Review: Clinical Trial Outcomes

CLINICAL INVESTIGATION

JAK2 inhibitors in the treatment of myeloproliferative neoplasms: rationale and clinical data

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The seminal discovery of the high prevalence of the JAK2-V617F mutation in essential thrombocythemia, polycythemia vera and myelofibrosis has led to a renewed interest in the science and therapy of Philadelphia chromosome-negative myeloproliferative neoplasms (MPNs). Within a short period of discovery, close to ten JAK2 inhibitor small molecules with different JAK2 kinase specificity have entered clinical trials for patients with MPNs. In myelofibrosis patients, these novel agents have been shown to decrease pathologic splenomegaly and improve disease-associated symptoms, as well as helping to improve cytopenias. Their ability to substantially impact disease progression, bone marrow histologic features and JAK2 allele burden remains to be shown. JAK2 inhibition in polycythemia vera and essential thrombocythemia appears to be promising in reducing myeloproliferation, constitutional symptoms and, possibly, thrombohemorrhagic events. Agents targeting alternative mechanisms, either used alone or in combination with JAK2 inhibitors, may further augment their clinical efficacy and broaden the therapeutic spectrum for patients with MPNs.

Keywords: acute leukemia • essential thrombocythemia • JAK2 inhibitor • myelofibrosis • myeloproliferative neoplasms • polycythemia vera • primary myelofibrosis

Classification & background of myeloproliferative neoplasms

According to the WHO 2008 criteria, the myeloid neoplasms are classified into five major entities with a variety of different subtypes. These classifications are summarized in Box 1. In this review we focus only on myeloproliferative neoplasms (MPNs), a detailed description of the other entities is beyond the scope of this review.

Among the MPNs, the Philadelphia chromosome negative (Ph[-]) and positive (Ph[+]) disease entities are distinguished. The Ph(+) entity is represented by chronic myelogenous leukemia (CML), while Ph(-) MPNs encompass; primary myelofibrosis (PMF), chronic neutrophilic leukemia, chronic eosinophilic leukemia, not otherwise classified, mastocytosis, MPN unclassifiable, essential thrombocythemia (ET), polycythemia vera (PV) and myelofibrosis (MF) (Box 1) [1]. MF itself includes either individuals with PMF or those in whom the disease has developed from an antecedent MPN, especially from ET or PV, then classified as post-ET MF or post-PV MF [2]. It was first assumed by Dameshek in 1951 that these myeloproliferative illnesses show a similar clinical phenotype, and that their pathogenesis is based on a generalized proliferation of bone marrow cells driven by unknown stimuli [3].

It is now well accepted that underlying pathogenesis of these disease entities is a clonal proliferation of multipotent haematopoietic progenitors cells, although ET is clonal in a minority of patients [4]. The specific phenotypic appearance is driven by distinct oncogenic events during pathogenesis of the various entities [5,6].

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Box 1. 2008 revision of classifications of myeloid neoplasms by the WHO.

- AML and related neoplasms
- MDS
- MPN
  - CML, BCR-ABL1 positive
  - PV
  - ET
  - PMF
  - CNL
  - CEL-NOS
  - Mastocytosis
  - MPN unclassifiable
- MDS/MPN
  - CMML
  - JMML
  - Atypical CML, BCR-ABL1 negative
  - MDS/MPN unclassifiable
  - Refractory anemia with ring sideroblasts associated with marked thrombocytosis (provisional entity)
- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB or FGFR1
  - Myeloid and lymphoid neoplasms associated with PDGFRA rearrangement
  - Myeloid neoplasms with PDGFRB rearrangement, five major entities
  - Myeloid and lymphoid neoplasms associated with FGFR1 abnormalities

AML: Acute myeloid leukaemia; CEL-NOS: Chronic eosinophilic leukemia, not otherwise classified; CMML: Chronic myelomonocytic leukaemia; CML: Chronic myelogenous leukaemia; CNL: Chronic neutrophilic leukaemia; ET: Essential thrombocythemia; JMML: Juvenile myelomonocytic leukaemia; MDS: Myelodysplastic syndrome; MPN: Myeloproliferative neoplasm; PMF: Primary myelofibrosis; PV: Polycythemia vera. Data from [1].

From a clinical point of view, MPNs appear as belonging to two major groups: patients with ET or PV, who in the earlier course of their disease are prone to develop thrombosis and hemorrhage being in the first group. Typically, after 10 years or more suffering from their illness, a subset of these patients convert to either post-ET, post-PV MF or acute myeloid leukemia (AML) that has evolved from a preceding MPN, often with a clinical picture resembling a leukemic blast phase [7]. The second group of MPN patients, those with MF, have a life-threatening illness presenting with more severe symptoms and more rapid progression. Patients with overt PMF or with secondary post-ET/PV associated MF are often clinically indistinguishable [8]. These individuals suffer, frequently from the time of diagnosis, from significant disease-associated symptoms, such as fatigue, night sweats, bone pain, fevers and chills. They also suffer from splenomegaly and anemia of a multifactorial origin, and show progressive MF with a predisposition to transform into AML [7].

The therapeutic management of MPNs was until now largely based on two different strategies:

- The first regimen is the control of thrombo-hemorrhagic events in patients with ET and PV by applying, for example, low-dose aspirin [9] or phlebotomy [10] in individuals with overt PV. These basic interventions were supplemented in patients with the highest risk for thrombotic events (age >60 years, prior thrombotic incidents, or other microvascular symptoms) [11] by the selected use of myelosuppressive agents such as hydroxyurea, anagrelide (in ET) or IFN-α [12].
- The second regime consisted of a palliative therapy aimed at ameliorating symptom burden and morbidity associated with MF. Specifically, agents such as thalidomide [13], prednisone [14] and androgens [15] that were known to improve anemia were administered. Other agents and interventions aimed to reduce splenomegaly, such as hydroxyurea [16], alkylator therapy [17], splenectomy [18] or even radiotherapy [19] were used. Patients with a serious risk of mortality from their illness and of suitable age, an acceptable health status and who had a concordant donor, would be considered for allogeneic stem cell transplantation [20]. Current focus of transplant is on intermediate-2 and high-risk patients with MF having a consideration of allogeneic stem cell transplant complying with the recommendations of the 'International Working Group for MF Research and Treatment' (IWG-MRT) [20]. However, there is currently no medication available that has been proven to halt the natural progression of any of the MPNs.

In the present review we focus on some genetic abnormalities that drive the proliferation of MPNs and evolving current and future therapeutic aspects.

Pathogenesis of MPNs

JAK2-V617F

Since the discovery of mutations in JAK2 in 2005 by several groups, signaling through the JAK-STAT pathway has been recognized as a hallmark of Ph(-) MPNs [21–25]. JAK2 is a cytoplasmic tyrosine kinase involved in a plethora of cellular responses (Figure 1). It plays a pivotal role in the signaling of the cytokine receptor superfamily by binding a variety of proteinaceous ligands implicated in proliferation and differentiation of hematopoetic progenitor cells to mature leukocytes, erythrocytes, thrombocytes and monocytes [26,27].

Initial evidence suggests that these signaling pathways are involved in PV, ET and PMF, as has been demonstrated in *ex vivo* cultures of primary samples from patients with MPNs. Cells from these patients showed both a hypersensitivity to hematopoetic cytokine stimulation as well as hematopoetic growth factor and cytokine-independent growth [28–30]. In JAK2 V617F mutant mouse models, a MPN phenotype resembling

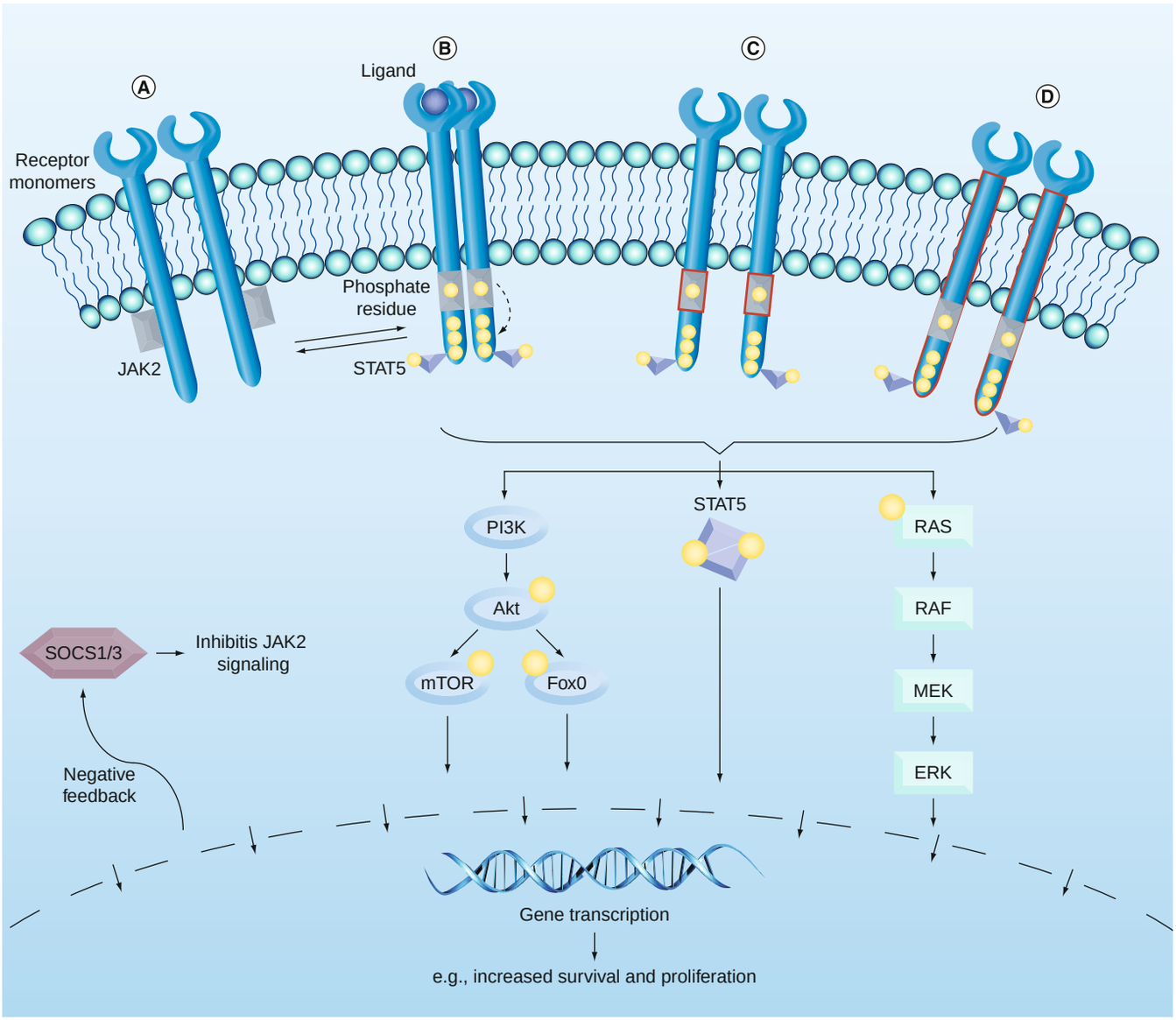

Figure 1. Signaling of the cytokine receptor superfamily, binding of proteinaceous ligands, activation and inactivation by JAK2 kinase. (A) When receptor monomeres are without ligand binding they associate with JAK2 monomeres. The kinase activity of the JH1 domain of JAK2 is inhibited by the JH2 domain of JAK2. JAK2 and the receptor are in the resting inactive state. **(B)** Ligand-binding results in homodimerization of the receptor and autophosporylation of JAK2 at its JH1 domain, thereby activating JAK2. Activated JAK2 phosporylates (dotted arrow) tyrosine residues in the cytoplasmic tail of the receptor. Autophosporylation of the JH1 domain is inhibited by the JH2 domain of JAK2 restoring JAK2 and the receptor to the inactive state. The phosphotyrosines in the receptor tail constitute docking sites recruiting cytoplasmic STAT5 and induce phosphorylation of STAT5. This is followed by STAT5 homodimerization and translocation into the nucleus facilitated by NPI-1 to activate gene transcription. Similarly, thyrosine phosphorylation activates other downstream signaling pathways including the MAPK and PI3K-Akt pathways. **(C)** JAK2 V617F and a JAK2 exon 12 mutation results in constitutive activation of the JAK2 kinase in the absence of ligand binding, as mutated JH2 domain is unable to inhibit autophosphorylation of JH1 domain. **(D)** MPLW515L/K mutant thrombopoietin receptor constitutively activates the wild-type JAK2 in the absence of ligand resulting in activation of signaling pathways downstream of JAK2. A negative feedback loop of JAK2 signaling is generated by SOCS1/3. The mutated JAK2 receptor escapes the negative inhibitory signal generated by SOCS1/3. Adapted with permission from [6].

the clinical picture is faithfully recapitulated, providing strong evidence that JAK2 mutations are indeed pathogenetic [31].

Further evidence came from the findings that the incidence of JAK2 V617F mutations is close to 100% (>95–97%) in PV, approximately 50–60% in ET and 50–60% in PMF [21–25]. In contrast, the frequency in other MPNs and hematopoetic malignancies is lower, with approximately 3–13% in chronic myelomonocytic leukemia, 3–5% in MDS (refractory anemia with ringed sideroblasts and thombocytosis) and <5% in AML (most of those arising out of MPNs) [32–34]. Interestingly, cells with JAK2 V617F mutations can become undetectable upon transformation to acute leukemic phase, suggesting an expansion of pre-existing non-JAK2 V617F mutated clones or acquisition of additional, yet unidentified, molecular aberrations contributing to leukemic transformation [35].

The percentage of homozygosity for JAK2V167F varies considerably, amounting to 21–52% in PV [36], 54% in MF and 2–4% in ET [37,38]; although it is now thought that most PV patients harbor homozygous clones [39]. There is insufficient knowledge regarding how a single gene mutation (JAK2) is responsible for the variety of phenotypic presentation of distinct MPN disease entities. Larsen *et al*. provided evidence that increasing JAK2 V617F allele burden from ET, PV to PMF is a principal determinant of an increasingly myeloproliferative phenotype with splenomegaly and associated clinical symptoms [40]. However, there are suggestions that additional genetic and epigenetic mechanisms, as well as tissue environmental factors, play pivotal roles [41].

Despite JAK2 mutation being a well-accepted diagnostic criterion for MPNs, its prognostic relevance for survival of patients is not fully established. However, the presence or absence of heterozygous or homozygous JAK2 V617F mutation influences clinical symptoms of MPN. For example, the presence of JAK2 V617F in ET and a high allele burden in both ET [42] and PV [38,43] are associated with an increased risk of thrombosis [42,44,45]. In a recent investigation, Trelinski *et al*. linked a higher risk of thrombosis in ET and PV patients to enhanced angiogenesis and other known thrombotic risk factors [27]. However, the levels of coagulation activation markers themselves were not affected by the JAK2 V617F mutational status. A general tendency for higher hemoglobin concentration and leukocyte counts was found in JAK2 V617F-positive ET, PV and MF, whereas lower platelet counts were reported for ET and PV patients, but in general not in patients with MF. Pruritus is often associated with JAK2 V617F positivity in ET and PV, and some studies found a positive association with leukemic transformation in MF patients [2,46,47].

In ET and PV JAK2 V617F, allele burden was associated with the progression and duration of disease, as well as with the severity of disease manifestation, such as higher hemoglobin levels, leukocyte counts, pruritis, splenomegaly and higher rates of fibrotic complications or transformation to leukemic phase or secondary MF [48–52]. Similarly, MF patients with higher allele burden were reported to suffer from more advanced disease stages with higher leukocyte counts, hemoglobin levels, splenomegaly and transformation to leukemia [47,49].

The findings are somewhat conflicting with respect to survival and JAK2 V617F mutational status, especially allele burden. Although in the general population JAK2 V617F mutation is a rare event with a prevalence of only 0.2%, it is associated with an increased risk of cancer (including hematologic and myeloproliferative cancer), and early death in a fraction of patients [53]. Some studies confirmed these epidemiologic observations and noted an inferior overall survival in mutated versus wild-type idiopathic MF or ET patients [54], however, others did not. Guglielmelli *et al*. [49] and Antonioli *et al*. [55] found that MF patients had a significantly shorter overall survival the lower their JAK2 V617F mutant allele burden was, especially in the lowest quartile. The authors offered two explanations: in these patients a myelodepletion rather than a myeloproliferation occurred and this was associated with a low allele burden and survival [49], or an overriding V617F-negative clone causing a more aggressive MF phenotype [55]. Despite the significant contribution to the development and maintenance of JAK2-positive MPNs, whether or not JAK2 V617F is indeed the underlying transforming event is still somewhat controversial [31].

Discovery of additional mutations in MPNs

Mutations can occur in receptors (JAK2 exon 12, MPL), adaptor proteins (LNK), genes and proteins downregulating activated protein-tyrosine kinases (C-CBL), genes and proteins involved in epigenetic control of transcription (TET2, ASXL1/2 and IDH1/2) and transcription factors (IKZF1). The impact of these genetic events for the manifestation of the various MPNs and their individual prognostic relevance is less well established as it is for JAK2 V617F. If sufficiently understood, their relationship to survival will briefly be mentioned within each section. Herein, only MPL, JAK2 exon 12 and LNK mutations will be discussed as these may be targetable by JAK2 inhibitors.

Additional genetic events altering JAK2

A majority of JAK2 V617F-negative PV patients (~50–80%) carry mutations in exon 12 of JAK2 [46,47]. Thus, almost all PV patients harbor some form of JAK2 mutational event. Several different exon 12 mutations

have been described, such as point mutations (i.e., K539L), double mutation (H538QK539L), deletion of two amino acids (N542-E543del,) and deletion of two amino acids replaced by corresponding insertions (F537-K539delinsL) [42,45]. In general, these mutations result in ligand-independent constitutive activation of JAK2 kinase (Figure 1) with a myeloproliferative picture, favoring a phenotype of erythrocytosis in the often younger patients [42,45,47]. This observation was supported by a JAK2 exon 12 murine model in which an erythroid-myeloproliferative phenotype developed with hyperplasia of erythroid and granulocytic lineages over lymphocytic or megakaryocytic lineages [45].

An important observation was the identification of a common JAK2 SNP 46/1 haplotype, that predisposes individuals to acquiring JAK2 V617F mutations [56,57] at an odds ratio of ~3.7 [56,57].

Finally, JAK2 is involved in several fusion products and translocations in myeloid and lymphoid malignancies [58], underscoring the importance of JAK2 signaling in hematological malignancies, however, these will not be discussed in detail here.

Mutations in the *MPL* gene (thrombopoetin receptor) are detected in ~1% of ET and up to 5–10% in PMF patients [59,60]. MPLW515L is the most frequent point mutation [60]. However, several other point mutations have been described, such as MPLW515K, MPLW515S and MPLS505N [5]. MPL mutations are usually found in JAK2 V617F-negative MPNs, although they can occur together with JAK2 V617F mutations [61,62]. MPL receptor mutations constitutively activate wild-type JAK2-STAT signaling in the absence of a ligand [60]. MPL mutations in ET often occur in older patients, confer increased platelet counts and lower hemoglobin concentrations, and patients frequently suffer from microvascular symptoms. Compared with MPL and JAK2 wild-type MPNs, a higher risk of arterial thrombosis has been reported in patients with MPL mutations [63]. So far, MPL mutations have had no significant impact on venous thrombotic or hemorrhagic events, transformation to MF or on survival. In PMF, MPL mutations are associated with a more severe phenotype, female gender, older age, lower hemoglobin levels and greater likelihood of requiring red cell transfusions [62].

LNK (SH2B3) is an adaptor protein negatively regulating receptor kinases engaged in the JAK-STAT signaling pathway (Figure 1) [64,65]. In LNK-deficient mice LNK was shown to play an important role in the development of erythroid, megakaryocyte and myeloid lineages [65]. LNK mutations disrupt its negative control function on the JAK-STAT signaling pathway, thereby promoting myeloproliferation in MPNs [65,66]. Mutations are found in blast-phase MPN and chronic phase MPNs and the discovery of these mutations has furthered our understanding of additional mechanisms leading to JAK-STAT pathway deregulation [64].

JAK2 inhibitors for advanced MPNs

Several JAK2 inhibitors are in clinical trials (Table 1) [67]. Most of the JAK2 inhibitors are not specific for the mutant JAK2 V617F and inhibit wild-type JAK2 tyrosine kinase [68], with the first mutant selective JAK2 inhibitors (i.e., LY2784544) now having entered trials [69]. The agents currently available inhibit additional kinases to varying degrees, possibly explaining the slightly varying responses as well as the different side-effects that were observed clinically.

INCB018424 (a JAK1/2 inhibitor) is the most advanced agent in clinical development. In the first, rather large, Phase I/II trial of 153 patients diagnosed with PMF and post-PV/ET MF, the recommended dose was 15 and 25 mg orally, twice daily [70]. Significant clinical benefit and symptomatic improvements were observed with a decrease in pruritus, weight gain and improvement in exercise capacity, measured by walking distance. Reduction in splenomegaly (>50% decrease in size according to IWG-MRT criteria) was seen for 44% of patients treated at all dose levels and 52% at the 15 mg twice daily dose schedule. The observed responses were long-lasting, often for more than 12 months in a number of patients, and the overall response rates ranged from 71 to 78%, depending on dose and schedule. Many of the responses were ongoing at publication, lasting for more than 2 years [70]. Improvement in leukocytosis was observed with a mean WBC count reduced from 29.8×10^9 to 16×10^9/l. Thrombocytosis decreased in 16 out of 17 patients, and 14% of patients that were transfusion-dependent became transfusion-independent. The median duration on therapy for all patients was greater than 14.7 months. Potential rebound hyperproliferation after discontinuing INCB018424 was seen. The dose-limiting toxicity and most common side-effect was thrombocytopenia followed by anemia, diarrhea and fatigue/asthenia. Responses were similar regardless of the JAK2 V617F mutation or underlying disease (PMF, post-PV or ET MF). The response and reduction in splenomegaly was objectively verified in 23 patients that underwent MRI evaluation: 52% achieved reduction in spleen length and 33% in spleen volume. Pharmacodynamic inhibition of the JAK-STAT pathway was demonstrated by a reduction in p-STAT3 levels after 28 days of treatment with INCB018424 to levels comparable to that of normal controls [70]. Inflammatory cytokine biomarkers decreased for the most part. However, there were only modest decreases in JAK2 allele burden, mostly less than 1 log.

TG101348 is a selective JAK2 inhibitor that also exhibits activity against other kinases, including FLT3

Table 1. Current status of clinical trial testing of JAK2 inhibitors for myeloproliferative neoplasms.

Drug and kinase target	Disease entity	Predominant efficacy	Main side-effects	Phase of clinical development	Ref.
INCB18424: JAK2, JAK1	MF, PV/ET	Splenomegaly symptoms	Anemia and thrombocytopenia	III	[70,82]
TG101348 (SAR302503): JAK2, FLT3, RET	MF	Splenomegaly symptoms	Anemia, thrombocytopenia and gastrointestinal	II	[71]
SB1518: JAK2, FLT3	MF	Splenomegaly symptoms	Gastrointestinal	II	[72]
CEP701: JAK2, FLT3, VEGFR, TRKA	MF, PV/ET	Splenomegaly symptoms	Gastrointestinal, anemia and thrombocytopenia	II	[83]
CYT-387: JAK1, JAK2, TYK2, (JNK, CDK2)	MF	Splenomegaly symptoms and anemia	First dose effect and cytopenias	I	[74]
LY2784544: JAK2	MF, ET/PV	NR	NR	I	[69]
AZD1480: JAK2, JAK3 (possibly JAK1), (TRKA, AURKA, RET, FGFR4, AX1, ALK4)	MF	NR	NR	I/II	[96]
BMS911543	MF	NR	NR	I	[79]
NS-018 JAK2	MF	NR	NR	I	[80,97]
PU-H71HSP90	Preclinical				[81]

ET: Essential thrombocythemia; MF: Myelofibrosis ; NR: No results in public domain on these ongoing trials; PV: Polycythemia vera.

and RET [68]. In 59 high- or intermediate-risk primary or post-PV/ET MF and PMF patients treated on a Phase I study, 47% achieved a spleen response by IWG-MRT after 12 months. Mean response duration was 315 days [71]. Leukocytosis and thrombocytosis normalized in 57 and 90% of patients. Early satiety, night sweats, cough, pruritus and fatigue resolved in 56, 89, 67, 50 and 63% of patients, respectively. JAK2 V617F allele burden was reduced [71].

SB1518 is a selective JAK2 inhibitor with low nanomolar activity against both wild-type and JAK2 V671F mutant JAK2 kinase as well as FLT3 [72]. Thirty-three PMF patients with splenomegaly that had received prior therapy were treated on a Phase I/II study, and 17 out of 30 patients (57%) demonstrated a 25% or greater reduction in spleen volume by MRI. Side-effects consisted of gastrointestinal symptoms, including diarrhea, nausea, vomiting, abdominal pain and fatigue, with only slight myelosuppression [72].

CYT387 inhibits JAK1/2, TYK2 and several other kinases (CDK2/cyclin A, MAPK8, PRKCN, PRKD1, ROCK2 and TBK1) [73]. By IWG-MRT criteria, 62% of patients responded in the initial Phase I/II study. Overall anemia response was 50%, with 57 and 69% of patients becoming transfusion-independent with increasing dosages at 150 and 300 mg, respectively. Importantly, responses were seen in five out of nine patients and five out of 12 patients that had received prior therapy with other JAK2 inhibitors and pomalidomide, respectively, indicating potential activity in pretreated patients. Splenomegaly was reduced in 47% and constitutional disease symptoms were controlled in the majority (>80%) of patients. For 88% of patients night sweats decreased, 80% had reduction in bone pain, 92% had reduction in pruritus and 100% had fevers resolved. Side-effects and DLTs observed were hyperlipasemia, transaminitis and headaches. A first-dose effect phenomenon was observed consisting of lightheadedness and hypotension. More severe thrombocytopenia, anemia and neutropenia (Grade 3 or 4) were seen in 27, 7 and 5% of patients, respectively. Results have been presented in abstract form and publication with the final data and clinical benefit of CYT387 is pending a full publication in manuscript form [74].

CEP701 is a JAK2 and FLT3 inhibitor that has been investigated in PMF and post-PV/ET MF patients and so far has shown only modest activity, with a rate of 27% clinical improvement. Side-effects of a gastrointestinal nature were frequent, as well as myelosupression. Pharmacodynamic activity was demonstrated by a reduction in phosphorylated STAT3 levels in responding patients, however, allele burden was unaffected [75]. The role of this agent needs to be compared and judged with other JAK2 inhibitors in development. CEP701 is also being investigated in AML [76].

Several other JAK2 inhibitors are in clinical development. Preclinical data for LY2784544 are available

and a clinical trial is open to accrual for patients [101]. LY2784544 is more selective for mutant JAK2 V617F with a 41-fold selectivity of mutant over wild-type JAK2 [69]. AZD-1480 is a targeted JAK2/3 inhibitor in early clinical study in patients with MF [101]. R723 targets JAK2 and JAK3 (IC50 = 2 and 24 nM, respectively) and inhibits cytokine-dependent and independent colony formation (CFU-E), as well as STAT5 activation in both JAK2 V617F mutant and wild-type background [77]. *In vivo* leukocyte, platelet and spleen responses were observed in mice, whereas anemia was unaffected in the mouse models [78]. BMS-911543 is a reversible JAK2 inhibitor with potent selectivity towards JAK2 (IC50 = 1 nM) versus JAK1 and 3, respectively [79]. This compound is active *in vitro* and *in vivo* and, due to its selectivity for JAK2 gene expression, analysis identified genes significantly inhibited after treatment with this JAK2 inhibitor, suggesting a possible specific JAK2 inhibitory transcriptional signature that may shed light on JAK2 inhibitory events in MPNs [79]. NS-018 is another novel potent JAK2 inhibitor with a 30–50-fold selectivity for JAK2 over other JAK-family kinases, such as JAK1, JAK3 and TYK2, and is active in *in vitro* and *in vivo* models [80]. The development of XL019 has been halted to our best knowledge.

Another target pursued is inhibition of HSP90 with PU-H71. This agent was active in mouse models, JAK2 allele burden was reduced *in vivo* and a modulation of the STAT5A transcriptional program by PU-H71 was observed [81].

JAK2 selective inhibitors for earlier MPNs

The initial development of JAK2 inhibitors centered around more advanced stages of MPNs and on PMF. In a second wave, increasing clinical data are assembled supporting substantial activity of JAK2 inhibitors in early-stage PV and ET patients. The agent that is farthest in development in clinical studies is INCB018424. In HU refractory or intolerant PV and ET patients, 82 and 72% of patients remain on study at a median follow-up of 21 months, indicating lasting responses [82]. Significant clinical activity was seen in PV, and 97 and 73% of patients normalized their hematocrit and white cell count, respectively. Approximately 80% of all patients had a spleen response and 56% showed a complete hematologic response. Similarly, patients with an ET of 79% achieved a substantial reduction in platelets and 49% a normalization of platelets. Symptoms improved for the majority of patients. JAK2 mutant allele burden was reduced but did not correlate with clinical responses. Side-effects were mild overall and mostly consisted of grade 1/2 myelosupression; anemia occurred in approximately 74% of PV and ET patients. Thrombocytopenia was somewhat less common and occurred in 29% of PV patients and leukopenia in 15 and 5% of PV and ET patients respectively [82]. Other agents, such as CEP701, have also shown spleen responses in PV patients and the same compounds that are under study for advanced MPNs are being investigated in chronic phase PV and ET patients [83]. Lastly, the EGF-receptor inhibitor erlotinib, as well as the BCR-ABL and Src inhibitor dasatinib, are being studied in patients with PV and the trial with dasatinib has completed accrual [101].

In summary, an emerging body of clinical data published in abstract and full manuscript form support the activity of JAK2 inhibitors in advanced as well as increasingly early-stage MPNs. Patients experience improvement in constitutional disease-associate symptoms in 70 to >90% of cases, and pruritus improves or resolves in approximately 50–90% of patients; further responses are long lasting, often 1 year or longer and approaching over 2 years in some studies. Spleen responses are seen in around 50–60% of patients, leukocytosis is reduced or normalized in approximately 50–60% and thrombocytosis improves in up to >90% of cases in early-stage MPNs. Improvement in cytopenias, especially anemia, was seen for some agents (i.e., CYT387), which may be a distinguishing factor amongst the various agents. However, these observations need further confirmation in larger clinical trials. For most JAK2 inhibitors, allele burden was only mildly to moderately decreased, although some (i.e., TG101348) demonstrated a greater reduction. However, the significance of this finding for long-term disease control is not currently clear. Side-effect profiles of the different JAK2 inhibitors vary, possibly due to their varying inhibitory potency on other kinases. For example, some agents show more gastrointestinal toxicities (i.e., CEP701, SB1518) various others have myelosupression and thrombocytopenia as their main side-effects. These differences may influence the therapeutic index and the overall clinical utility of a particular JAK2 inhibitor.

Non-JAK2 targeting agents in MPNs

To fully assess the clinical activity and importance of JAK2 inhibitors in MPNs, they need to be compared with agents that have shown activity but do not target JAK2. For example, PEGylated IFNα-2a has shown a remarkable 95% complete response rate in 40 PV patients, complete molecular responses were seen that lasted after discontinuation of therapy [10]. Another study demonstrated high complete hematological remissions in 70 and 76% of patients with advanced PV and ET, respectively. The molecular response rates were quite encouraging: 38 and 54% of ET and PV patients achieved any molecular response and 6 and 14% of ET and PV patients achieved a

complete molecular response. Constitutional symptoms resolved in 57%, hemoglobin and white cell count normalized in 47 and 59% of patients, and a number of patients became transfusion-independent [84]. Complete responses with platelet normalization were achieved in 52% [85] and for both patient groups therapy was tolerated very well.

Agents that have been successfully used in other hematological malignancies, such as mTor inhibitors, have also been assessed in MPNs. RAD001 demonstrated clinical activity in PMF and post-PV/ET MF patients. Partial and complete responses for spleen size reduction were reported to be 46 and 8%. Approximately half of the patients (52%) experienced complete resolution of systemic constitutional symptoms and in 74% pruritus resolved [86]. Improvements in anemia and platelet complete remission were demonstrated and side-effect profile was low [86].

HDAC inhibitors have been studied in small clinical trials. In a 12-patient study with LBH589, two patients, one JAK2 V617F-positive and one negative, experienced clinical improvement, and four patients had stable disease as their best response [87]. Another HDAC inhibitor, ITF2357, induced complete and partial responses in ET and PV and major responses in PMF patients. Spleen size reduced in six out of eight patients with splenomegaly and pruritus being relieved in most patients with overall good tolerance of ITF2357 [88].

Immunomodulatory agents such as thalidomide, lenalidomide and the clinically tested agent pomalidomide improve symptoms in patients with MPNs, and their activity is greatest in lower risk patients and those with anemia and thrombocytopenia [14,89].

Experimental therapies & bone marrow transplantation

For patients progressing to aggressive stages of MPNs, including MPN in blastic phase (MPN-BP), a presentation resembling that of acute leukemias, aggressive treatments such as leukemia-type induction chemotherapy or even allogeneic hematopoietic stem-cell transplantation (allo-HSCT) are options. However, these need to be carefully judged against the patients overall health and possibly tolerance of an allo-HSCT versus other targeted therapies [90]. For MPN-BP, several papers report rather good outcome with reduced intensity conditioning regimens and allo-HSCT is one option at disease transformation [90].

There seems to be a strong component of epigenetic regulation in MPNs and, in a recent study, 54 MPN patients who had progressed to AML or MDS were treated with 5-azacitidine with an overall response rate of 52% (24% complete response rate) [91]. Median duration of response was 9 months. Responses may be lower in chronic phase MPNs [92]. These clinical observations may indicate a different disease and target biology in chronic versus leukemic phase of MPNs. To improve on the efficacy of 5-azacytidine, our laboratory has identified that combining 5-azacytidine with BCL-XL inhibitors (BH3 mimetics) has a strong preclinical rationale and we generated data in primary MPN patient samples showing potent activity of 5-azacytidine with ABT-737 [93,94]. ABT-263 is the clinically developed form of ABT-737 and we have proposed a clinical trial in combination with 5-azacytidine or IFNα-2a. Other novel agents targeting putative disease biology are smoothened inhibitors, which inhibit activation through the hedgehog pathway. IPI-926, a smoothened inhibitor, will enter clinical trials soon [Mesa R, Pers. Comm.]. Agents under development for MPNs are GSK3β and TGFβ inhibitors. Survivin inhibitors have been tested in hematological malignancies with clinical activity in lymphomas as well as in a patient with CML and AML in a Phase I trial performed by our group [95]. Survivin may also represent a novel target in MPNs.

Strategies of integrating JAK2 inhibitors into therapies for patients with MPNs

JAK2 inhibitors have been clearly shown to control counts and reduce spelnomegaly in PV, ET and PMF patients, and some agents can reduce allele burden. However, eradication and complete remission of the disease as seen with BCR-ABL-specific inhibitors in CML is not encountered. The reasons for this are still unknown and the field of targeting JAK2 is still at an early stage. The currently available JAK2 inhibitors target JAK2 to varying degrees. Most of the agents in development inhibit the wild-type JAK2 kinase and the first JAK2 mutant specific inhibitors are being tested clinically. It will be interesting to await data on whether these agents may eradicate mutant clones more completely. Furthermore, most agents also inhibit other receptor and nonreceptor kinases, which may compromise on dose and thus limit their activity by the side-effect profile (Table 1). Hence, more selective, mutant-specific inhibitors may show greater activity. Clearly, JAK2 inhibitors have not been the breakthrough as hoped from CML observations, and CML may be a more 'monogenetic' disease with MPNs beng activated through a host of cytokines and other factors [31].

Clinically, JAK2 inhibitors bring benefits to patients and a common theme are improvements in splenomegaly and constitutional symptoms. Perhaps the greatest feature of how the current JAK2 inhibitors differentiate themselves is in their ability to affect cytopenias. It is important to note that, so far, no significant proof has been delivered that these inhibitors lead to either

molecular remissions or improvement of marrow histologic features. Whether JAK2 inhibition might alter the natural history of these disorders will be demonstrated over time and needs to be put into the context of benefit with other treatment approaches and historical controls. In addition, JAK2 inhibiton needs to be compared with some of the non-JAK2 targeting agents that have shown activity in MPNs.

Given these remaining uncertainties, what could the role be for JAK2 inhibitors? To answer that question we need to consider the two main groups of MPN patients that present with distinct clinical phenotypes separately. First, with the more mature data for advanced and MPNs in MF, it seems evident that JAK2 inhibitors will be a reasonable therapeutic choice and an important component in the overall treatment strategy of palliating the disease, such as reducing suffering from splenomegaly and constitutional symptoms. Most patients with advanced myelofibrotic-stage MPNs clearly experience clinical benefit with JAK2 inhibitors. For patients with an early-stage of the disease, and with minimal symptoms, they may not need or experience the same degree of benefit, and careful use of these agents needs to be exercised. For individuals whose severity of illness mandates more aggressive therapy approaches immediately with stem cell transplantation (intermediate 2 or high-risk disease and an appropriate transplant candidate), these patients should proceed to transplant. For patients with early/chronic-phase PV and ET, the role of JAK2 inhibition is currently too early for us to judge. JAK2 inhibitors need to be carefully judged against, and demonstrate therapeutic equivalency with, hydroxyurea and other agents currently in use; they must also adequately prevent vascular events and help to control patients' blood counts. The benefit beyond hydroxyurea at which adopting JAK2 inhibitors as front-line therapy for PV/ET would be considered is if they reliably improve disease-associated symptoms and if they improve splenomegaly, which is likely to be a clinically relevant issue in only a minority of patients. One of the most important long-term considerations for JAK2 inhibitor therapy in early PV and ET as front-line therapy is whether these agents alter the natural history of the diseases. If this would indeed be the case then this would indicate a major benefit over the currently used approaches, which mainly consist of myelosuppressive agents. Utilization of JAK2 inhibitors as second-line treatments for patients resistant or refractory to hydroxyurea or other myelosuppressive therapy would initiate a more straightforward developmental path for JAK2 inhibitors. For first-line treatment, randomized studies comparing JAK2 inhibitors to standard of care agents, such as hydroxyurea, would need to be considered prior to implementing JAK2 inhibitors in front-line therapy. Competing in this mix for the same group of patients is PEGylated-IFN-α-2a, which can also help to control the risk of thrombosis and thrombocytosis and erythrocytosis in patients with PV.

Future perspective

The discovery of the JAK2 V617F mutation, now more than 5 years ago, and the subsequent discoveries and our increased understanding of pathogenetic mechanisms of MPNs, together with the availability of a series of targeted agents, have already greatly affected and benefited individuals with MPNs. At this juncture, we envision two main potential avenues of further development of the therapeutic armamentarium. In the first scenario, according to the current understanding that the JAK2 mutation itself may not be the initiating step in the pathogenesis of MPNs, but rather an intermediate step in the progression, it is natural to continue to seek alternative targets that may be superior to those of JAK2. We envision that alternative pathways, mechanisms and targets will be identified that are able to either selectively, or more effectively, inhibit the underlying proliferative and transforming processes in MPNs. The second line of development would be combination strategies with agents currently in development, such as the addition of agents that have demonstrated improvements in anemia such as pomalidomide, lenalidomide, erythropoietin and androgens, to JAK2 inhibitors. Further sequencing of active agents and therapeutic strategies, such as the use of a JAK2 inhibitor to improve performance status and splenomegaly with a subsequent stem cell transplantation. Or sequencing of agents with alternative mechanisms that are active by themselves, and using these in combination or sequence, such as the use of IFNα, which works at a stem cell level, followed, or concurrently with, JAK2 inhibitors.

We are hopeful that over the coming years there will be substantial progress into the treatment and management of individuals with MPNs. Careful assessment as to the overall impact of JAK2 inhibitors and other novel agents on patients with MPNs, assessment of the clinical net benefit, exploration of combination strategies as well as novel targets in MPNs give promise for more and more treatment options in the near future for patients afflicted with these diseases.

Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

- In 2011 no medication has been proven to halt the natural progression of myeloproliferative neoplasms (MPNs).
- The JAK2 V617F mutation is an almost obligatory genetic abnormality in polycythemia vera (PV) and frequent (~40–60%) in essential thrombocythemia and primary myelofibrosis, however, it may not be the initial underlying driving genetic event.
- The exact role of JAK2 V617F mutation and allele burden for disease presentation and manifestation are still being defined.
- In JAK2 V617F-negative PV patients, exon 12 mutations are frequently found.
- Several additional mutations, in varying frequency, in genes such as *MPL*, *LNK*, *TET2*, *IDH 1/2*, *C-CBL*, *ASXL1* and *IKZF1* have been described in PV, essential thrombocythemia and primary myelofibrosis.
- These mutational events are contributing to disease pathogenesis and may represent additional targets in MPNs.
- Early clinical data support the activity of experimental JAK2 inhibitors in advanced, as well as increasingly early-stage MPNs.
- Experimental JAK2 inhibitors show differences in their response and side-effect profile that may influence their therapeutic activity and index.
- Significant clinical activity of JAK2 inhibitors with improvement of constitutional symptoms has been seen in up to 70 to >90% of patients, pruritus in 50–90%, splenomegaly in ~50–60%, leukocytosis in ~50–60% and thrombocytosis in up to 90% of patients.
- Improvement of constitutional symptoms in patients with advanced MPNs is one clinical avenue of development for JAK2 inhibitors.
- For early-stage MPNs, the hurdle is higher and these agents would need to add significant benefit over current therapies and upfront randomized trials would likely be required.
- Alteration of the natural history of the disease would be a major determinant of the utility of JAK2 inhibitors.

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