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Review Article

JAK2 mutation and treatment of JAK2 inhibitors in Philadelphia chromosome-negative myeloproliferative neoplasms

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ABSTRACT

The Philadelphia chromosome-negative (Ph-negative) myeloproliferative neoplasms (MPNs) polycythaemia vera (PV), essential thombocythaemia (ET) and primary myelofibrosis (PMF) are clonal disorders of multipotent haematopoietic progenitors. The genetic cause of these disorders was not fully defined until a somatic activating mutation in the JAK2 non-receptor tyrosine kinase, JAK2V617F, was identified in most patients with PV and a considerable proportion of patients with ET and PMF. The discovery of JAK2 mutation has changed the molecular reclassification of MPNs and served as a genomic target for therapeutic implication. A number of JAK2 inhibitors have been developed and tested for MPNs. Several JAK2 inhibitors have reached the phases of clinical trial and included patients with intermediate-risk or high-risk MF. This population of MF is the best candidate for trials because currently it has no effective therapy besides patients' poor survival. Considering all clinical data on Ph-negative MPNs, JAK2 inhibitors have shown a clinical benefit and reduced symptoms in the vast majority of MF cases. The most developed among JAK2 inhibitors is Ruxolitinib, which has demonstrated clinical improvement with well tolerated toxicities. However, JAK2 inhibitor was equally active in patients with and without JAK2 mutation. Other JAK2 inhibitors are less developed, but showed a similar clinical benefit. Furthermore, its effect on the natural course of MPNs in treating patients needs to be investigated.

Keywords: *myeloproliferative neoplasms – JAK2 mutation – JAK2 inhibitors.*

INTRODUCTION

Myeloproliferative disorders (MPD), recently renamed as myeloproliferative neoplasms (MPNs), are clonal disorders of transforming multipotent hematopoietic stem cells which manifest clinically by uncontrolled myeloid proliferation. This group of disorders include chronic myeloid leukaemia (CML), polycythaemia vera (PV), essential thrombocythaemia (ET) and primary myelofibrosis (PMF), as well as chronic eosinophilic leukaemia (CEL), chronic myelomonocytic leukaemia (CMML), and systemic mastocytosis (SM) and others.[1] Although each of the MPN is identified as a distinct clinicopathological existence, these disorders share common characters that discriminating them from other myeloid malignancies such as myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML). These features include biology and clinical characteristics. Biologically, MPNs involve a multipotent hematopoietic stem cell with a dominance of the transformed clone over non-transformed progenitors and hypercellularity of the marrow, with unstimulated over production of one or more of the formed elements of blood. Clinically, they have an increased risk of thrombosis and bleeding and spontaneous transformation to acute leukemia and marrow fibrosis.[2]

Detection of mutant alleles in CML, CMML, CEL and SM,[3-6] led to observations that constitutive activation of tyrosine kinase signalling was induced by the particular mutation. A clear example is the product of the Philadelphia (Ph) chromosome, the BCR (breakpoint cluster region)–ABL (Abelson murine leukemia) fusion tyrosine kinase in CML.[3] In contrast to the cause of CML the pathogenesis of other MPNs is less unravelled. A key feature of Ph-negative MPNs is cytokine-independent blood colony formation, a process that normally relies on cytokine-dependent signaling. This character was firstly observed in PV, where there was a presence of endogenous erythroid colonies, erythroid progenitors that form colonies *in vitro*, in the absence of exogenous erythropoietin (EPO). Moreover, several kinase inhibitors, including a non-selective JAK inhibitor AG-490, inhibit EPO-independent colony formation from patients with PV.[7] This indicated that Janus kinase 2 (JAK2) is constitutively active in PV progenitor cells and is a proto-oncogene in Ph-negative MPNs. The identification of somatic mutations that activate JAK2 signalling in patients with PV, ET and PMF provided important insight into pathogenesis and diagnostic aspect of MPNs. This also lays a ground for the development of molecular treatment targeting JAK2 kinase.[8-11] This review elaborates the understanding of the genetic basis of PV, ET and PMF in association with the role of JAK2 activation and the recent advances of JAK2-targeted therapy in MPNs.

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Identification and pathogenesis of JAK2 mutation

Blood cell production is regulated by certain protein growth factors and cytokines which play roles in cell survival, proliferation and differentiation. These molecules bind to cell surface receptors that are closely associated with cytoplasmic non-receptor tyrosine kinases of the Janus kinase (JAK) family. There are four mammalian JAKs namely JAK1, JAK2, JAK3 and TYK2. JAK2 and the other members of the JAKs normally function as intermediates between membrane receptors and intracellular signaling molecules through their association with the cytoplasmic domains of receptors.[12] Cell activation generally occurs when the binding of a ligand (eg, erythropoietin/EPO or thrombopoetin/TPO) induces JAK phosphorylation and activation, cytokine receptor phosphorylation, recruitment and phosphorylation of signal transducer and activators of transcription (STAT) proteins and the activation of downstream signalling proteins. The activated STAT molecules then enter the nucleus, where they act as transcription factors by binding specific regulatory sequences to activate or repress the transcription of target genes. The roles of JAK family members are overlapped, as most signaling pathways involve more than one JAK. JAK1 transduces signaling of a number of proinflammatory cytokines, often in association with other JAK family members. JAK2 is used primarily by receptors for hematopoietic growth factors, such as EPO and TPO. JAK3 mediates immune function by transmitting interleukin (IL)-2 generated signals. Tyk2 associates with JAK2 and JAK3 to transduce signaling of cytokines such as IL-12 and IL-23.[13,14]

The identification of JAK2 mutation in MPNs was a major breakthrough in the understanding of the pathogenesis of MPNs. In 2005, five groups independently reported the presence of a single mutation in the JAK2 tyrosine kinase in different patients with non-CML MPNs in an about similar frequency.[8-11,15] These studies identified a guanine (G) to thymidine (T) alteration at position 1849of JAK2 protein coding gene resulting inan altered protein structure where valin is substituted by phenylalanine at amino acid 617 (V617F)of its pseudokinase (JH2) domain. The mutation is not present in the germ line, consistent with the concept that JAK2V617Fis acquired as a somatic disease allele in the hematopoietic compartment.[11] Different experiment groups analysed the identical mutation in JAK2 using a variety of genetic, functional and genomic approaches. The observation of Vainchenker *et al*. on small moleculeor siRNA-mediated inhibition of JAK2 in PV hematopoietic progenitors that abrogated EEC formation has stimulated the investigation of JAK2 genetic variationin patients with PV. Baxter *et al*. used candidate gene resequencing followed by allele-specific PCR to identify the JAK2V617F allele in PV, ET and PMF.[10] Based on a major finding of Prchal *et al*. acquiring that uniparental disomy (UPD) of chromosome 9p24 is common in PV[16] Kralovics *et al*. sequenced the genes in the minimal region of UPD to identify the JAK2V617F allele.[11] Later on, Levine *et al*. performed a systematic survey of the tyrosine kinome in PV using high-throughput DNA resequencing. This test identified recurrent somatic missense mutation JAK2V617F.[8]

The JAK2V617F protein has constitutive kinase activity and when expressed *in vitro* JAK2V617F is constitutively phosphorylated. This activity promotes oncogenic transformation and possibly contributes to the growth and survival advantage of the abnormal clone. The acquired point mutation arises in a multipotent progenitor and may generate both erythroid and myeloid lineages.[8,17] *In vitro* and *in vivo* studies have shown that JAK2 mutation has the ability to transmit signals from EPO, TPO and G-CSF receptor in haematopoetic cells more efficiently.[18] However, an observation of significant correlation between JAK2V617F and disease duration indicates that JAK2V617F occurs after the appearance of the MPN phenotype as a mutation associated with disease progression but is not sufficient to cause the phenotype. The correlation between the presence of the mutation and increased frequency of disease complications could also be linked to a more aggressive phenotype mediated by the mutation. Taken together, these data underline the notion that MPNs are genetically heterogeneous with unclear molecular basis.

The frequency of JAK2 V617F in different MPNs is displayed in Table 1.[10,19,20] These frequency of JAK2 V617F mutations was assessed using sensitive, allele-specific assay in different malignancies. Generally, testing for the JAK2 V617F mutation includes allele-specific polymerase chain-reaction (PCR) assay, pyro sequencing, restriction-enzyme digestion, and real-time PCR. The assays are sensitive to detect the presence

Table 1. Frequency of the JAK V617F allele in myeloid disorders.[8]

Disease	Frequency
Polycytemia vera	81-99%
Essential thrombocytosis	41-72%
Primary myelofibrosis	39-57%
Chronic myelomonocytic leukemia	3-9%
Myelodysplasia*	3-5%
Acute myeloid leukemia#	<5%

*Most common in patients with refractory anaemia with ringed sideroblasts and thombocytosis, a clinically distinct subtype of myelodysplastic syndromes. #Most common in patients with a previous history of polycythaemia vera, essential thrombocytopaenia and primary myelofibrosis.

of a heterozygous mutation in as few as 5 to 10% of cells and have low rates of false positivity, making them pivotal for diagnostic purpose.[10, 21, 22, 20]

After the finding of V617F JAK mutant, many other mutations have been detected in JAK2-negative MPNs, both in chronic phase (exon 12 mutations of JAK2, MPL, TET2, LNK, EZH2) and blast phase (NF1, IDH1, IDH2, ASXL1, CBL, Ikaros). Some of the mutations involve JAKSTAT signaling activation while others involve chromatin remodeling and leukemic transformation. One well characterized mutation is the cytokine transmembrane receptor MPL (MPL W515) which is found in 3% patients of ET and about 10% cases with JAK2-negative PMF, but not in PV. The mutated gene expression results in factor-independent growth and constitutive activation of downstream signaling proteins leading to gene transcriptions.[17,23] Other mutation, JAK2 exon 12 mutation, may bind cytokine receptors and is phosphorylated in the absence of ligand, allowing ligand-independent activation of downstream signalling pathways.[17]

Furthermore, activation of signalling by the JAK2V617F kinase might partly be due to loss of negative-feedback mechanisms. JAK activity is negatively regulated by the suppressor cytokine signaling (SOCS) family of proteins, which normally bind to the JAK kinases resulting in their degradation. Two important proteins, SOCS1 and SOCS3, can bind JAK2 and inhibitJAK2 catalytic activity.[17] Expression of SOCS1 results in JAK2V617F degradation and inhibition of kinase activity whereas the expression of SOCS3 results in increased JAK2V617F protein stability, increased SOCS3 phosphorylation and increased JAK2V617F phosphorylation.[24]

Rational concept of JAK2 inhibitor treatment in MPNs

Despite incomplete understanding of the molecular basis of MPNs, JAK2 remains an attractive option for drug development. The character of resistance to apoptosis was supported by *in vitro* studies demonstrating that small molecule inhibitors of JAK2 inhibit the proliferation of cell lines carrying JAK2V617F. This inhibition is dose dependent and reduces the phosphorylation of JAK2 and STAT5 downstream signaling pathways resulting in induction of apoptosis.[8] Even in patients without a confirmed JAK2 mutation, the detection of STAT activation indicates dysregulated JAK2 activity. Thus, regardless of the mutational status of JAK2, the malignant cells retain their responsiveness to JAK2-activating cytokines and/or growth factors and might benefit from JAK2 inhibition.

Generally the developed JAK2 inhibitors can be categorized into two classes, JAK2-selective inhibitor (class I) and non-JAK2 selective inhibitor (class II). Class I inhibitors primarily target JAK2 kinase. Pharmacological strategy was to refine an existing tyrphostin (tyrosine phosphorylation inhibitor) scaffold based on available molecular structural data for JAK2 and JAK3 kinase domains,[25] to design compounds that selectively bind to the ATP-binding JAK2 kinase catalytic site by competing with the ATP.[26] Class II inhibitors were developed for non-MPNs indications but also have a therapeutic implication in MPNs.[25]

Clinical evidence of JAK2 inhibitor in MPNs

Clinical trials with small-molecule JAK2 inhibitors and drugs targeting other targets are displayed in Table 2.

Table 2. Current therapies for Ph-negative MPN patients.(modified from[53, 54])

	Drug	Target	Phase	Disease	Efficacy	JAK2V617F load	Toxicity	Refs
JAK2 inhibitors	INCB018424 Ruxolitinib	JAK2 JAK1	III	MF PV/ET	>50% reduction in splenomegaly and constitutional symptoms	JAK2V617F load marginally reduced	Anemia Thrombocytopenia	[51]
	TG101348 or SAR302503	JAK2, FLT3	II	MF	Reduction in splenomegaly	JAK2V617F load significantly reduced	Anemia Thrombocytopenia Gastrointestinal	[52]
	CYT387	JAK2, JAK1, TYK2			In a murine model, normalized erythrocytes, leukocytes, spleen size, and levels of inflammatory cytokines	JAK2V617F load reduced		[55]
	CEP-701 Lestaurtinib	JAK2, FLT3	II	MF PV/ET	Reduction in splenomegaly	JAK2V617F load no reduced	Gastrointestinal Anemia Thrombocytopenia	[56]
Other targets	LBH-589	HDAC	II	MF	Splenomegaly anemia	unknown	Anemia Thrombocytopenia Gastrointestinal	Reviewed in [53]
	RAD-001	mTOR	II	MF	Splenomegaly symptoms	unknown	Minimal	
	Pomalidomide	IMID	III	MF	Anemia	unknown	Minimal	
	Pegylated Interferon Alpha-2a	Biological	III	PV/ET	Erythrocytosis Thrombocytosis symptoms	unknown	Myelosuppression Depression	[57] [58]

The best study subjects for JAK2 inhibitor trial are intermediate-risk or high-risk PMF since trials cannot be applied directly to unselested patients. Intermediate- or high-risk PMF cases commonly present with significant morbidity such as cachexia, hepatosplenomegaly, symtomatic anemia, and a poor prognosis with a median survival of two to three years.[35] There is no effective therapy for these individuals despite the introduction of immunomodulatory drugs. The conventional treatment is only palliative intent to prevent thrombohemorrhagic event and do not alter disease natural history or prevent clonal evolution.[36,37] In contrary, patients with ET or PV have a much better life expectancy with median survival more than 20 years using conventional treatment in combination with phlebotomy and low dose aspirin and hydroxyurea.[38,39]

INCB18424,[29] TG101348,[30] and CEP-70[32] are drug compounds currently tested in the most advanced clinical phases, in which INCB018424 and TG101348 are shown the most promising.[27,28,40] In 2010 Verstovsek *et al*. published comprehensive data on INCB018424 (Ruxolitinib), a potent andoral selective inhibitor of JAK1 and JAK2. This trial conducted a phase I-II trial to compare the treatment in 153 patients with JAK2V617F−positive and JAK2V617F−negative PMF, post–ET MF and post–PV MF. With a 15-mg twice-daily starting dose, followed by individualized dose titration, 52% patients had a rapid objective response (≥ 50% reduction of splenomegaly) which was durable for 12 months or more. This study showed well tolerability where only less than 10% of patients experienced a grade 3 or grade 4 adverse events, mainly myelosuppression. Clinical improvement was demonstrated by a marked reduction of levels of circulating inflammatory cytokines (e.g. IL-6) that are commonly elevated and responsible for the induction of constitutional symptoms in MF.[29]

TG101348is an oral JAK2 inhibitor that has been tested in a phase I trial on 59 patients with PMF or post-PV, post-ET MF being unresponsive to standard treatments. The subjects presented with thrombocytopenia (platelet less than 50 $x10^9$/L) and neutropenia (actual netrophil count less than 1,000 $x10^9$/L). Dose chosen for a phase II/III trial was 400 mg or 500 mg daily. Based on International Working Group (IWG) criteria of response, 59% of patients achieved reduction of spleen size at 6 months. The majority of patients with constitutional symptoms, fatigue and pruritus had a persisted improvement. However, in these patients there were no improved effects on cytokines. Between different doses, leukocytosis and thrombocytosis were normalized at 12 months in 57% and 90% of patients. Response rate did not make any difference in term of JAK2V617F mutational status. The toxicities included grade 3-4 hematologic events such as anemia (in 35% of 37 patients who were RBC transfusion-independent at baseline), thrombocytopenia (24%) and neutropenia (10%). The main non-hematologic adverse events included all grades nausea (69%), diarrhea (64%) and vomiting (58%), which were self-limiting and could be managed by symptomatic treatments. About 25% patients showed asymptomatic increase of lipase, AST, ALT and creatinine.[30]

CEP-701 (Lestaurtinib) is a non-selective small-molecule inhibitor of TRKA that was previously developed for treatment of prostate cancer. Based on *in vitro* result showing its inhibition of JAK2 kinase,[32] a phase II study tested its efficacy in PMF patients.[37,41] Santos *et al*. reported CEP-701 treatment in 22 JAK2V617F-positive MF patients (80 mg orally twice daily). Most patients (90%) were previously treated and the median number of previous therapies was 3 (ranged from 0-6). As much as 90% of patients presented with splenomegaly with19 cm of median size from left costal margin (ranged from 0-30 cm). Six patients (27%) responded by IWG criteria (clinical improvement in all cases). Clinical responses included reduction in spleen size only (n = 3), transfusion independency (n = 2), and reduction in spleen size with improvement in neutrophil counts and platelets (n = 1). Median time to response was 3 months and duration of response was ≥ 14 months. No improvement was found in bone marrow fibrosis or JAK2V617F allele burden. Eight patients (36%) experienced grade 3 or 4 toxicity and 6 patients (27%) required dose reduction. Main side effects were myelosuppression (anemia and thrombocytopenia) and gastrointestinal disturbances (diarrhea, nausea, and vomiting). Putting all data together, CEP-701 demonstrated only modest efficacy but a relative well tolerated toxicity in MF patients.[41]

Based on molecular aspect, patients with other MPNs showed a various response to different treatment. As much as 40-50% of the patients with PMF and ET who carried JAK2V617F mutation had decreased proportion of JAK2-mutated DNA after treatment. About 20% of the PMF and ET patients who carried MPL mutations had no decreases in proportion of MPL-mutated DNA when treated with JAK2 inhibitors.[42]

As new alleles are identified, either alone or in conjunction with JAK2 mutations, additional drugs may evolve targeting these alleles. Recently, other drugs targeting alternative pathways were reported on trials including inhibitors of chromatin remodeling proteins such as histone deacetylase inhibitors and HSP90 inhibitors,[43,44] or the epigenetic drugs acting through remodeling chromatin (givinostat, panobinostat, vorinostat),[27,40] pegylated interferon alpha-2a,[33,34] mammalian target of rapamycin-inhibitor/mTOR (everolimus), the MAPK (erlotinib) and the

NF-Kb (bortezomib) pathways. Combination of JAK2 inhibitors with other regimens which show synergy and other biological properties are promising as effective treatment in MPNs.[27,28]

As the understanding of the mechanism of transformation by JAK2V617F is incomplete, the data showing that JAK2V617F-negative AML occurs frequently in patients with a JAK2V617F-positive MPN[45] may raise the possibility that JAK2 inhibitor therapy might increase the risk of leukemic transformation. Longer observation on this drug is thus required to determine whether it can prolong survival with reduced risk of thrombotic events and transformation to AML. These findings will therefore change the natural history of PMF. In population of patients with PV management using conventional therapies such as phlebotomy, aspirin and hydroxyurea has a reasonable outcome at relatively low cost with prolonged survival. A cost-benefit analysis of potentially expensive long-term JAK2 inhibitor treatment is also needed.[46,47]

Summary

A growing number of data derived from genetic, biochemical and functional studies has demonstrated important insight into the pathogenesis of Ph-negative MPNs. The discovery of the JAK2V617F mutation also has a strengthened link between the three diseases. However, the role of a single disease allele in three related but clinically distinct phenotypes is not well understood. Future studies should explore additional mutations that contribute to the pathogenesis of these MPNs. The identification of the JAK2V617F mutation has provided a pivotal basis for the development of JAK2-targeted therapies. Recently several trials have tested JAK2 inhibitor aiming to determine the safety and activity of these agents. Ruxolitinib (INCB018424) has been proven safe and effective at reducing spleen size and improving clinical symptoms in patients with MF. Similar results in PMF have been obtained with TG101348 and CYT387. Improvements of cytopenias and bone marrow fibrosis, as well as complete molecular responses in PMF patients still need to be further tested. Nevertheless, the dramatic improvement in quality of life and splenomegaly achieved by JAK2 inhibitors in patients with PMF urges it to become the new standard of therapy in PMF. Furthermore, other trials have also proven its effectiveness in patients with PV and ET.

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