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# How does JAK2V617F contribute to the pathogenesis of myeloproliferative neoplasms?

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**A decade on from the discovery of the JAK2V617F mutation in the majority of patients with myeloproliferative neoplasms (MPNs), JAK2V617F is now firmly installed in the hematology curriculum of medical students and the diagnostic-testing algorithm of clinicians. Furthermore, the oral JAK1/JAK2 inhibitor ruxolitinib, rationally designed to target activated JAK2 signaling in MPN, has been approved by the Food and Drug Administration (FDA) of the United States for the past 3 years for the treatment of intermediate- and advanced-phase myelofibrosis. Notwithstanding this, JAK2V617F continues to stimulate the MPN research community and novel insights into understanding the mechanisms by which JAK2V617F contributes to the pathogenesis of MPN are continually emerging. In this chapter, we focus on recent advances in 4 main areas: (1) the molecular processes coopted by JAK2V617F to induce MPN, (2) the role that JAK2V617F plays in phenotypic diversity in MPN, (3) the functional impact of JAK2V617F on hematopoietic stem cells, and (4) therapeutic strategies to target JAK2V617F. Although great strides have been made, significant deficits still exist in our understanding of the precise mechanisms by which JAK2V617F-mutant hematopoietic stem cells emerge and persist to engender clonal hematopoiesis in MPN and in developing strategies to preferentially target the JAK2V617F-mutant clone therapeutically. Critically, although myelofibrosis remains arguably the greatest clinical challenge in JAK2V617F-mediated MPN, the current understanding of myelofibrosis-specific disease biology remains quite rudimentary. Therefore, many important biological questions pertaining to JAK2V617F will continue to engage and challenge the MPN research community in the coming decade.**

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**Learning Objective**

- To understand the role played by the JAK2V617F mutation in the development of MPNs: specifically, to outline its role in perturbing signal transduction, engendering distinct clinical disease phenotypes, altering hematopoietic stem cell function, and serving as a rational therapeutic target in MPN

## Introduction

The chronic myeloproliferative neoplasms (MPNs) encompass a spectrum of clonal neoplastic disorders characterized by overproduction of terminally differentiated cells of the myeloid lineage. A common genetic basis for the *BCR-ABL*-negative MPN disorders polycythemia vera (PV), essential thrombocythemia (ET), and myelofibrosis (MF) was elucidated in 2005 with the identification of the JAK2V617F mutation in the majority of MPN patients.[1-4] The discovery of JAK2V617F had a dramatic impact on the diagnosis and treatment of MPN. Testing for JAK2 mutations is now embedded in the World Health Organization (WHO) criteria for the diagnosis of MPN and, in 2011, the oral JAK1/JAK2 kinase inhibitor ruxolitinib became the first Food and Drug Administration (FDA)-approved drug for the treatment of MF. In this review, we focus on 4 questions surrounding the role of JAK2V617F in the pathogenesis of MPN: (1) what are the molecular processes coopted by JAK2V617F to instigate the malignant state?, (2) how does JAK2V617F cause distinct clinical phenotypes in MPN?, (3) what are the effects of JAK2V617F on hematopoietic stem cell (HSC) function?, and (4) how can we leverage our understanding of JAK2V617F biology for therapeutic targeting?

## What are the molecular processes coopted by JAK2V617F?

### *Structural biology of activating JAK2 mutations*

It has long been surmised that aberrant cytokine signaling plays a key role in the pathogenesis of MPN. In his seminal article in 1951, William Dameshek proposed that an "undiscovered stimulus" that causes overproliferation of BM cells underlies the clinical manifestations of MPN.[5] Subsequent studies using *in vitro* cultures showed that hematopoietic progenitor cells derived from MPN patients exhibited hypersensitivity to cytokines including erythropoietin, insulin-like growth factor-1 (IGF-1), IL-3, and GM-CSF, with the ability to form erythroid and megakaryocytic colonies in the presence of reduced levels, or the complete absence, of cytokines.[6]

In 2005, activating mutations in JAK2 kinase were uncovered in a majority of MPN patients, thus cementing the fundamental role of hyperactive cytokine signaling in MPN pathophysiology. Because many cytokine receptors lack intrinsic catalytic activity, the transduction of extracellular cues requires molecules that physically associate with receptors at the cell surface and activate downstream effector proteins in the cytosol and nucleus, with proper coordination of these signaling pathways being essential for homeostatic production of the different hematopoietic lineages. JAK2 belongs to the Janus family of nonreceptor tyrosine kinases and plays a fundamental role in hematopoiesis as one of these key signaling intermediates. Under normal conditions, ligand binding induces conformational changes in cytokine receptors that lead to the activation of receptor-associated JAK molecules and the phosphorylation of specific tyrosine residues within the intracellular domain of

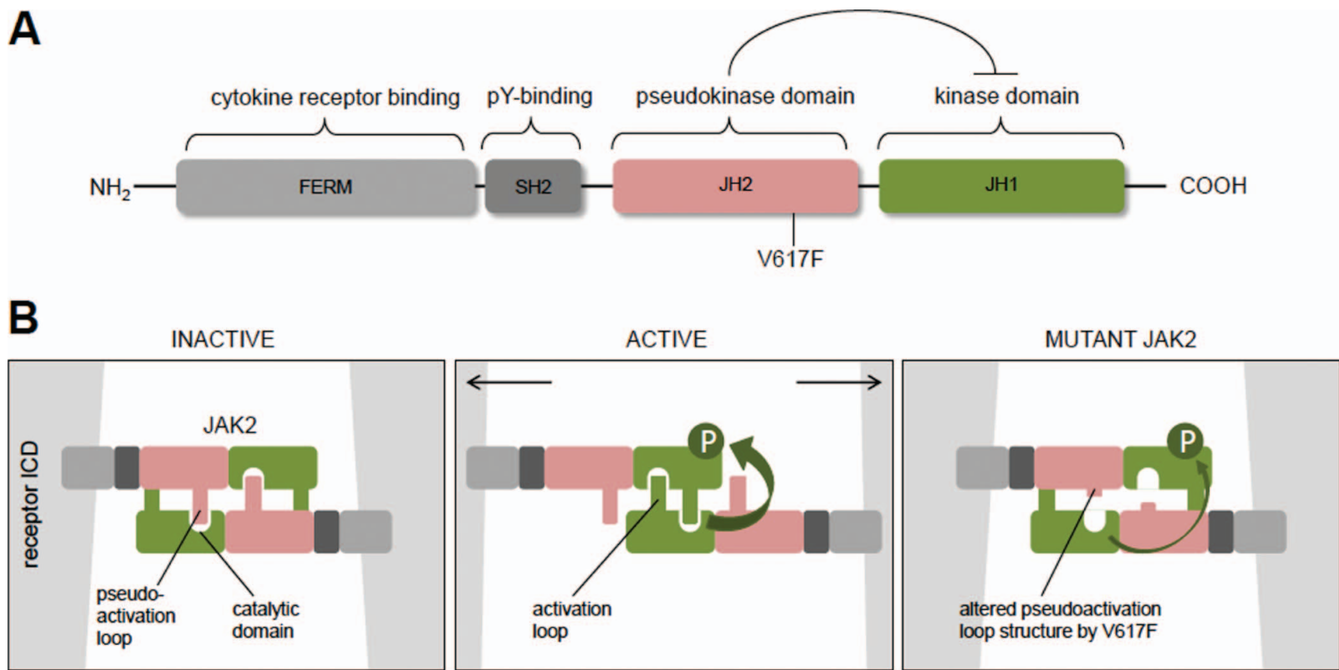


**Figure 1. JAK2 structure and signaling.** (A) Domain structure of JAK2. JAK2 contains a tyrosine kinase domain (JH1), a pseudokinase domain (JH2), an SH2-like domain, and a FERM domain that is responsible for attachment to the intracellular domain of cytokine receptors. (B) Model for JAK2 activation at the cytokine receptor. Left, Intermolecular JH1–JH2 interactions keeps JAK2 in an inactive state. Center, Ligand binding induces increased separation of cytokine receptors and movement of JAK2 dimers leads to apposition of kinase domains of the 2 JAK2 molecules and facilitates mutual phosphorylation of specific tyrosine residues in *trans*. Right, Consequences of the JAK2V617F mutation on the "sliding model" of JAK2 activation remains unclear, but is likely to involve diminished repression of the catalytic activity of the JH1 domain due to decreased stability of JH1–JH2 interaction.

the cognate receptor. These phosphotyrosine residues on the intracellular domain of the receptor serve as docking sites for downstream signaling proteins that harbor either a Src homology-2 (SH2), or a phosphotyrosine-binding domain. Upon recruitment to the receptor, these messengers are phosphorylated by JAK kinases, leading to their activation. In this way, extracellular signals can be transmitted via receptor proximal events into the activation of multiple downstream effector processes, including STAT transcription factors, the Ras/MAPK pathway, and the PI3K/AKT pathway.

MPN-associated JAK2 mutations include the V617F mutation, which is found in ~95% of individuals with PV and between 50% and 60% of those with ET and MF,[1-4] or a heterogenous set of complex mutations clustered in exon 12 of the *JAK2* gene, which is specific to a subset of PV patients associated with an isolated erythrocytosis.[7] Additional rare variants in exons 12-15 have also since been identified.[8]

Biochemically, most is known about the JAK2V617F mutation. The V617 amino acid residue is located within the JAK2 JH2 pseudokinase domain, which normally exerts an inhibitory effect on the adjacent JH1 kinase domain, thus keeping JAK2 in an inactive conformation (Figure 1A). The prevailing model in the field is that the V617F mutation disrupts JH1/JH2 interactions through steric interference, thus abrogating the JH2-inhibitory effect. Crystal structures of the JAK2 JH2 pseudokinase domain with or without the V617F substitution have now been reported to support and clarify this idea.[9] As predicted, the JH2 domain adopts a prototypical protein kinase fold but possessing a short, nonphosphorylatable activation loop.[9] The V617F mutation induces only a subtle effect on the JH2 structure, but is thought to modify at least 2 properties of the pseudokinase domain. First, V617F rigidifies the alpha-C ($\alpha$C) structure within the JH2 domain, which coincides with impaired ability of the pseudokinase domain to keep the kinase domain in an inactive state.[9-11] Secondly, V617F abolishes low-level dual-specific kinase activity of the pseudokinase domain that functions to autophosphorylate residues S523 and Y570, 2 modifications that are important for facilitating the JH1–JH2 interaction.[12] Combined, these effects result in diminished repression of JH1 kinase activity by the JH2 pseudokinase domain, culminating in a constitutively active JAK2 molecule.

A structure for the full-length JAK2 protein or for a fragment containing both JH1 and JH2 domains remains elusive, so the details of JH1 inhibition by the JH2 domain at an intermolecular level remain unclear. A recent molecular modeling study has provided some interesting insights into the nature of JH1 inhibition by the JH2 domain. JAK2 bound to the growth hormone receptor, the archetypal type I homodimeric receptor that belongs to the same receptor class as the erythropoietin receptor (EPOR) and thrombopoietin receptor, was shown to exist as a dimer and is held in an inactive state through an intermolecular interaction between the kinase domain of one molecule with the pseudokinase domain of the other (Figure 1B, left).[13] Receptor activation is mediated by ligand binding, which prompts physical separation of cytokine receptor intracellular domains to produce a sliding movement of the 2 JAK2 molecules such that the kinase domains become apposed and can activate each other in *trans* (Figure 1B, center). Such a mode of JAK2 activation is consistent with evidence that mutant JAK2 proteins retain a dependence on cytokine receptors for ectopic signaling and the induction of cytokine-independent growth.[14-17] Importantly, when the JAK2 binding to EPOR is disrupted by mutagenesis of a critical residue on the EPOR or disruption of the cytokine-binding region of JAK2 (FERM domain), the transforming

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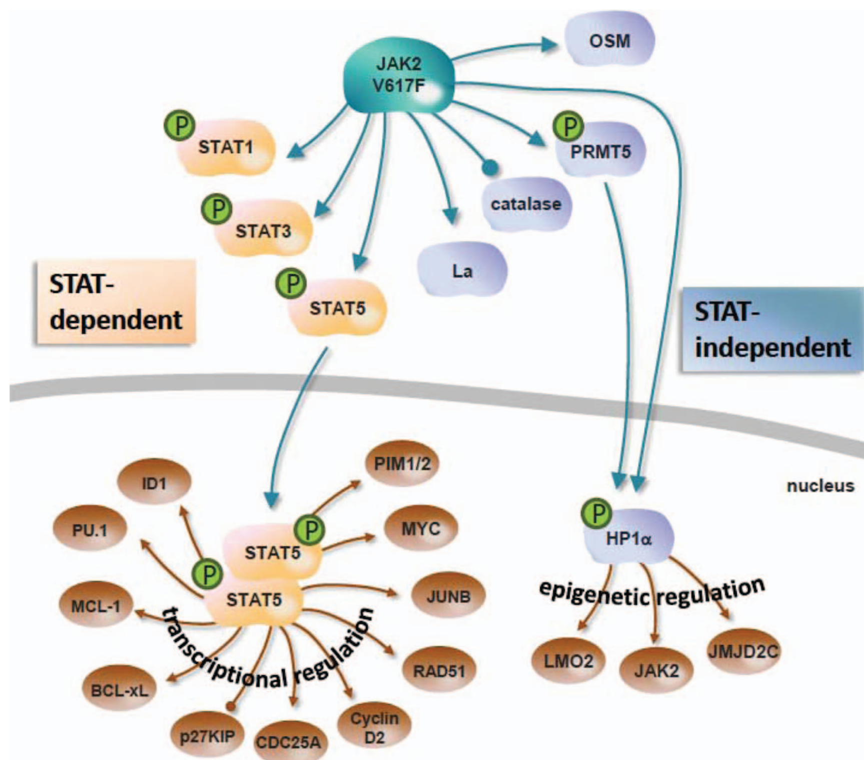


**Figure 2. Downstream targets of JAK2V617F in MPN.** JAK2V617F can activate a variety of downstream targets through both STAT-dependent and STAT-independent pathways. Arrows indicate proteins with function or expression that is activated; blunt ends designate inhibited targets. OSM indicates oncostatin M.

ability of JAK2V617F is abolished.[14,18] How the V617F mutation might affect the "sliding model" of JAK2 activation remains to be studied (Figure 1B, right).

### *Aberrant downstream consequences of JAK2V617F*

The downstream consequences of oncogenic JAK2 molecules remain topics of intense investigation (Figure 2). Of the known downstream effectors of JAK2V617F, the STAT5 transcription factor plays a critical role in disease pathogenesis. Numerous STAT5 transcriptional targets have been reported in the literature, which, when dysregulated, can promulgate the malignant state in MPN. These include: (1) the PIM kinases PIM-1 and PIM-2, which stimulate cell proliferation and impair apoptosis[18]; (2) c-MYC and JUNB, which provide an immediate early pro-proliferative response to growth stimuli[19,20]; (3) CYCLIN D2, p27KIP, and CDC25A, which can disrupt the $G_1$/S checkpoint and increase cell cycling[21,22]; (4) the PU.1 and ID1 transcription factors, which function to increase myeloid differentiation[23,24]; (5) BCL-XL and MCL-1, which inhibit apoptosis and promote cytokine-independent growth and erythropoietin-independent colony formation[25,26]; and (6) RAD51, which increases rates of DNA repair and maintains genomic stability to facilitate rapid cell cycling.[27] Consistent with the central role of STAT5 in MPN pathophysiology, Jak2V617F expression is incapable of producing disease in a Stat5a/b-deficient mouse.[28,29] Although some *in vitro* studies have suggested that active STAT3 can confer resistance to apoptosis caused by cytokine withdrawal,[30] murine studies have shown Stat3 is in fact not required for myeloid expansion induced by Jak2V617F.[29]

In addition to STAT proteins, several other direct downstream consequences of JAK2V617F have been identified that may engender MPN. These include: (1) phosphorylation of PRMT5 by mutant JAK2, which suppresses its arginine methyltransferase activity and leads to altered chromatin remodeling concomitant with increased erythroid colony formation and cell growth[31]; (2) up-regulation of the La autoantigen by JAK2V617F, which leads to impaired p53 activity and contributes to cytokine hypersensitivity[32]; (3) decreased expression of catalase downstream of the PI3K-AKT-FOXO3a signaling axis, which leads to increased levels of intracellular reactive oxygen species[33]; (4) elevated oncostatin M expression and secretion into the BM microenvironment, which causes specific features of MF, including growth of fibroblasts and increased production of profibrogenic cytokines[34]; and (5) phosphorylation of the Y41 residue of histone H3 by mutant JAK2, which leads to displacement of HP1 protein from chromatin and increased gene transcription at loci of known protooncogenes.[35,36]

## How does JAK2V617F cause distinct clinical phenotypes in MPN?

An important question is how an identical mutation contributes to the development of distinct clinical phenotypes in MPN (eg, PV, ET, and MF). In this section, we discuss specific factors that may contribute to phenotypic diversity in JAK2-mutated MPN.

### *JAK2V617F allele burden and clinical phenotype*

Loss of heterozygosity at 9p24 (9pLOH) encompassing the *JAK2* locus is a common feature of MPN.[37] In contrast to tumor suppressors, in which LOH is commonly the result of a hemizygous deletion of the remaining wild-type copy of the gene, 9pLOH in MPN is the result of uniparental disomy such that patients are left with either 2 paternal or 2 maternal copies of this region after mitotic recombination. This cytogenetic abnormality leads to some



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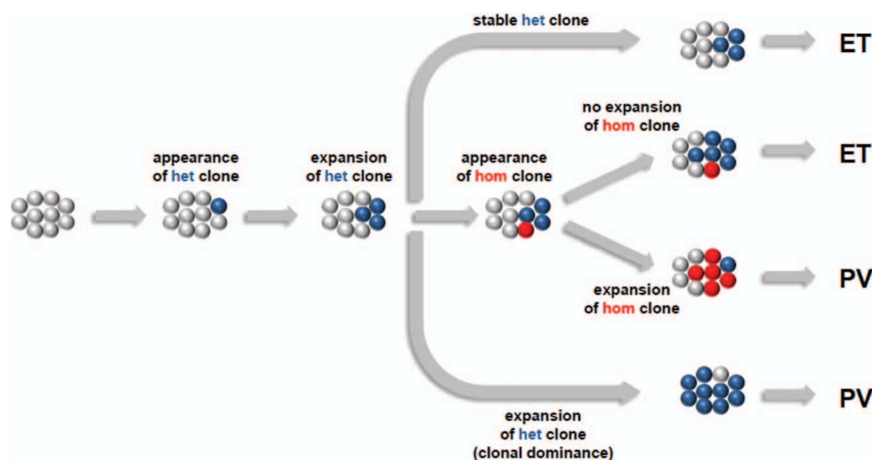


**Figure 3. Interplay among JAK2V617F copy number, clonal composition, and disease phenotype.** Clonal composition in ET and PV patients can be varied and complex and can have a direct impact on disease phenotype. Analysis of clonally derived erythroid progenitors demonstrated that the clonal composition of ET patients consists of a population of JAK2V617F-heterozygous cells with either no or a small and unexpanded population of JAK2V617F-homozygous cells. In contrast, the clonal composition of PV patients can consist of either near to complete dominance of the entire cell compartment with JAK2V617F-heterozygous cells, or a population of JAK2V617F-heterozygous cells with an expanded population of JAK2V617F-homozygous cells.

cells harboring 2 copies of the JAK2V617F mutation. Indeed, "homozygous" sequencing pattern (>50% JAK2V617F allelic burden) in granulocyte DNA is seen in >25% of PV and MF patients.[2,3] Although mitotic recombination would also theoretically yield a cell with 2 copies of wild-type JAK2, this clone has never been reported in patients, suggesting that it lacks sufficient selective advantage for clonal growth.[38]

Strikingly, homozygosity for JAK2V617F as detected in the granulocyte compartment is a much more prevalent phenomenon in PV than in ET.[3] In PV, JAK2V617F homozygosity is associated with higher hemoglobin levels, higher WBC counts, lower platelets, more splenomegaly, and a greater need for cytoreduction. These associations are present irrespective of whether the JAK2V617F mutation is considered as a discrete variable ("heterozygous" versus "homozygous") or as a continuous variable (quantitation of allele burden by qPCR).[39,40] This has led to the hypothesis that homozygous JAK2V617F drives an erythroid phenotype. This hypothesis is consistent with murine studies showing that low levels of JAK2V617F causes thrombocytosis with a slight elevation in hematocrit, a phenotype reminiscent of that seen in ET patients, whereas higher levels of JAK2V617F elicits marked erythrocytosis and leukocytosis without associated thrombocytosis.[41] In addition, the JAK2 exon 12 mutations, which are "stronger" alleles capable of inducing more robust activation of downstream STATs, are seen only in patients with PV and idiopathic erythrocytosis and not ET.[7] Therefore, it is likely that the differences in signaling between JAK2V617F heterozygous and homozogyous cells contribute to phenotypic variability in MPN.

An issue of quantitating allelic burden within a bulk tissue compartment (such as granulocytes) is the inability to ascertain what proportion of cells within the tissue has a given genotype. For example, a patient with a 50% allele burden can have either 100% heterozygous cells, 50% homozygous cells, or a combination of heterozygous and homozygous cells that combine to a final allelic burden of 50%. Therefore, analyses of clonally derived erythroid progenitors have been fruitful in providing insights into the effects of JAK2V617F copy number on disease phenotype. Somewhat surprisingly, when PV and ET patients were analyzed at a single-cell level, a high proportion of both PV and ET patients harbored homozygous clones (~80% in PV and ~50% in ET).[42] However, the use of microsatellite markers to map the LOH breakpoint revealed that PV patients were distinguished from ET patients by the presence of a clonal expansion of a single homozygous clone. This is consistent with a model that expansion of a homozygous subclone drives erythrocytosis in a majority of PV patients, whereas the infrequent homozygous cells in patients with ET are insufficient to drive erythrocytosis to a similar extent (Figure 3). It remains unclear what drives the homozygous clone to expand in PV, but it could be due to either additional genetic or epigenetic events or non-cell-autonomous selective pressures such as low levels of circulating erythropoietin in the context of elevated hematocrit.

### *Differential signaling consequences of JAK2V617F in PV, ET, and MF*

Strength of JAK2 signaling is unlikely to be the complete story, because many PV patients have relatively low V617F allele burdens. Accordingly, multiple studies have suggested that there are qualitative differences downstream of JAK2V617F in a disease-dependent manner. Analysis of gene expression signatures from paired normal and JAK2V617F heterozygous samples revealed cell-intrinsic changes that were common to both PV and ET or unique to one disease or the other. This analysis revealed that STAT5 activation is omnipresent in both disease subtypes, whereas STAT1 activation is significantly more pronounced in mutant cells from ET patients compared with those from PV patients.[43] Increased STAT1 activity was shown to promote megakaryopoiesis and constrain erythropoiesis of cord blood–derived $CD34^+$ cells, a finding that can partly explain the phenotypic differences of specific MPN subtypes.[44] Consistent with this idea, attenuated STAT1 activity produced a more erythroid phenotype in JAK2V617F-positive progenitors derived from ET patients[43] and in a JAK2V617F mouse model of MPN.[45] Other qualitative differences in signaling downstream of mutant JAK2 have been reported in bulk tissues. Analysis of $CD34^+$ cells taken from patients with PV, ET, and MF for a panel of signaling pathways downstream of JAK2V617F by intracellular flow cytometry revealed higher STAT3 and STAT5 (but not ERK) phosphorylation in MF patients relative to PV and ET patients,[46] and immunohistochemical analysis of >100 MPN BM trephines reveal distinct patterns in STAT3/5 staining in distinct MPN subtypes, with STAT3/5 being higher in PV than in ET.[47] The

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mechanisms that account for such qualitative differences in signaling remain unknown.

### *Role of constitutional genetic modifiers in MPN phenotype*

Phenotypic diversity in MPN may also reflect differences in constitutional genetic modifiers. A limited study of 32 single nucleotide polymorphisms in a cohort of 179 patients revealed disease-specific associations with several single nucleotide polymorphisms in *JAK2* and *EPOR*.[48] Consistent with a modifying role for host genetic variation in disease phenotype, significantly lower JAK2V617F allele burden has been reported in women with MPN compared with men, suggestive of a role for sex in influencing the rate of loss of heterozygosity and/or the extent of clonal expansion.[49] Moreover, larger numbers of homozygous colonies can be seen in male patients with PV and in female patients with ET, suggesting that sex may also modulate the phenotypic consequences of homozygosity.[50] These final studies highlight a potentially important concept, that the 3 variables in disease presentation (JAK2V617F copy number, qualitative differences in signaling, and constitutional genetic modifiers) are not mutually exclusive, but rather are likely to be heavily interdependent.

## What are the effects of JAK2V617F on HSC function?

### *Cell of origin in JAK2V617F-mediated MPN*

Soon after the discovery of JAK2V617F, it was shown that the mutation is detectable in primitive $CD34^+$ $CD38^-$ HSC[51] and can be found in all mature cell lineages.[52,53] Although it seems intuitive that JAK2V617F must confer a competitive advantage at the level of the HSC to engender clonal hematopoiesis, it has not been unambiguously demonstrated that this is the case, and the mechanisms by which JAK2V617F-mutant HSCs emerge and persist to cause clonal hematopoiesis are not well understood. A study of 41 JAK2-mutated MPN patients found no expansion of the $CD34^+$ $CD38^-$ HSC compartment in PV and ET, whereas a significant expansion was seen in MF.[54] Consistent with this, a separate study showed that the JAK2V617F allele burden was higher in the $CD34^+$ cell compartment of patients with MF compared with those with PV and ET.[55] Interestingly, this expansion of JAK2 mutant cells in the $CD34^+$ compartment in MF was found to be independent of JAK2V617F homozygosity, suggesting that clonal expansion in the $CD34^+$ compartment in MF is driven by other somatic genetic alterations enriched in MF compared with PV or ET (eg, loss-of-function mutations in genes involved in epigenetic regulation). In fact, whether JAK2V617F alone is sufficient to cause MPN or if additional genetic or epigenetic alterations are required is a question that arises with the observation that the JAK2V617F mutation is detectable at low frequency in the general population and in individuals without overt clinical manifestations of MPN.[56,57]

Data from MPN patients and primary samples indicate that JAK2V617F is not a strong driver of clonal expansion in HSCs. In ET and PV, the JAK2V617F-mutant clone size in HSCs is often small,[58] whereas in ET, JAK2V617F-heterozygous clones can remain stable over years[59] and recent evidence suggests that homozygous JAK2V617F clones do not necessarily expand.[42] Consistent with this, ET and PV are diseases generally characterized by clinical stability over decades. Nevertheless, clonal evolution and transformation to acute myelogenous leukemia can occur, particularly in MF.[60] Interestingly, acute myelogenous leukemia that arises out of JAK2V617F-mutant MPN retains the JAK2V617F allele only ~50% of the time,[61,62] suggesting that JAK2V617F is not a strong clonal driver of cell-intrinsic mechanisms of leukemic transformation. Potential explanations for the propensity of JAK2 wild-type HSCs for leukemic transformation in JAK2V617F-mutant MPN include a permissive BM microenvironment, selection pressure imposed by treatment interventions, inherited genetic variants that predispose to myeloid neoplasms, and toxic environmental exposures that predispose to myeloid neoplasms.

### *Functional studies of JAK2V617F-mutant HSCs in mice*

The effects of the JAK2V617F mutation in HSCs have been extensively modeled in mice using retroviral, transgenic, knock-in, and xenotransplantation approaches.

The functional impact of JAK2V617F on HSCs has been assessed in xenografts. Peripheral blood $CD34^+$ cells from patients with MF engraft NOD/SCID mice and show clonal hematopoiesis with myeloid skewing.[63] More recently, it has been shown that splenic $CD34^+$ cells from MF patients demonstrate sustained engraftment in NOD/SCID/IL2Rγ-null mice and can be serially transplanted into secondary recipients.[64] Conversely, JAK2V617F mutant $CD34^+$ cells from patients with PV and ET demonstrate relatively poor engraftment[65] and, consistent with this the ratio of JAK2V617F to *JAK2* wild-type SCID-repopulating cells (SRCs), has been shown to be higher in MF compared with PV.[66] Functionally, JAK2V617F SRCs do not gain a proliferative advantage over wild-type SRCs over time in CD122-depleted NOD/SCID mice.[66] In aggregate, this suggests that JAK2V617F alone does not significantly enhance SCID-repopulating activity and that the superior engraftment of $CD34^+$ cells from MF patients compared with those from PV or ET patients is likely driven by the presence of additional genetic abnormalities. In fact, TET2-JAK2V617F-comutated $CD34^+$ cells have been shown to have an increased capacity over JAK2V617F-mutated $CD34^+$ cells to repopulate NOD-SCID mice.[67] One important caveat in the interpretation of the results of xenograft studies in MPN is that incompatibilities between human cytokine receptors (expressed on transplanted $CD34^+$ cells) and murine cytokines (produced by the recipient murine BM) may affect the results given that interaction with a cell surface cytokine receptors (eg, MPL) is required for JAK2V617F to transform. In general, reliable xenotransplantation studies in MPN require additional optimization.

To circumvent the issue of species incompatibility for some cytokines and cytokine receptors, several investigators have used syngeneic genetic murine models (both knock-in and transgenic) to assess the impact of JAK2V617F on HSC function. In general, there has been considerable variability in the findings of these studies, likely as a result of differences in the targeting strategy of different MPN murine models (eg, promoter, oncogene expression level, murine vs human protein).

Four separate JAK2V617F knock-in models have been generated and, in each model, the disease is transplantable into secondary recipients, indicating, as expected, that JAK2V617F-mediated MPN is cell autonomous. Mullally et al conditionally expressed murine Jak2V617F from the endogenous murine *Jak2* promoter and found that Jak2V617F conferred minimal competitive repopulating advantage at 16 weeks.[68] By transplanting sorted populations of stem and progenitor cells, they further demonstrated that the MPN disease-propagating cell population is contained exclusively in the long-term HSC (LT-HSC) compartment and that expanded Jak2V617F progenitor cell populations such as megakaryocytic erythroid progenitor cells are incapable of reconstituting MPN in a transplanted animal.[68,69] These findings indicate that Jak2V617F does not confer self-renewal upon non-self-renewing hematopoietic cells and that,

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to maintain disease, JAK2V617F must occur in cells that have inherent self-renewal capability. Hasan et al also conditionally expressed murine Jak2V617F from the endogenous murine *Jak2* promoter, but found a stronger competitive advantage for Jak2V617F-mutant HSC.[70] The differences in these results may reflect differences in mutant Jak2V617F expression level between the models. Li et al conditionally expressed human JAK2V617F from the endogenous murine *Jak2* promoter and found decreased competitive reconstitution in heterozygote JAK2V617F mutant mice that was further exacerbated in homozygote JAK2V617F animals.[71,72] Competitive transplantation of 10 LT-HSCs from JAK2V617F mice also demonstrated a competitive disadvantage that was sustained through serial transplantation, indicating that JAK2V617F-expressing LT-HSCs have impaired self-renewal function in this model.[73] Interestingly, when a human JAK2V617F transgene was expressed from the endogenous human *JAK2* promoter and crossed with Mx1-Cre-transgenic mice to induce JAK2V617F expression *in vivo*, competitive repopulation experiments found a strong advantage for the JAK2V617F-mutant expressing cells.[74] Approximately 6-9 copies of the JAK2V617F transgene are present after Cre recombination in this model, although the ratio of mutant human JAK2V617F to wild-type murine Jak2 expression is approximately 1:1.[41] The differences in the findings with respect to human JAK2V617F expression *in vivo* in mice may relate to different promoter (murine vs human), differences between the signaling characteristics of murine and human proteins, and/or to differences inherent to knock-in versus transgenic approaches.

Finally, although MPN animal models accurately recapitulate human disease in mice and have been an important tool for the study of MPN biology and therapy, it is important to remember that several differences remain. First, although many of the murine models recapitulate physiological Jak2V617F expression *in vivo*, in primary animals, the mutant Jak2 allele is simultaneously expressed in all hematopoietic cells rather than in a single clone as typically occurs in humans. Another relevant point is that, although virtually all JAK2V617F genetic murine models develop an MPN phenotype, suggesting that JAK2V617F alone is sufficient to induce MPN, hematopoiesis is polyclonal in these models, in contrast to the clonal hematopoiesis that occurs in MPN patients. This point is also pertinent to competitive hematopoietic reconstitution of Jak2V617F-mutant hematopoietic cells, which is also polyclonal and also occurs in a BM niche that has been perturbed by irradiation.

In aggregate, studies on the effects of JAK2V617F on HSC function indicate that, by itself, it is not a strong driver of clonal expansion in the primitive HSC compartment. This implies that the differential molecular dependencies of JAK2-mutant HSCs may be subtle and that selectively targeting them therapeutically may prove challenging. However, in PV and ET, in which the JAK2-mutant clone is generally small, if effective strategies to selectively target JAK2-mutant HSCs were to emerge, instituting these early in the disease course could plausibly eradicate the malignant hematopoietic clone to definitively cure MPN.

## How can we target JAK2V617F?

Although many novel agents are under investigation in JAK2V617F-mutant MPN, we have restricted the focus of this section to those therapeutic approaches that either directly target JAK2 itself or those, such as IFN, that appear to preferentially target JAK2V617F-mutant HSCs.

### *Inhibiting JAK2 kinase activity*

The discovery of JAK2V617F, an activating mutation in a kinase present in the majority of patients with MPN, was immediately recognized as an attractive therapeutic target and JAK2 kinase inhibitors were rapidly developed. In fact, within 6 years of the identification of JAK2V617F, the FDA approved ruxolitinib, an oral JAK1/JAK2 inhibitor, for the treatment of patients with intermediate- and advanced-phase MF (ruxolitinib is now also approved in Europe and Canada). Approval was granted on the basis of 2 randomized phase 3 studies, COMFORT-I and COMFORT-II, which compared ruxolitinib with placebo and best-available therapy, respectively, and found significant reductions in splenomegaly and improvement in constitutional symptoms.[75,76] With a median follow-up of 2 years, Kaplan-Meier survival estimates demonstrated an improved overall survival probability in the ruxolitinib treatment group (hazard ratio = 0.58) in the COMFORT-I study.[77] Follow-up at 3 years was recently reported for the COMFORT II study.[78] The median change in the JAK2V617F allele burden from baseline was −8.0% at 72 weeks, indicating that ruxolitinib does not preferentially target the JAK2V617F mutant clone to any significant extent, a finding also seen in clinical trials of other JAK inhibitors.[78,79] A notable feature of the JAK inhibitor clinical trials is the fact that pharmacodynamic data (eg, STAT5 phosphorylation status) has generally not been reported, so the level of JAK2 inhibition achieved during treatment in patients has not been well documented. Anemia and thrombocytopenia were among the most common adverse effects observed in the COMFORT II study[78] and, in conjunction with the JAK2V617F allele burden data indicating a nonclonally selective effect of ruxolitinib, these have been interpreted as "on-target" toxicities related to inhibiting unmutated JAK2. It is interesting that not all JAK inhibitors behave the same in terms of their propensity to induce anemia, with some actually capable of rendering patients transfusion independent,[79] a finding that may relate to the level of JAK2 and/or JAK1 inhibition achieved during treatment with different JAK inhibitors.[80] Another notable feature of the COMFORT II study is that only 45% of those originally randomized to ruxolitinib remained on treatment at 3 years, with 15% patients discontinuing the drug due to disease progression. Despite this, there was an ∼50% reduction in the risk of death in the ruxolitinib arm compared with the best-available therapy arm (the median overall survival was not reached after 3 years of follow-up).[78] Guglielmelli et al recently evaluated the impact of molecular abnormalities in a subset of the COMFORT-II cohort (166 of the 219 total patients were evaluated) by genotyping 14 MF-associated prognostically significant mutations, but did not identify any molecular predictors of response to ruxolitinib.[81] In aggregate, these results indicate that any survival benefit of ruxolitinib in patients with intermediate- or advanced-phase MF is not occurring as a result of selectively targeting the malignant hematopoietic clone. It is plausible that increased dietary intake and enhanced performance status as a result of improved constitutional symptoms and reduced splenomegaly could contribute to the improved Kaplan-Meier survival estimates for patients treated with ruxolitinib.[78] It is also possible that stabilization or improvement in fibrosis as a result of a reduction in inflammatory cytokines could be a factor, but additional studies with longer follow-up will be required to validate this preliminary finding.[82]

### *Targeting JAK2 protein for degradation*

There are multiple potential contributing factors to the nonclonal selectivity of JAK2 inhibitors in the treatment of JAK2V617F mediated MPN. These include an absence of strong oncogene addiction to mutant JAK2, the presence of concomitant genetic

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alterations in patients with MF, and a narrow therapeutic index for inhibiting JAK2 given its critical function in normal erythropoiesis (patients with MF are often anemic at the time of initiating JAK2 inhibitor therapy). The potential for hematologic toxicity from on-target JAK2 inhibition has been highlighted by recent murine studies in which hematopoietic-specific conditional genetic deletion of Jak2 in adult mice resulted in severe cell-intrinsic defects in HSC function, impaired hematopoiesis, and reduced survival,[83-85] suggesting that, even if more potent and selective JAK2 inhibitors were available, these would not be well tolerated in patients. It is important to note, however, that genetically deleting Jak2 in mice results in a total loss of Jak2 protein, rather than just inhibiting Jak2 kinase activity. Consistent with an absence of clonal selectivity for the JAK2 inhibitors, JAK2 resistance mutations have not been identified in treated patients,[86] likely because there is insufficient selective pressure for them to emerge. Although a small number of JAK2 resistance mutations have been identified under strong selection pressure conditions *in vitro*,[87,88] a recent JAK2 kinase inhibitor saturation mutagenesis screen did not identify second-site JAK2 mutations in JAK2 kinase-resistant clones, but rather found that JAK2V617F-mutant cells that persisted despite chronic JAK2 inhibition remained dependent on JAK2 protein expression.[86] This has led to the strategy of targeting JAK2 protein for degradation using HSP90 inhibitors (JAK2 is an HSP90 chaperone client), an approach that has been demonstrated to be efficacious in murine MPN models using an HSP90 inhibitor either alone[89] or in combination with JAK2 kinase inhibition.[90] These preclinical studies have led to a clinical trial of the HSP90 inhibitor AUY922 in patients with MF.

### *Preferentially targeting JAK2V617F-mutant HSCs using IFN*

IFNα has a long history of clinical efficacy in the treatment of PV and ET.[91,92] More recent clinical trials have demonstrated that, in addition to achieving hematological remissions, IFNα can render the JAK2V617F-mutant clone undetectable by PCR.[93,94] Furthermore, long-term molecular responses after discontinuation of treatment have been reported, suggesting that JAK2V617F-mutant HSCs are eradicated by IFNα,[95] although molecular relapse after cessation of IFNα has also been observed.[96] Murine studies indicate that Jak2V617F-mutant HSCs are preferentially sensitive to IFNα treatment[70,97] and suggest a potential mechanism for molecular remission in MPN patients through activated cell cycling within the HSC compartment, resulting in preferential depletion of JAK2V617F-mutant HSCs.[97]

## Concluding remarks

The JAK2V617F mutation is the most frequent somatic lesion in MPN, and selectively targeting mutant JAK2 remains a laudable goal for definitive curative therapy. Although the JAK inhibitors demonstrate clinical efficacy in MF, their non-selectivity for the JAK2V617F-mutant clone can result in dose-limiting anemia and may impair their ability to alter the natural history of MPN. The recent elucidation of the crystal structure of the JAK2 JH2 domain (in which the V617F mutation occurs) advances the potential for the development of mutant-specific JAK2 inhibitors. Efforts at preferentially targeting the JAK2-mutated clone would also be advanced by a better understanding of the mechanisms by which JAK2V617F-mutant HSCs "out-compete" normal HSCs to engender clonal hematopoiesis in MPN. Furthermore, the biological mechanisms underlying disease evolution in MPN, in particular the role of the JAK2V617F-mutant hematopoietic clone in driving fibrotic transformation in the bone marrow stromal compartment and in promoting leukemic transformation, remain poorly understood. Because these complications can significantly reduce the survival of patients with MPN, they remain important areas for future research.

## Disclosures

Conflict-of-interest disclosures: The authors declare no competing financial interests. Off-label drug use: None disclosed.

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