Blood Reviews 28 (2014) 189–196

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/blre

REVIEW

# The future of JAK inhibition in myelofibrosis and beyond

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ARTICLE INFO

*Keywords:*
Myeloproliferative neoplasms
Myelofibrosis
Polycythemia vera
JAK
Ruxolitinib

ABSTRACT

The identification of aberrant JAK–STAT signaling in patients with myeloproliferative neoplasms has served as the basis for the development of a new class of targeted agents. Ruxolitinib, the first-in-class oral small molecule JAK1/2 inhibitor, has demonstrated clinical efficacy and shown a potential overall survival benefit in two randomized phase III clinical trials. However, this agent has not been associated with improvements in cytopenias, molecular remissions, or resolution of bone marrow fibrosis. Therefore, further translational research is needed to improve the understanding of the pathogenetic mechanisms driving this myeloid malignancy to ultimately address remaining unmet clinical needs. A number of novel JAK inhibitors are being evaluated in ongoing clinical trials and the full clinical potential of these newer agents remains incompletely understood. The use of JAK inhibition in combination therapy approaches, as well as mono- and combination therapies in the treatment of advanced forms of polycythemia vera are also under active investigation. This review will update the reader on the current understanding of oncogenic JAK–STAT pathway activity in the pathogenesis of myeloproliferative neoplasms and the current success and limitations of anti-JAK therapy.



## 1. Introduction

The Janus family of protein tyrosine kinases (JAKs) plays an integral role in essential physiological processes, such as innate and adaptive immunity, inflammatory response, hematopoietic cell differentiation and development, tissue growth, and embryonic development. These kinases, namely JAK1, JAK2, JAK3, and TYK2, are known to mediate cytokine and growth factor signaling cascades lacking intrinsic kinase activity. While JAK2 alone plays a predominant role in erythropoietin (Epo), thrombopoietin (TpoR), granulocyte macrophage colony-stimulating factor (GM-CSF), interleukin (IL)-3 and IL-5 signaling, both JAK1 and JAK2 mediate signaling through IL-6, IL-10, IL-11, IL-19, IL-20, IL-22, and IFN-γ [1,2]. Binding of these ligands to their cognate transmembrane receptors results in activation of receptor-associated JAKs, which in turn activate signal transducers and activators of transcription (STATs) and other signaling pathways such as PI3K (phosphoinositide 3-kinase) and RAS, which are known to promote cell survival and proliferation. For instance, the activation of JAK2 is known to promote recruitment and phosphorylation of STAT3 and STAT5, which can form homo- as well as heterodimers that upon translocation to the nucleus, mediate transcription of genes that regulate cell proliferation, differentiation, and apoptosis (e.g., Bcl-xL, cyclin D1, and PIM1) [2–4].

It is therefore not surprising that overactivation of the JAK–STAT pathway has been observed in malignancies, including solid and hematologic neoplasms [2]. In solid tumors, such as breast, lung, and head and neck cancers, persistent phosphorylation of STAT1, STAT3, and STAT5 is mediated by an increase in cytokine levels, in both autocrine and paracrine manners, and by enhanced expression of cytokine receptors [5]. In Hodgkin lymphoma and primary mediastinal B-cell lymphoma, the JAK–STAT pathway is often activated due to amplification of the JAK2 gene [6–8]. Activation of the JAK–STAT pathway is also known to mediate resistance to BCR–ABL1 kinase inhibitors in chronic myeloid leukemia (CML) [9]. In high-risk acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML), upregulation of the JAK–STAT pathway mediated by mutated JAK1, JAK2, and JAK3 proteins has been reported. On the other hand, somatic mutations in the *JAK3* gene and chimeric fusion transcripts involving *JAK2* have been associated with ALL, CML, AML, multiple myeloma (MM) and non-Hodgkin lymphoma (NHL) [10–14]. Similarly, dysregulation of the JAK–STAT pathway due to a mutation in the JAK2 protein has been documented to play a crucial role in polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) [15–19].

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http://dx.doi.org/10.1016/j.blre.2014.06.002
0268-960X/

Indeed, the discovery of aberrant regulation of the JAK–STAT pathway in myeloproliferative neoplasms (MPNs) such as PV, ET, and PMF has been crucial in facilitating an improved understanding of this group of malignancies characterized by unchecked proliferation of terminally differentiated myeloid cells arising from hematopoietic stem or progenitor cells. While PV and ET display an increased tendency to develop thrombotic and hemorrhagic complications and a risk for progression to myelofibrosis (MF), all MPNs can potentially progress to AML or transform into myelodysplasia (MDS). In addition, MPN patients suffer from a number of debilitating symptoms that significantly impair their quality of life. Until recently, none of the available treatment options for MF patients could improve survival, which has been a dismal 5 years based on poor knowledge of the molecular pathogenesis of MPNs [2,20,21]. Therefore, altering the deregulated JAK–STAT pathway using JAK inhibitors represents an attractive therapeutic approach for MPN patients. This review will focus on the potential of JAK inhibition in MPNs and other disorders with an emphasis on the approved JAK inhibitor ruxolitinib and other emerging agents.

## 2. Mechanisms of JAK deregulation

In 2005, a recurrent somatic point mutation (G → T) in exon 14 of the *JAK2* gene was reported in >95% of PV and 50%–60% of ET and MF patients [22]. The mutation results in substitution of valine at codon 617 to phenylalanine in the expressed JAK2V617F protein in MPN patients. Since this mutation occurs in the autoinhibitory JH2 pseudokinase domain of the JAK2 protein, it is expected to result in constitutive activation of the JH1 kinase domain and thus result in activation of the JAK–STAT pathway and PI3K, RAS, and MAPK downstream pathways [16]. In addition, JAK2V617F escapes negative regulation mediated by SOCS3 (suppressors of cytokine signaling) [23] and also impairs the ability of PRMT5 to methylate histones H2A and H5 [24]. This deregulated activation of JAK2 finally results in cytokine-independent activation of the JAK–STAT pathway in hematopoietic cells, thereby resulting in cytokine hypersensitivity and cytokine-independent proliferation and growth.

Various mutations in exon 12 of *JAK2* are complex insertion/deletion events that have been reported in 1%–2% of PV patients (Fig. 1). Similar to JAK2V617F, the mutations in JAK2 exon12 result in deregulated activation of the JAK2 protein [25–27]. Thus dysregulation of the JAK–STAT pathway is primarily mediated by direct somatic mutations in the *JAK2* gene in a majority of MPN patients. On the other hand, indirect dysregulation of the JAK–STAT pathway via somatic mutations in proteins that play a role in its regulation has also been reported. These include activating mutations in *MPL* exon 10 resulting in base substitutions in the tryptophan 515 of the TpoR (W515L, W515K, or W515A). While found in a minority of ET and PMF patients, these mutations facilitate autonomous activation of TpoR, thereby activating JAK2 and its downstream signaling pathways [28,29].

Similarly, mutations in the *SH2B3* gene affecting the adaptor protein LNK, which is known to negatively regulate JAK2 activation, are found in a subset of MPN patients [30–32]. Mutations in *CBL*, another negative regulator of tyrosine kinase signaling, have been observed in PMF patients. Thus, direct as well as indirect activation of the JAK2 pathway constitutes a central theme in the pathogenesis of MPNs [33,34]. Recently mutations in *CALR* have been reported in the majority of ET and PMF cases that test negative for *JAK* and *MPL* mutations [35,36]. Currently it is unclear how these mutations give rise to myeloproliferation and, although mutant CALR has been suggested to activate JAK/STAT signaling in myeloid cells it is not yet known if this is the primary driver of the disease phenotype in these cases [37].

Mutations in epigenetic regulators and mRNA splicing regulators are also seen in a minority of MPN patients particularly MF [22]. Mutated epigenetic regulators include *TET2*, *IDH1/2*, *ASXL*, *EZH2* and *DNMT3A* [38–40]. While the functional consequence of these mutations in epigenetic regulators remains unclear, some, notably *ASXL1* and *EZH2*, as well as mutations in *TP53* have been associated with an adverse prognosis and progression of PV and ET to MF and blastic phases [41]. It has been clearly demonstrated that the blasts of transformed *JAK2*V617F-positive MPN are often V617F-negative, suggesting that there might be important genetic changes that precede the acquisition of the *JAK2* mutation [42]. Indeed, subsequent work demonstrated that *TET2* mutations are often (but not always) acquired prior to *JAK2*V617F [43]. Thus, clonal hematopoiesis may be established in some cases at least prior to *JAK2*V617F, but in these cases it appears that the *JAK2* mutation is the primary driver of the MPN phenotype.

Despite these different observed genetic changes and the presence of JAK2V617F in a majority of MPN cases, why some patients develop

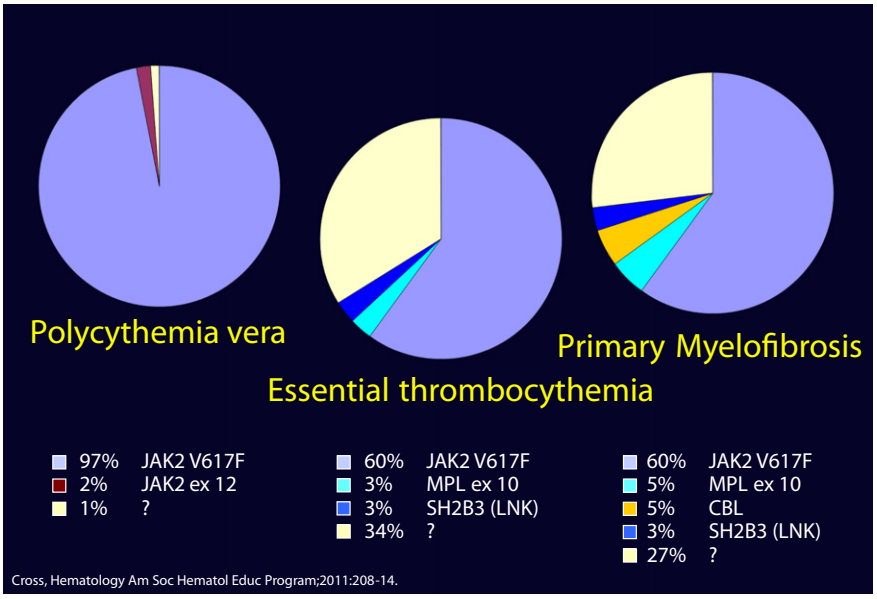


**Fig. 1.** Mechanisms of JAK2 activation in MPN. JAK2 is activated by acquisition of the V617F mutation in the majority of MPN cases, or occasionally by exon 12 mutations in PV. In ET and PMF, JAK2 signalling may also be activated by upstream mutations in the thrombopoietin receptor (encoded by the MPL gene) or downstream mutations in the negative signalling regulators CBL and LNK (encoded by the gene SH2B3). Most ET and PMF cases without abnormalities in these genes test positive for CALR mutations; currently it is unclear if JAK2 signaling is also activated in CALR-mutated cases.