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 significant proportion of patients with ET and PMF. The discovery of JAK2 mutation has changed the molecular reclassification of MPNs. This also provideda genomic target for therapeutical approach. A number of JAK2 inhibitors have been developed and tested for MPNs. Several JAK2 inhibitors have reached the stage of clinical trial and includedpatients with intermediate-risk or high-risk MF. This population of MF is best candidate for trials because currentlyit has no effective therapy and thesurvival is significantly poor. Considering all clinical data on Ph-negative MPNs, JAK2 inhibitors give a clinical benefit of spleen reduction in approximately 40-50% of patients and abolished symptoms in 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. The effect on the natural course of MF in treated patients needs to be further investigated.

INTRODUCTION

Myeloproliferative disorders (MPD), recently renamed as myeloproliferative neoplasms (MPNs), are clonal disorders of transformed**multipotent hematopoietic stem cells**which manifest clinically by uncontrolled myeloid proliferation.These proliferative syndromes 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.Although each of the MPN is identified as a distinct clinicopathological existence, these disorders share common features that discriminate them from other myeloid malignancies such as myelodysplastic syndromes (MDS) and acute myeloid leukaemia (AML). These featuresincludebiology and clinical characteristics. Biologically, MPNs involve a multipotent hematopoietic stem cell with a dominance of the transformed clone over nontransformed progenitors and hypercellularity of the marrow, with apparently unstimulated overproduction 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.

Detection of mutant alleles in CML, CMML, CEL and SM, led to observations that constitutive activation of tyrosine kinase signalling was induced by the mutation. A clear example of these mutated kinases is the product of the Philadelphia (Ph) chromosome, the BCR (breakpoint cluster region)–ABL (Abelson murine leukemia) fusion tyrosine kinase in CML.Accumulating dataafterwarddemonstrated that tyrosine kinase signalling activation is a common pathogenetic mechanism in MPNs and that these mutated kinases might serve as targets for molecular treatment approaches.

In contrast to the cause of CML the pathogenesis of other MPNs is less clear.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 firstlyobserved 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 inhibitorAG-490,, inhibit EPO-independent colony formation from patients with PV.This indicatedthat Janus kinase 2 (JAK2) is constitutively active in PV progenitor cellsand is an ideal candidate proto-oncogene in Ph-negative MPNs.The identification of somatic mutations that activate JAK2 signalling in most patients with PV and significant proportions of patients with ET and PMF provided important insight into pathogenesis and diagnostic aspectof MPNs. This also became a ground for the development of oral small-molecule inhibitorsthat selectively target JAK2 kinase.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 MPD.

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 JAKsnormally function as intermediates between membrane receptors and intracellular signaling moleculesthrough their association with the cytoplasmic domains of receptors.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 seems to be overlapped, as most signaling pathways involve more than one JAK. JAK1 transducessignaling of a number of proinflammatory cytokines, oftenin association with other JAK family members. JAK2 is used primarily by receptors for hematopoietic growth factors, such as EPO and TPO. JAK3 has a primary role in mediating immune function by transmitting interleukin (IL)-2 generated signals. Tyk2 functions in association with JAK2 and JAK3 to transduce signaling of cytokines such as IL-12 and IL-23.

The identification of JAK2 mutation in MPNs was a major breakthrough in the understanding of the pathogenesis of MPNs that can be compared with identification of BCR-ABL fusion gene in the pathogenesis of CML. 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.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 notion that *JAK2V617F*is acquired as a somatic disease allele in the hematopoietic compartment.The different groups analysed the identical mutation in *JAK2 using a variety* 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 formationhas prompted the investigation of *JAK2 genetic variation*in 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.**Sequence traces showing wild-type sequence and G to T mutation in *JAK2 was demonstrated in figure 1.*** Based on amajor finding of Prchal et al.acquiring that uniparental disomy (UPD) of chromosome 9p24 is common in PV,Kralovics et al. sequenced the genes in the

minimal region of UPD to identify the *JAK2V617F*allele.Built on identification of activating mutations in tyrosine kinases previously done in patients withMPDs, Levine et al. performed a systematic survey of the tyrosine kinome in PV using high-throughput DNA resequencing. This testidentified recurrent somatic missense mutation JAK2V617F.

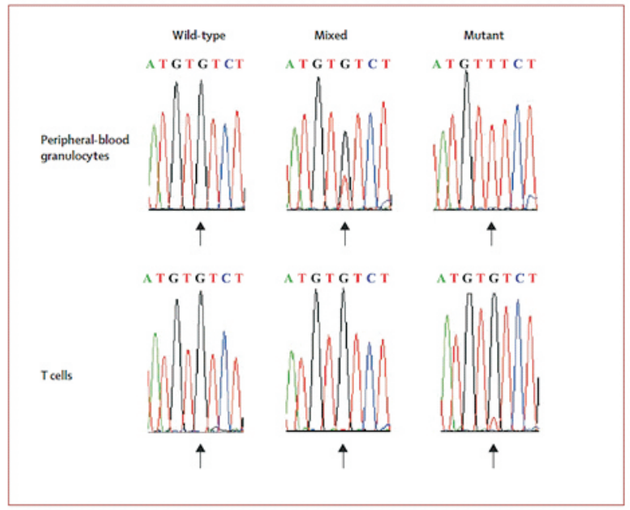


Figure 1. Sequence traces showing wild-type sequence and G→T mutation in *JAK2.*

Wild-type pattern in a patient with idiopathic myelofibrosis. Mixed and mutant sequences in two patients with polycythaemia vera. Arrows indicate the relevant base.

Each of the JAKs has seven homologous domains (JH1–7), which contain the catalytic kinase domain (JH1)and a catalytically inactive pseudokinase domain (JH2).N-terminal JH7 domain has been shown to be essential for association with cytokine receptors.The *JAK2V617F* point mutation lieswithin the pseudokinase domain of JH2, which has been observed to serve as an autoinhibitory function similar to the juxtamembrane domain of receptor tyrosine kinases, thus negatively regulates JH1 kinase activity.This observation was supported by a computational model of the structure of JAK2 suggesting that valine 617 closely associates with the JH1 activation loop and has an important role in mediating JAK2 kinase autoinhibition and is important for the maintenance of the kinase domain of JAK2 in an inactive conformation.Replacement of valine 617 by phenylalanine destabilizes this inhibitory interaction and might abrogate autoinhibition leading to a constitutive kinase activity.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 are capable of giving rise to both erythroid and myeloid lineages.*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. Furthermore, *in vivo* expression of JAK2V617Fin a murine bone marrow transplantation model produces an MPNphenotype resembling PV.

The frequency of JAK2 V617F in different MPNs is displayed in Table 1. These frequency of JAK2 V617F mutations was assessed using sensitive, allele-specific assay in different malignancies.

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

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.*

Generally, testing for the *JAK2* V617F mutation includes allele-specific polymerase chain-reaction (PCR) assay, pyrosequencing, restriction-enzyme digestion, and real-time PCR. The assays are sensitive to detect the presence of a heterozygous mutation in as few as 5 to 10% of cells and have low rates of false positivity. These properties make them useful for diagnostic purpose.

After the finding of V617F JAK mutant, many other mutations have been observed in JAK2V617F-negative MPNs, both inchronic 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). MPL W515 mutationis found in 3% patients of ET and about 10% cases with JAK2V617F-negative PMF, but not in PV. The mutated gene expression results in factor-independent growth and constitutive activation of downstream signaling proteins including the STAT, MAP kinase, and phosphatidylinositol 3-kinase transduction pathways. Similar to JAK2V617F mutation JAK2 exon 12 mutant kinases bind cytokine receptors and are phosphorylated in the absence of ligand, and lead to ligand-independent activation of downstream signalling pathways.

Furthermore, activation of signalling by the JAK2V617F kinase might partly be due to loss of negative-feedback mechanisms which is important in altering JAK2 signalling. 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, have capabilities in binding to JAK2 and inhibiting JAK2 catalytic activity. Expression of SOCS1 results in JAK2 and 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.

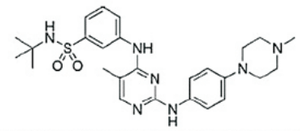
An observation of significant correlation between JAK2V617F and disease duration indicates that JAK2V617F occurs after the appearance of the MPN phenotype as a mutation associated with disease progression but is not sufficient to cause the phenotype. The correlation between the presence of the mutation and increased frequency of disease complications could also be linked to a more aggressive phenotype mediated by the mutation. Taken together, MPNs are genetically heterogeneous with unclear molecular basis.

Rational concept of JAK2 inhibitor treatment in MPD

Despite incomplete understanding of the molecular basis of MPNs, JAK2 remains an attractive target for drug development. Mutations with a gain of function of *JAK2*, *MPL*, *CBL* and those with a loss of function of *LNK* and *NF1* activate the JAKSTAT pathway leading to a final phenotype of MPN with alteration of immune response, inflammation, angiogenesis, proliferation and resistance to apoptosis.The character of resistance to apoptosis was supported by *in vitro* studies demonstrating that smallmolecule 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. 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.

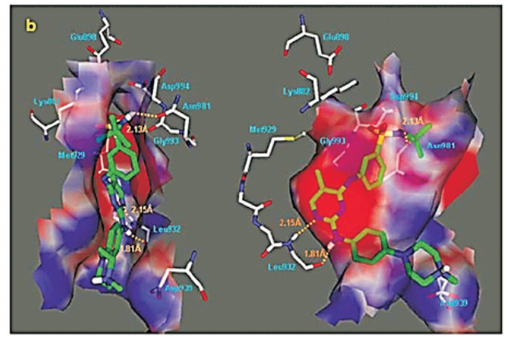
Generally JAK2 inhibitorscan be categorized into two classes, JAK2-selective inhibitor (class I) and non-JAK2 selective inhibitor (class II). Class I inhibitors primarily target JAK2 kinase (including JAK2V617F) whereas class II inhibitors were developed for non-MPD indications but still have therapeutic potential in MPD given their significant 'off-target' JAK2 kinase-inhibitory activity. For class I inhibitors, pharmacological strategy has been to refine an existing tyrphostin (tyrosine phosphorylation inhibitor) scaffold based on available molecular structural data for JAK2 and JAK3 kinase domains,to design compounds that selectively bind to the JAK2 (relative to JAK3) kinase catalytic site at low nanomolar concentrations,as displayed in figure 5. As a consequence,these compounds can potently inhibitboth wild-type (JAK2WT) and mutant (JAK2V617F) alleles. A cell-based screen was developed to identify allelespecific inhibitors of JAK2V617F-negative. These have the potential for a more optimal therapeutic window because the developed compound will only inhibit the disease allele.Vast majority of JAK2 inhibitors are small molecules that act by competing with ATP for the ATP-binding catalytic site in the kinase domain.

a



N-tert-Butyl-3-{5-methyl-2-[4-(4-methyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-ylamino}-benzenesulfonamide

b



c

	JAK2	FLT3	RET	JAK3
IC_{50} (nM)	6	25	17	169

Figure 5. Molecular structure of TG101209 and its selective inhibition of JAK2 kinase. (a) Chemical structure of TG101209 (molecular formula,C26H35N7O2S; molecular weight, 509.7; melting point 2431C). (b) Molecular model depicting docking of TG101209 in the JAK2 ATP pocket. The shaded surface illustrates hydrophobic (red) and hydrophilic (blue) portions of the protein. Key inhibitor–protein interactions, including the hydrogen bond with the hinge Leu932 residue, the hydrophobic contacts in the shallow angular pocket lined by residues Met929, the methylene groups of Lys882, and the initial portion of the DFG (AspPheGly) (Asp994 shown) activation loop, as well as the hydrogen bond with the NH of the sulfonamide from TG101209 with Asn981 are shown. (c) TG101209 inhibitory activity (IC50) against select kinases in the in vitro kinase assay.

Clinical evidence of JAK2 inhibitor in MPD

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)

	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 candidates for JAK2 inhibitor trial are intermediate-risk or high-risk PMF considering that thecases commonly present with significant disease-related morbidity such as cachexia or other constitutional symptoms, hepatosplenomegaly, extramedullary hematopoesis, symtomatic anemia, and a poor prognosis with a median survival of 2-3 years.Currently there is no effective therapy for these population despite the introduction of immunomodulatory drugs. Few treatment options exist for patients with myelofibrosis (MF), and their survival is significantly shortened.The intention of conventional treatment is only palliative such as to prevent thrombohemorrhagic event and do not alter the natural history of PMF or preventing clonal evolution.In contrary, patients with ET or PV have a strikingly better life expectancy with median survival more than 20 years using conventional treatment in combination with phlebotomy and low dose aspirin and hydroxyurea.In consequence, primary trial of JAK2 inhibitors in unselected ET or PV patients are not applicable until the safety and tolerability of these drugs has been confirmed in higher risk MPN cases.

INCB18424, TG101348,and CEP-701 are the drugs that are currently in the most advanced phases of testing. INCB018424 and TG101348 are the most promising JAK2 inhibitors with published results.The most integruing data on JAK2 inhibitor came from a report published in 2010 by Verstovsek et al. on INCB018424 (Ruxolitinib),a potent, selective, and orally bioavailable inhibitor of JAK1 and JAK2. Based on the fact that about half of patients with myelofibrosis carry *JAK2*V617F mutant, this trial was conducted as a phase I-II trial to compare patients with *JAK2*V617F−positive or *JAK2*V617F−negative PMF, post–ET MF and post–PV MF. This study was carried out in consideration that among other MPNs MF most associates with cytopenias, splenomegaly, poor quality of life and shortened survival. In a total of 153 patients with a median duration of more than 14.7 months, a dose-dependent suppression of phosphorylated signal transducer and STAT 3 was demonstrated in either patients with wild-type JAK2 or with JAK2 V617F mutation. With a 15-mg twice-daily starting dose, followed by individualized dose titration,17 of 33 patients (52%) had a rapid objective response (≥ 50% reduction of splenomegaly) which persisted for 12 months or more. This therapy was associated with grade 3 or grade 4 adverse events (mainly myelosuppression) in less than 10% of patients. In additional, clinical improvement was associated with a marked reduction of levels of circulating inflammatory cytokines that are commonly elevated in MF.Later on, an MF-specific instrument called Myelofibrosis Symptom Assessment Form was proposed to help characterize the symptomatic improvements observed in trials on MF patients.The reduced constitutional symptoms have been attributed to dual activity of Ruxolitinib against JAK1 and JAK2. JAK1 inhibitory activity of Ruxolitinibmay contribute to the suppression of signaling from several cytokines (e.g., IL-6), which is thought to be responsible for the induction of constitutional symptoms.

TG101348is an oral JAK2 inhibitor that has been tested in a phase I trial on 59 patients withPMF or post-PV, post-ET MF. This study recruited intermediate and high-risk patients 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). The maximum tolerated dose was 680 mg/day and dose-limiting toxicity was a reversible and asymptomatic increase in the serum amylase level. Dose chosen for a phase II/III trial was 400 mg or 500 mg daily. Based on (International Working Group)IWG-MRT criteria of response, 59% of patients achieved reduction of spleen size by palpation at 6 months. The majority of patients with constitutional symptoms, fatigue and pruritus had a durable improvement. However these decreased parameters were not associated with a measurable effect on cytokines. Between different doses, leukocytosis and thrombocytosis were normalized at 12 months in 57% and 90% of patients. There were no differences in term of response rate according to *JAK2*V617F mutational status. Of all patients, 39% cases with more than 20% *mutant*JAK2V617F/total JAK2 ratio (allele burden) at baselineshowed decreased mutation loadmore than 50% at 12 months. The toxicities included grade 3-4 hematologic events such as anemia (in 35% of 37 patients who were not RBC

transfusion dependent at baseline), thrombocytopenia (24%) and neutropenia (10%). The main non-hematologic adverse events included all grades nausea (69%), diarrhea (64%) and vomiting (58%). These reactions were all self-limiting and controlled by symptomatic treatments. Asymptomatic increase of lipase, AST, ALT and creatinine have beenobserved in about 25% patients.

CEP-701 (Lestaurtinib) is a non-selective small-molecule inhibitor of TRKA that was initially developed for treatment of prostate cancer. *In vitro*it inhibited JAK2 kinase, the proliferation of progenitor cells and JAK2STAT5 signaling from patients with MPNs. A phase II study investigated the efficacy of CEP-701 in PMF patients.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 (range from 0-6). Splenomegaly was present in 90% of patients with 19 cm of median size from left costal margin (range from 0-30 cm). Median allele burden was 53.5%.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 monthsand duration of response was ≥ 14 months. No improvement was seen in bone marrow fibrosis or JAK2V617F allele burden. Phosphorylated STAT3 levels decreased from baseline in responders during therapy. Eight patients (36%) experienced grade 3 or 4 toxicity and 6 (27%) required dose reduction. Main side effects were myelosuppression (grade 3 or 4 anemia, 18%; and thrombocytopenia, 18%) and gastrointestinal disturbances (diarrhea, any grade, 68%; grade 3 or 4, 9%; nausea, grade 1 or 2 only, 50%; vomiting, grade 1 or 2 only, 27%). Overally, CEP-701 resulted in modest efficacy and and relatively well tolerated toxicity in MF patients.

Based on the underlying genetic mechanism, patients with MPNs showed a various response to different treatment. As much as 40-50% of the patients with PMF and ET who carried *JAK2*V617F mutation had decreases in 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.

As new alleles are identified, either alone or in conjunction with JAK2 mutations, additional drugs may evolve that target these alleles. There is also space for development of drugs that have been empirically used. Recently, other drugs targeting alternative pathways which are critical for MPN development were reported on trials. These include inhibitors of chromatin remodeling proteins such as histone deacetylase inhibitors and HSP90 inhibitors,or the drugs acting through remodeling chromatin with a key role in epigenetics (givinostat, panobinostat, vorinostat),pegylated interferon alpha-2a,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 biologicalproperties than *JAK2*inhibitors holds promise as effectivetreatment in these disorders.

Expert opinions and further considerations

The genetic, biochemical and functional studies have described important insight into the pathogenesis of PV, ET and PMF as Ph-negative MPNs. The discovery of the JAK2V617F mutation also have strengthened the association between the three diseases. However, there are still essential questions regarding the molecular basis of disorders. The role of a single disease allele in three related but clinically distinct phenotypes is not well understood. Thus, future studies will undoubtedly discover additional mutations that contribute to the pathogenesis of these MPNs. The identification of the JAK2V617F mutation hasprovided a pivotal basis for the development of JAK2-targeted therapies.Recently a growing number of trials have tested JAK2 inhibitor aimed at determining the safety and activity of these agents. Ruxolitinib (INCB018424) has proven safe and has been shown to be effective at reducing spleen size and improving clinical symptoms in patients with MF. Ruxolitinib has also proven very effective in patients with PV and ET. Similar results in PMF

have been obtained with TG101348 and CYT387. Furthermore, improvements in cytopenias and bone marrow fibrosis, as well as complete molecular responses in JAK2V617F-positive patients still need to be further explored.The role of JAK2V617F mutation in ET and PMF as the driving force in disease mechanism is somewhat unclear. Nevertheless, the dramatic improvement in quality of life and splenomegaly achievedby JAK2 inhibitors in patientswith PMF supports the notion that these agents may become the new standard of therapy in PMF. 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, may raise the possibility that JAK2 inhibitor therapy might increase the risk of leukemic transformation.Longer investigation will define whether treatment with JAK2 inhibitor can prolong survival, reduce the risk of thrombotic events and transformation to AML. These findings may therefore change the natural history of PMF.In population of patients with PV management using conventional therapies such asphlebotomy, aspirin and hydroxyurea has a reasonable outcome at relatively modest cost. Acost-benefit analysis of potentially expensive long-term targeted therapy is thus highly needed.

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