boultonwood J, Grand FH, Cross NC. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid disorders. *Nat Genet*. 2010; 42:722–6. [PubMed: 20601953]
 118. Guglielmelli P, Biamonte F, Score J, Hidalgo-Curtis C, Cervantes F, Maffioli M, Fanelli T, Ernst T, Winkelmann N, Jones AV, Zoi K, Reiter A, Duncombe A, Villani L, Bosi A, Barosi G, Cross NC, Vannucchi AM. EZH2 mutational status predicts poor survival in myelofibrosis. *Blood*. 2011; 118:5227–34. [PubMed: 21921040]
 119. Abdel-Wahab O, Pardanani A, Patel J, Wadleigh M, Lasho T, Heguy A, Beran M, Gilliland DG, Levine RL, Tefferi A. Concomitant analysis of EZH2 and ASXL1 mutations in myelofibrosis, chronic myelomonocytic leukemia and blast-phase myeloproliferative neoplasms. *Leukemia*. 2011; 25:1200–2. [PubMed: 21455215]
 120. Fisher CL, Lee I, Bloyer S, Bozza S, Chevalier J, Dahl A, Bodner C, Helgason CD, Hess JL, Humphries RK, Brock HW. Additional sex combs-like 1 belongs to the enhancer of trithorax and polycomb group and genetically interacts with Cbx2 in mice. *Dev Biol*. 2010; 337:9–15. [PubMed: 19833123]
 121. Scheuermann JC, de Ayala Alonso AG, Oktaba K, Ly-Hartig N, McGinty RK, Fraterman S, Wilm M, Muir TW, Muller J. Histone H2A deubiquitinase activity of the Polycomb repressive complex PR-DUB. *Nature*. 2010; 465:243–7. [PubMed: 20436459]
 122. Carbuccion N, Murati A, Trouplin V, Brecqueville M, Adelaide J, Rey J, Vainchenker W, Bernard OA, Chaffanet M, Vey N, Birnbaum D, Mozziconacci MJ. Mutations of ASXL1 gene in myeloproliferative neoplasms. *Leukemia*. 2009; 23:2183–6. [PubMed: 19609284]
 123. Tefferi A, Guglielmelli P, Lasho TL, Rotunno G, Finke C, Mannarelli C, Belachew AA, Pancrazzi A, Wassie EA, Ketterling RP, Hanson CA, Pardanani A, Vannucchi AM. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: an international study of 570 patients. *Leukemia*. 2014; 28:1494–500. [PubMed: 24496303]
 124. Makishima H, Visconte V, Sakaguchi H, Jankowska AM, Abu Kar S, Jerez A, Przychodzen B, Bupathi M, Guinta K, Afable MG, Sekeres MA, Padgett RA, Tiu RV, Maciejewski JP. Mutations in the spliceosome machinery, a novel and ubiquitous pathway in leukemogenesis. *Blood*. 2012; 119:3203–10. [PubMed: 22323480]
 125. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, Sato Y, Sato-Otsubo A, Kon A, Nagasaki M, Chalkidis G, Suzuki Y, Shiosaka M, Kawahata R, Yamaguchi T, Otsu M, Obara N, Sakata-Yanagimoto M, Ishiyama K, Mori H, Nolte F, Hofmann WK, Miyawaki S, Sugano S, Haeflacher C, Koeffler HP, Shih LY, Haeflacher T, Chiba S, Nakauchi H, Miyano S,

- Ogawa S. Frequent pathway mutations of splicing machinery in myelodysplasia. *Nature*. 2011; 478:64–9. [PubMed: 21909114]
126. Lasho TL, Jimma T, Finke CM, Patnaik M, Hanson CA, Ketterling RP, Pardanani A, Tefferi A. SRSF2 mutations in primary myelofibrosis: significant clustering with IDH mutations and independent association with inferior overall and leukemia-free survival. *Blood*. 2012; 120:4168–71. [PubMed: 22968464]
 127. Papaemmanuil E, Cazzola M, Boulton J, Malcovati L, Vyas P, Bowen D, Pellagatti A, Wainscoat JS, Hellstrom-Lindberg E, Gambacorti-Passerini C, Godfrey AL, Rapado I, Cvejic A, Rance R, McGee C, Ellis P, Mudie LJ, Stephens PJ, McLaren S, Massie CE, Tarpey PS, Varela I, Nik-Zainal S, Davies HR, Shlien A, Jones D, Raine K, Hinton J, Butler AP, Teague JW, Baxter EJ, Score J, Galli A, Della Porta MG, Travaglino E, Groves M, Tauro S, Munshi NC, Anderson KC, El-Naggar A, Fischer A, Mustonen V, Warren AJ, Cross NC, Green AR, Futreal PA, Stratton MR, Campbell PJ. Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. *N Engl J Med*. 2011; 365:1384–95. [PubMed: 21995386]
 128. Malcovati L, Papaemmanuil E, Bowen DT, Boulton J, Della Porta MG, Pascutto C, Travaglino E, Groves MJ, Godfrey AL, Ambaglio I, Galli A, Da Via MC, Conte S, Tauro S, Keenan N, Hyslop A, Hinton J, Mudie LJ, Wainscoat JS, Futreal PA, Stratton MR, Campbell PJ, Hellstrom-Lindberg E, Cazzola M. Chronic Myeloid Disorders Working Group of the International Cancer Genome Consortium and of the Associazione Italiana per la Ricerca sul Cancro Gruppo Italiano Malattie Mieloproliferative. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. *Blood*. 2011; 118:6239–46. [PubMed: 21998214]
 129. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, Werner L, Sivachenko A, DeLuca DS, Zhang L, Zhang W, Vartanov AR, Fernandes SM, Goldstein NR, Folco EG, Cibulskis K, Tesar B, Sievers QL, Shefler E, Gabriel S, Hacohen N, Reed R, Meyerson M, Golub TR, Lander ES, Neuberger D, Brown JR, Getz G, Wu CJ. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med*. 2011; 365:2497–506. [PubMed: 22150006]
 130. Quesada V, Conde L, Villamor N, Ordonez GR, Jares P, Bassaganyas L, Ramsay AJ, Bea S, Pinyol M, Martinez-Trillos A, Lopez-Guerra M, Colomer D, Navarro A, Baumann T, Aymerich M, Rozman M, Delgado J, Gine E, Hernandez JM, Gonzalez-Diaz M, Puente DA, Velasco G, Freije JM, Tubio JM, Royo R, Gelpi JL, Orozco M, Pisano DG, Zamora J, Vazquez M, Valencia A, Himmelbauer H, Bayes M, Heath S, Gut M, Gut I, Estivill X, Lopez-Guillermo A, Puente XS, Campo E, Lopez-Otin C. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. *Nat Genet*. 2011; 44:47–52. [PubMed: 22158541]
 131. Lasho TL, Finke CM, Hanson CA, Jimma T, Knudson RA, Ketterling RP, Pardanani A, Tefferi A. SF3B1 mutations in primary myelofibrosis: clinical, histopathology and genetic correlates among 155 patients. *Leukemia*. 2012; 26:1135–7. [PubMed: 22064353]
 132. Harutyunyan A, Klampfl T, Cazzola M, Kralovics R. P53 Lesions in Leukemic Transformation. *N Engl J Med*. 2011; 364:488–90. [PubMed: 21288114]
 133. Klampfl T, Harutyunyan A, Berg T, Gisslinger B, Schalling M, Bagienski K, Olcaydu D, Passamonti F, Rumi E, Pietra D, Jager R, Pieri L, Guglielmelli P, Iacobucci I, Martinelli G, Cazzola M, Vannucchi AM, Gisslinger H, Kralovics R. Genome integrity of myeloproliferative neoplasms in chronic phase and during disease progression. *Blood*. 2011; 118:167–76. [PubMed: 21531982]
 134. Rampal R, Ahn J, Abdel-Wahab O, Nahas M, Wang K, Lipson D, Otto GA, Yelensky R, Hricik T, McKenney AS, Chiosis G, Chung YR, Pandey S, van den Brink MR, Armstrong SA, Dogan A, Intlekofer A, Manshouri T, Park CY, Verstovsek S, Rapaport F, Stephens PJ, Miller VA, Levine RL. Genomic and functional analysis of leukemic transformation of myeloproliferative neoplasms. *Proc Natl Acad Sci U S A*. 2014; 111:E5401–10. [PubMed: 25516983]
 135. Laurie NA, Donovan SL, Shih CS, Zhang J, Mills N, Fuller C, Teunisse A, Lam S, Ramos Y, Mohan A, Johnson D, Wilson M, Rodriguez-Galindo C, Quarto M, Francoz S, Mendrysa SM, Guy RK, Marine JC, Jochemsen AG, Dyer MA. Inactivation of the p53 pathway in retinoblastoma. *Nature*. 2006; 444:61–6. [PubMed: 17080083]

136. Ley TJ, Ding L, Walter MJ, McLellan MD, Lamprecht T, Larson DE, Kandoth C, Payton JE, Baty J, Welch J, Harris CC, Lichti CF, Townsend RR, Fulton RS, Dooling DJ, Koboldt DC, Schmidt H, Zhang Q, Osborne JR, Lin L, O'Laughlin M, McMichael JF, Delehaunty KD, McGrath SD, Fulton LA, Magrini VJ, Vickery TL, Hundal J, Cook LL, Conyers JJ, Swift GW, Reed JP, Alldredge PA, Wylie T, Walker J, Kalicki J, Watson MA, Heath S, Shannon WD, Varghese N, Nagarajan R, Westervelt P, Tomasson MH, Link DC, Graubert TA, DiPersio JF, Mardis ER, Wilson RK. DNMT3A mutations in acute myeloid leukemia. *N Engl J Med.* 2010; 363:2424–33. [PubMed: 21067377]
137. Walter MJ, Ding L, Shen D, Shao J, Grillot M, McLellan M, Fulton R, Schmidt H, Kalicki-Veizer J, O'Laughlin M, Kandoth C, Baty J, Westervelt P, DiPersio JF, Mardis ER, Wilson RK, Ley TJ, Graubert TA. Recurrent DNMT3A mutations in patients with myelodysplastic syndromes. *Leukemia.* 2011; 25:1153–8. [PubMed: 21415852]
138. Stegelmann F, Bullinger L, Schlenk RF, Paschka P, Griesshammer M, Biersch C, Kuhn S, Schauer S, Dohner H, Dohner K. DNMT3A mutations in myeloproliferative neoplasms. *Leukemia.* 2011; 25:1217–9. [PubMed: 21537334]
139. Abdel-Wahab O, Pardanani A, Rampal R, Lasho TL, Levine RL, Tefferi A. DNMT3A mutational analysis in primary myelofibrosis, chronic myelomonocytic leukemia and advanced phases of myeloproliferative neoplasms. *Leukemia.* 2011; 25:1219–20. [PubMed: 21519343]
140. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med.* 2013; 368:2059–74. [PubMed: 23634996]
141. Bejar R, Stevenson K, Abdel-Wahab O, Galili N, Nilsson B, Garcia-Manero G, Kantarjian H, Raza A, Levine RL, Neuberg D, Ebert BL. Clinical effect of point mutations in myelodysplastic syndromes. *N Engl J Med.* 2011; 364:2496–506. [PubMed: 21714648]
142. Ding Y, Harada Y, Imagawa J, Kimura A, Harada H. AML1/RUNX1 point mutation possibly promotes leukemic transformation in myeloproliferative neoplasms. *Blood.* 2009; 114:5201–5. [PubMed: 19850737]
143. Georgopoulos K. Haematopoietic cell-fate decisions, chromatin regulation and ikaros. *Nat Rev Immunol.* 2002; 2:162–74. [PubMed: 11913067]
144. Mullighan CG, Miller CB, Radtke I, Phillips LA, Dalton J, Ma J, White D, Hughes TP, Le Beau MM, Pui CH, Relling MV, Shurtleff SA, Downing JR. BCR-ABL1 lymphoblastic leukaemia is characterized by the deletion of Ikaros. *Nature.* 2008; 453:110–4. [PubMed: 18408710]
145. Jager R, Gisslinger H, Passamonti F, Rumi E, Berg T, Gisslinger B, Pietra D, Harutyunyan A, Klampfl T, Olcaydu D, Cazzola M, Kralovics R. Deletions of the transcription factor Ikaros in myeloproliferative neoplasms. *Leukemia.* 2010; 24:1290–8. [PubMed: 20508609]
146. Zhao R, Follows GA, Beer PA, Scott LM, Huntly BJ, Green AR, Alexander DR. Inhibition of the Bcl-xL deamidation pathway in myeloproliferative disorders. *N Engl J Med.* 2008; 359:2778–89. [PubMed: 19109573]
147. Marty C, Lacout C, Droin N, Le Couedic JP, Ribrag V, Solary E, Vainchenker W, Villeval JL, Plo I. A role for reactive oxygen species in JAK2 V617F myeloproliferative neoplasm progression. *Leukemia.* 2013; 27:2187–95. [PubMed: 23558526]
148. Plo I, Nakatake M, Malivert L, de Villartay JP, Giraudier S, Villeval JL, Wiesmuller L, Vainchenker W. JAK2 stimulates homologous recombination and genetic instability: potential implication in the heterogeneity of myeloproliferative disorders. *Blood.* 2008; 112:1402–12. [PubMed: 18515659]
149. Schaub FX, Jager R, Looser R, Hao-Shen H, Hermouet S, Girodon F, Tichelli A, Gisslinger H, Kralovics R, Skoda RC. Clonal analysis of deletions on chromosome 20q and JAK2-V617F in MPD suggests that del20q acts independently and is not one of the predisposing mutations for JAK2-V617F. *Blood.* 2009; 113:2022–7. [PubMed: 19047681]
150. Vannucchi AM, Lasho TL, Guglielmelli P, Biamonte F, Pardanani A, Pereira A, Finke C, Score J, Gangat N, Mannarelli C, Ketterling RP, Rotunno G, Knudson RA, Susini MC, Laborde RR, Spolverini A, Pancrazzi A, Pieri L, Manfredini R, Tagliafico E, Zini R, Jones A, Zoi K, Reiter A, Duncombe A, Pietra D, Rumi E, Cervantes F, Barosi G, Cazzola M, Cross NC, Tefferi A. Mutations and prognosis in primary myelofibrosis. *Leukemia.* 2013; 27:1861–9. [PubMed: 23619563]

151. Tefferi A, Guglielmelli P, Larson DR, Finke C, Wassie EA, Pieri L, Gangat N, Fjerza R, Belachew AA, Lasho TL, Ketterling RP, Hanson CA, Rambaldi A, Finazzi G, Thiele J, Barbui T, Pardanani A, Vannucchi AM. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. *Blood*. 2014; 124:2507–13. quiz 2615. [PubMed: 25037629]
152. Guglielmelli P, Lasho TL, Rotunno G, Score J, Mannarelli C, Pancrazzi A, Biamonte F, Pardanani A, Zoi K, Reiter A, Duncombe A, Fanelli T, Pietra D, Rumi E, Finke C, Gangat N, Ketterling RP, Knudson RA, Hanson CA, Bosi A, Pereira A, Manfredini R, Cervantes F, Barosi G, Cazzola M, Cross NC, Vannucchi AM, Tefferi A. The number of prognostically detrimental mutations and prognosis in primary myelofibrosis: an international study of 797 patients. *Leukemia*. 2014; 28:1804–10. [PubMed: 24549259]
153. Patel KP, Newberry KJ, Luthra R, Jabbour E, Pierce S, Cortes J, Singh R, Mehrotra M, Routbort MJ, Luthra M, Manshouri T, Santos FP, Kantarjian H, Verstovsek S. Correlation of mutation profile and response in patients with myelofibrosis treated with ruxolitinib. *Blood*. 2015; 126:790–7. [PubMed: 26124496]
154. Vannucchi AM, Guglielmelli P, Rotunno G, Pascutto C, Pardanani A, Ferretti V, Pacilli A, Pancrazzi A, Lasho T, Hanson CA, Ketterling R, Gangat N, Tagliafico E, Manfredini R, Artusi V, Bernardis I, Pietra D, Rumi E, Maffioli M, Rosti V, Salmoiraghi S, Delaini F, Bosi A, Cilloni D, Cervantes F, Passamonti F, Barosi G, Rambaldi A, Barbui T, Cazzola M, Tefferi A. Mutation-Enhanced International Prognostic Scoring System (MIPSS) for Primary Myelofibrosis: An AGIMM & IWG-MRT Project. *Blood*. 2014; 124:405.
155. Tefferi A, Guglielmelli P, Finke C, Lasho TL, Gangat N, Ketterling R, Hanson CA, Pardanani A, Vannucchi AM. Integration of Mutations and Karyotype Towards a Genetics-Based Prognostic Scoring System (GPSS) for Primary Myelofibrosis. *Blood*. 2014; 124:406.
156. Vaidya R, Caramazza D, Begna KH, Gangat N, Van Dyke DL, Hanson CA, Pardanani A, Tefferi A. Monosomal karyotype in primary myelofibrosis is detrimental to both overall and leukemia-free survival. *Blood*. 2011; 117:5612–5. [PubMed: 21450904]
157. Barbui T, Thiele J, Vannucchi AM, Tefferi A. Problems and pitfalls regarding WHO-defined diagnosis of early/prefibrotic primary myelofibrosis versus essential thrombocythemia. *Leukemia*. 2013; 27:1953–8. [PubMed: 23467025]
158. Barosi G. Essential thrombocythemia vs. early/prefibrotic myelofibrosis: why does it matter. *Best Pract Res Clin Haematol*. 2014; 27:129–40. [PubMed: 25189724]
159. Tefferi A, Vardiman JW. Classification and diagnosis of myeloproliferative neoplasms: the 2008 World Health Organization criteria and point-of-care diagnostic algorithms. *Leukemia*. 2008; 22:14–22. [PubMed: 17882280]
160. Hernandez-Boluda JC, Pereira A, Gomez M, Boque C, Ferrer-Marin F, Raya JM, Garcia-Gutierrez V, Kerguelen A, Xicoy B, Barba P, Martinez J, Luno E, Alvarez-Larran A, Martinez-Lopez J, Arbelo E, Besses C. Grupo Espanol de Enfermedades Mieloproliferativas Filadelfia Negativas. The International Prognostic Scoring System does not accurately discriminate different risk categories in patients with post-essential thrombocythemia and post-polycythemia vera myelofibrosis. *Haematologica*. 2014; 99:e55–7. [PubMed: 24488561]