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## Targeting JAK2 in the therapy of myeloproliferative neoplasms

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### Abstract

**Introduction**—Myeloproliferative neoplasms (MPNs) are a group of stem cell diseases, including polycythemia vera, essential thrombocythemia and primary myelofibrosis. Currently, there is no curative therapy for these diseases other than bone marrow transplant; therefore there is an apparent need for palliative treatment. MPNs are frequently associated with activating mutations in Janus Kinase 2 (JAK2); small molecule drugs targeting this molecule have entered clinical trials.

**Areas covered**—In this review novel JAK2 inhibitors will be discussed and alternative approaches to inhibiting their transforming potential will be highlighted.

**Expert opinion**—Current clinical approaches do not only aim at blocking JAK2 activity, but also at reducing its stability and expression. Inhibition of heat shock protein 90 (HSP90) and deacetylase inhibitors (DACi) have the potential to significantly enhance the efficacy of JAK2 inhibitors. Preliminary results from clinical trials indicate the feasibility and efficacy of JAK2 targeted approaches. However, JAK2 inhibitor treatment is limited by dose-dependent toxicity and combination treatment might be required. The discovery of JAK2 mutations that cause secondary resistance *in vitro* would further highlight the need for the development of next generation JAK2 inhibitors and novel synergistic approaches.

### Keywords

Myeloproliferative neoplasms; JAK2; signaling; metabolism; targeted therapy

### 1. Introduction

In Philadelphia chromosome negative (Ph<sup>-</sup>) myeloproliferative neoplasms (MPNs), the non-receptor tyrosine kinase Janus kinase 2 (JAK2), is frequently found to contain an activating V617F mutation [1-5]. This mutation is present at a high frequency in patients with polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (MF) as well as infrequently in other MPNs, myelodysplastic syndromes and acute myeloid leukemia (see for review [6]). Even though the V617F mutation can occur spontaneously, there appears to be a three- to fourfold greater risk for carriers with the so called '46/1' haplotype,

encoded by a region in the 3' of *JAK2* [7-9]. Additional genetic abnormalities have been found in the *JAK2* gene (exon12-15) in a variety of hematologic malignancies but with lower frequencies [10]. The carboxy-terminal kinase domain in *JAK2* can also be activated as part of an oncogenic fusion, involving breakpoints in the JH2-JH5 domain. For example, a t(9;12)(p24;p13) or variant translocations in patients with a chronic myeloproliferative disease or acute lymphoblastic leukemia fuses the *ETV6* to the *JAK2* gene [11,12]. There are additional rare translocations that involve *JAK2* and lead to the formation of a constitutive activation of the kinase (see for review [10]) (Figure 1). *JAK2* is also directly involved in the transformation by oncogenic receptors. In MPNs, the thrombopoietin (TPO) receptor MPL, which requires *JAK2* for signaling, is an infrequent target of activating mutation, in particular at amino acid W515 [13,14]. Also, in acute lymphoblastic leukemias (ALL), activating CRFL2 (cytokine receptor-like factor 2) mutations and rearrangements and activating *JAK2* mutations are frequently found [15], suggesting that this pathway is important for the disease process. Thus, *JAK2* targeted approaches may not only be beneficial for the treatment of MPNs, but may also help in the treatment of other malignancies with a constitutively active *JAK2* signaling pathway.

## 2. *JAK2* - structure and function

*JAK2* belongs to the family of related non-receptor Janus tyrosine kinases, including *JAK1-3* and *TYK2* [16]. There is a considerable degree of homology between these kinases that can be defined to specific JAK homology (JH) domains. The carboxy terminus contains the kinase domain (JH1) and the related pseudokinase domain (JH2) (Figure 1). The latter is structurally similar to the JH1 domain except for a DFG motif in the activation loop, which results in lack of kinase activity [17]. This distinct architecture of JAKs gave them their name, according to the two-faced Roman god Janus. The JH2 domain plays an important role in regulatory functions of Janus kinases [18,19]. This domain is thought to negatively regulate the kinase activity through interaction with the JH1 domain and the V617F mutation in the JH2 domain found in MPNs has been suggested to overcome these inhibitory constraints. [2,3]. A Src homology 2 (SH2)-like domain (JH3-4) is adjacent to the pseudokinase domain and the amino-terminal region (JH6-7) contains the FERM (4.1 protein, ezrin, radixin, moesin) domain [16]. This domain together with the SH2-like domain form the amino-terminus of *JAK2* that is essential for upregulation of surface expression of cytokine receptors such as EpoR [20]. A proline rich eight amino acid motif (box1) in the cytoplasmic portion of membrane-associated receptors typically recruits the FERM domain [21]. Disruption of this interaction, such as in the case of a Y114A substitution in the FERM domain, results in loss of *JAK2* activation, independent of the *JAK2*V617F activating mutation [22,23]. Thus, an intact FERM domain is necessary for phosphorylation and activation of *JAK2* signaling pathway [23]. This domain may also promote cell surface localization of the thrombopoietin receptor and thus upregulation of the downstream signaling of *JAK2* [22]. However, erythroid progenitors in PV show hypersensitivity to erythropoietin or factor independent growth [24,25], suggesting that *in vivo* *JAK2*V617F may, at least in part, require ligand stimulation for signaling.

### 3. Regulation of cellular functions by JAK2 signaling pathways

JAK2 acts as a kinase for cytokine receptors that lack an intracellular tyrosine kinase domain. Mice with JAK2 gene disruption are embryonically lethal, due in part to ineffective erythropoiesis. JAK2 is also indispensable for functions of various cytokines, such as interleukin 3, thrombopoietin and erythropoietin [26,27]. JAK mediated tyrosine phosphorylation of receptors forms docking sites for intracellular effectors, such as STATs (signal transducers and activators of transcription). STAT proteins are phosphorylated at the receptor and translocate in its active form to the nucleus to initiate transcription of their target genes [28]. In JAK2V617F expressing cells, STAT5 is constitutively phosphorylated [2]. Constitutive activation of STAT5 was shown to be sufficient to induce growth factor independent cell proliferation [29]. Also, disruption of the STAT5 genes demonstrated a role in anti-apoptotic signaling, immature hematopoiesis and fetal erythropoiesis *in vivo* [30,31]. Signaling and activation of STAT5 by JAK2V617F is crucial for JAK2V617F-dependent growth factor independent proliferation and transformation *in vivo* and required the functional expression of a cytokine receptor such as the erythropoietin receptor [23,32,33]. Recently, a high throughput chemical screen for small molecule inhibitors identified pimozone as a potential STAT5 targeting drug [34]. Treatment of CML cell lines with pimozone resulted in decreased phosphorylation and expression of STAT5 and was associated with increased apoptosis and cell-cycle arrest. However, the exact mode of action for pimozone is not clear. Furthermore, active STAT5 can induce the expression of the serine/threonine kinases PIM1 and PIM2, which are required for optimal growth in JAK2V617F transformed cells [23]. Early clinical trials with the PIM inhibitor SGI-1776 (SuperGen) [35] for the treatment of refractory leukemias, lymphomas and prostate cancer were terminated due to toxicity and there is a need for safer compounds. In addition to cellular targets, JAK2 was also shown to phosphorylate nuclear histone H3 on tyrosine 41 (H3Y41) in the nucleus of hematopoietic cells [36]. Phosphorylation of H3Y41 leads to release of transcriptional repressor HP1 $\alpha$  from the chromatin and concomitant expression of genes repressed by HP1 $\alpha$ . The mechanism of JAK2 activation in the nucleus still needs to be determined [37]. Surprisingly, only JAK2 protein of MPN patients that carry the V617F mutation accumulates in the nucleus of CD34+ progenitor cells and not JAK2 from patients that carry the wildtype protein [38]. The functional significance of this difference is unclear. JAK2 has additional downstream targets such as the erythropoietin receptor [39], VAV [40], p27KIP [41], PAK1 [42], SH2-B $\beta$  [43], PRMT5 [44] and other proteins may significantly contribute to JAK2 dependent malignancies and are potential candidates for targeted approaches (Figure 2).

Signaling through the JAK2V617F gain-of-function mutation is not limited to a set of specific JAK2 target proteins. The expression of JAK2V617F is thought to confer hypersensitivity to growth factors as well as factor independent growth, thus aberrantly activating growth and survival pathways that can normally be stimulated by signaling through cytokine and growth factor receptors. In addition to the STAT pathway, the PI3K (phosphatidylinositol -3'-kinase) and MAPK (mitogen activated protein kinase) pathways are also prominent targets of activation in JAK2V617F expressing cells [45,46]. The importance of the PI3K downstream target mTOR (mammalian target of rapamycin) has

recently been demonstrated in phase 1/2 clinical trials of patients with MF [47]. mTOR activity within the multi-functional mTORC1 or mTORC2 complex was inhibited by everolimus. mTOR is thought to function as a nutrient and energy sensor as well as a regulator of cell growth, in part through phosphorylation of the translation initiation factor 4E-BP1 and the ribosomal p70S6 kinase [48]. Everolimus was overall well tolerated and in about 50% of the patients a reduction of splenomegaly was observed with the majority of the patients experiencing complete resolution of their clinical symptoms [47]. Interestingly, the response was independent of the presence of JAK2V617F. Larger cohorts will be needed to further evaluate the efficacy of this approach but these initial results would indicate that targeting mTOR with everolimus may be a viable option for the treatment of MF and related diseases. At present, it is not known whether concomitant inhibition of JAK2 and mTOR is beneficial for this patient group. The importance of the MAPK pathway in JAK2V617F signaling has not been worked out in great detail. However, *in vitro* experiments with CD34+ cells from PV patients suggest that inhibition of p38MAPK with SB203580 does not suppress growth of JAK2V617F-positive hematopoietic progenitor cells [49]. Interestingly, in these experiments interferon- $\alpha$  targeted this cell population and the growth inhibition could be overwritten by SB203580 treatment.

#### 4. Targeting JAK2 in myeloproliferative neoplasms

Activating JAK2 mutations in MPNs have been suggested to be an ideal target for inhibition due to their prevalence and apparent role in the disease phenotype. Initial clinical trials have been focusing specifically on MF due to a limited number of efficient targeted approaches and immediate therapeutic need. Clinical trials are in the process of being expanded to all MPNs and include PV and ET as well (see also <http://clinicaltrials.gov>). The current treatment options for MF patients depend on individual circumstances and can include drug treatment (such as hydroxyurea, danazol, thalidomide, corticosteroids or others), splenectomy, radiotherapy (locally for extramedullary hematopoiesis) or stem cell transplant. Aggressive treatment options are frequently a last resort and may be accompanied by significant side effects or even death [50]. Even though MPNs can often be managed for years with little complications, the onset of pain, splenomegaly and anemia are some of the main intermediate concerns that require additional palliative care. There are several small molecule drugs currently in clinical trials that attempt to target JAK2 function at different levels (Figure 3).

##### 4.1. JAK2 small molecule kinase inhibitors

The tremendous success of small molecule tyrosine kinase inhibitors targeting BCR-ABL in chronic myeloid leukemia (CML) has led to the development of related drugs that specifically inhibit kinases required for transformation in various malignancies. In general, these drugs prevent interaction of ATP with its binding pocket in the catalytic site of the kinase. High affinity interactions with amino acids in this hydrophobic groove determine the specificity for each drug. Currently, there are seven JAK2 kinase inhibitors in clinical trials (Table 1, Figure 4). The JAK2 inhibitor ruxolitinib was recently approved by the FDA (U.S. Food and Drug Administration) for the treatment of intermediate or high-risk myelofibrosis. Even though these inhibitors were developed to target JAK2, they have been shown to have

activity against some other kinases as well. Notably ruxolitinib (INCB018424), one of the first drugs tested for its efficacy in the treatment of MF, also inhibits the related JAK1 [51,52]. Similarly, the JAK2 inhibitors SAR302503 (TG101348), AZD1480 and CYT-387 display activity against JAK1 as well [53-56]. Surprisingly, the effect on JAK2V617F allele burden with ruxolitinib did not match the clinical outcome and it had been speculated that its clinical efficacy might be in part due to inhibition of JAK1-dependent cytokine signaling. Ruxolitinib demonstrated significant and dose-dependent efficacy in MF with dose-limiting thrombocytopenia [51]. There may be additional specific toxic effects that are caused by inhibiting JAK2 or secondary targets, such as dose-limiting hyperamylasaemia with SAR302503 or other effects with related drugs [57,58]. The JAK2 inhibitor lestauritinib (CEP-701) belongs to a different group of compounds and is structurally related to the pan protein kinase inhibitor staurosporine with additional activity against FLT3, RET and Trk family members [59-62]. The JAK2 inhibitor SB1518 is a pyrimidine-based macrocycle with additional activity against FLT3 [63], whereas the EGFR inhibitor erlotinib is also effective against JAK2 [64], but little is known about the JAK2 inhibitor LY2784544. Despite these additional activities, all inhibitors tested for MF, including ruxolitinib, lestauritinib, CYT-387, SB1518 and SAR302503, lead to reduction in splenomegaly in some patients, further validating JAK2 as an important therapeutic target in MF (see for detailed review of clinical trials [58]). The toxicity profile of each individual JAK2 inhibitor may be an important determinant for its success and it will be interesting to see whether this correlates with the known off-target effects.

#### 4.2. HSP90 (heat shock protein 90) inhibitors

Heat shock proteins (HSPs) are family of related proteins that are involved in the maintenance of proper folding and stability of its client proteins. Their expression can be significantly increased not only upon heat exposure but also in response to a variety of other stress conditions. HSP90 is a member of this family with ATP dependent chaperone activity, towards several cellular proteins [65]. In myeloid malignancies, BCR-ABL and active forms of FLT3 or JAK2 are among prominent targets that are dependent on HSP90 for their functional conformation [66-68]. Thus, HSP90 plays a crucial role in the proper folding and activation of these oncoproteins and their downstream signaling pathways. HSP90 may not only act on the native oncoprotein but also mutated forms that are resistant to targeted approaches, such as BCR-ABL mutations in CML. In MPNs, JAK2 kinase inhibitors have only recently been introduced into clinical trials and drug resistant point mutations are not expected to emerge at this stage but may be of significant relevance in the near future. Nevertheless, recent approaches suggest that HSP90 inhibitors may show efficacy against active JAK2. Marubayashi et al. have shown that the novel HSP90 inhibitor PU-H71, is effective *in vitro* as well as *in vivo* using preclinical models of PV and ET [69]. Direct interaction between HSP90/PU-H71 and JAK2 protein in isogenic and leukemic cell lines that led to reduced growth was demonstrated. Mouse bone marrow transplant models and MPN primary samples confirmed the HSP90 inhibition mediated JAK2 degradation and further downregulation of its downstream signaling pathway. Targeting HSP90 may also overcome drug resistance. Fiskus et al. found that cells resistant to the JAK2 inhibitor TG101209 were sensitive towards the HSP90 inhibitor AUY922. Further, AUY922 in combination with TG101209 exerted synergistic *in vitro* activity in human CD34<sup>+</sup> MF-MPN

hematopoietic progenitors [68]. AUY922 depleted JAK2V617F protein expression in a dose dependent manner, thus effectively further inhibiting its downstream signaling and reducing the phosphorylation of STAT5, AKT and ERK1/2 *in vitro*. As would be predicted, this effect was enhanced by co-treatment with TG101209. Combination of both drugs in TG101209 resistant cells expressing JAK2V617F led to higher apoptosis as compared to each drug alone. Thus, combination treatment may enhance the efficacy of JAK2 targeted approaches in MPN patients.

### 4.3. Deacetylase inhibitors (DACi)

Histone deacetylase (HDACs) inhibitors have shown efficacy in preclinical models of MPNs [70,71]. Increased HDAC function was found in MF, even though no correlation was observed between JAK2 mutational status and its activity [72]. It is not known whether inhibition of histone deacetylation contributes to the efficacy of these drugs or whether inhibition of deacetylation of non-histone proteins by HDACs is more prominent, in particular HSP90 [73]. Currently, there are four deacetylase inhibitors (vorinostat, panobinostat, givinostat and SB939) (Figure 3) in clinical trials for the treatment of MPNs. In a limited study of patients with JAK2V617F positive MF, vorinostat (suberoylanilide hydroxamic acid, SAHA, MK-0683; Merck) showed clinical efficacy [74]. This drug binds to the Zinc ion in the catalytic domain to inhibit HDAC activity [75]. It recently gained FDA approval for the treatment of T cell lymphomas [76,77] and is currently in phase II clinical trials for the treatment of MPNs. Givinostat (ITF 2357; Italfarmaco, Milan, Italy), is currently in phase II clinical studies for the treatment of PV and permitted in the European Union for its treatment as an orphan drug. A phase IIA study in MPN patients with JAK2V617F showed hematological response in most PV patients [78]. It has also been shown to selectively target the cells from patients expressing JAK2V617F, but not wildtype JAK2. The phosphorylation levels of JAK2V617F, STAT5 and STAT3 were reduced in response to givinostat treatment [70]. Panobinostat (Novartis) was developed as a pan-deacetylase inhibitor and is currently in phase II trial for the treatment of MPNs either as a single agent or in combination with ruxolitinib in phase I study. Panobinostat treatment resulted in disruption of signaling downstream of JAK2V617F as well as HSP90 mediated folding of JAK2V617F *in vitro*. The efficacy of panobinostat was synergistically increased in CD34+ MPN cells when treated in combination with TG101209 [79]. Another deacetylase inhibitor, SB939 (S\*BIO, Singapore) is currently in phase II clinical trials for the treatment of MPNs. This inhibitor has been shown to specifically accumulate to tumor tissue compared to healthy tissues in a colorectal cancer mouse model [80].

## 5. JAK2 inhibitor resistance

With the introduction of imatinib in CML and related tyrosine kinase inhibitors in other cancers, drug resistant mutations that interfere with the binding of the inhibitors rapidly emerged. For example, the T315I mutation in ABL shows imatinib resistance by interfering with drug binding [81,82]. Therefore, it would be of great clinical value to predict potential JAK2 inhibitor resistant mutations in MPNs. Recently, in a screen of randomly mutagenized JAK2V617F libraries, five non-synonymous point mutations in its JH1 domain were identified that led to ruxolitinib resistance *in vitro* [83] (Figure 4). These Y931C, I960V,

G935R, E985K and R938L point mutations were either predicted to interact with ruxolitinib or were in proximity to the binding pocket. In particular mutation at tyrosine 931 would offer drug resistance as a result of loss of  $\pi$ - $\pi$  interaction between the tyrosine ring and the inhibitor structure. JAK2V617F containing mutations also showed cross-resistance to the structurally unrelated JAK2 inhibitors CYT-387, SAR302503, AZD1480 and lestaurtinib [83], indicating an overlap in the interaction of these drugs with the ATP binding pocket. Similarly, ruxolitinib resistant mutations were identified *in vitro* in the JAK1 kinase domain. Homologous to the JAK1 F958C mutation identified in this screen, a Y931C mutation was introduced into JAK2 and not only led to cytokine independent growth of cells expressing JAK2 but also caused ruxolitinib resistance independent of the JAK2 mutation status [84]. Surprisingly, this screen did not identify a homologous ‘gate-keeper’ mutation that has been linked to secondary drug resistance in active forms of ABL, EGFR and KIT [82,85,86]. Introduction of a M929I mutation at the gatekeeper site in JAK2V617F showed only four-fold increase in EC50 for ruxolitinib compared to native JAK2V617F but not to CYT-387, SAR302503, AZD1480 and lestaurtinib [83]. It will be important to carefully look for potential JAK2 inhibitor resistance in MPN patients that have undergone prolonged treatment.

## 6. Metabolic reprogramming as an opportunity for targeted approaches

One of the hallmarks of malignant cells is dysregulated glucose metabolism, compared to their healthy counterparts. Transformed cells tend to utilize glycolysis and fermentation as a major energy generating process, even in the presence of oxygen instead of more efficient oxidative phosphorylation. This so-called ‘Warburg effect’ [87], named after its discoverer, is a result of increased expression, localization and/or activity of the genes involved in glucose transport and metabolism. Cancer cells exploit this altered metabolism to provide precursors for biomolecules needed for their uncontrolled proliferation and to evade apoptosis [88]. Even though there may be significant overlap between mechanisms that induce the Warburg effect in different types of cancers, little has been done to specifically investigate this effect in malignancies driven by active JAK2. For instance, it has been shown that active JAK2V617F was sufficient for increased cell surface expression of glucose transporter Glut1, concomitant increase in glucose uptake and increased lactate production [89]. In addition, JAK2 expressing cells also showed elevated expression of phosphofructokinase/fructose-bisphosphatase 3 (PFKFB3), a rate-limiting enzyme that affects phosphofructokinase-1 (PFK-1) activity in the glycolytic pathway. The metabolic end product of PFKFB3, fructose-2,6-bisphosphate is an allosteric activator of PFK1, that allows continuous activity, even in the presence of ATP, which normally suppresses PFK1 activity. The role of PFKFB3 in JAK2V617F mediated transformation was further confirmed *in vivo* using a murine xenograft model with knockdown of PFKFB3 [89]. PFKFB3 could also be targeted with the small molecule inhibitor 3-PO (3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one) [90], resulting in reduced cell growth and reversal of the Warburg effect [89]. Another important aspect of hyperactive metabolism associated with JAK2V617F is an excess production of mitochondrial reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl radicals. ROS are a by-product of the electron transport chain and transiently elevated levels are required for some normal cellular

functions such as cell growth, migration and signaling and may contribute to genomic instability [91]. In JAK2V617F expressing cells ROS are constitutively elevated [92] (Figure 5). In leukemia stem cells, the naturally occurring compound parthenolide further increased ROS levels and triggered apoptosis [93]. Additional strategies to target ROS are currently under investigation [94]. A minor source for superoxide production in metabolically active myeloid cells transformed by JAK2V617F and other oncogenic tyrosine kinases are NADPH oxidases [95]. Interestingly, knockdown of the NADPH oxidases resulted in decreased cell growth and migration [95], consistent with a localized production of superoxide. Targeting NADPH production or development of specific NADPH oxidase inhibitors may provide synergy to JAK2 inhibition.

## 7. Conclusion and expert opinion

The discovery of activating JAK2 mutations in MPNs has underlined the importance of the associated signaling pathway for the disease process. However, the lack of activating JAK2 or receptor mutations in a proportion of MPNs suggests that there may be additional activities that are independent of this pathway. Nevertheless, early results from clinical trials demonstrate efficacy of JAK2 inhibitors, even in MPNs without apparent JAK2 pathway mutations. These findings do not indicate whether the JAK2 signaling pathway is constitutively active in MPN patients, even though this would be an intriguing possibility. It is likely that in these MPNs, JAK activation through cytokine receptor stimulation plays a greater role than previously thought. Initial clinical trials with JAK2 inhibitors demonstrate dose-dependent efficacy that is limited by toxicity. The importance for JAK2 in the hematopoietic compartment may leave a small therapeutic window for treatment, thus causing adverse effects at higher inhibitor concentrations. It can also not be entirely excluded that JAK2 with activating mutation is insufficient to cause addiction to this oncogene. *In vivo* mouse models suggest that JAK2V617F by itself can initiate a myeloproliferative disease. However, this picture is somewhat more complicated in patients, where JAK2V617F is frequently associated with other mutations that have epigenetic effects. A two-hit model adapted for MPNs transformation (see for review [96]) suggests that in MPNs the activation of signaling molecules (such as JAK2V617F) may not be sufficient for the full transforming potential and that changes in the activity of epigenetic regulators (such as TET2) may be required for the disease process. Also, JAKV617F more efficiently supports late hematopoiesis and not expansion of stem cells, whereas TET2 mutations benefit the progenitor pool. Thus, additional therapeutics for co-treatment may well be required to efficiently inhibit MPNs associated with JAK2V617F and could have differential effects on the disease phenotype. Further, oncogene-targeted therapies in combination with additional signaling-targeted therapies have the potential to increase the efficacy of current approaches and further identification and characterization of signaling pathways that drive MPNs may lead to novel therapeutics. Lesson learned from imatinib and other ABL kinase inhibitors would suggest that even under optimal circumstances, JAK2 inhibition by itself is insufficient to lead to a curative therapy but may be sufficient to stabilize clinical symptoms in a large proportion of the patient pool for a long period of time. Additional characterization of the genetic alterations associated with these diseases and a better understanding of the affected genes may be required to achieve this goal. Whole



genome sequencing has brought a new understanding about these changes. Nevertheless, analysis of future results needs to be further refined to obtain useful insights into the evolution of different malignant clones and the mutations that truly drive their expansion. These data will likely identify genes that provide growth advantages and help to prioritize target development. In this context it is also important to note that similar to other kinase inhibitors, it may be necessary to monitor for secondary drug resistance. Preliminary data hint at potential JAK2 inhibitor resistant mutations in the JAK2 kinase domain and this knowledge will help to develop next generation JAK2 inhibitors or drugs that target proteins such as HSP90 and DAC to overcome JAK2 inhibitor resistance. Similar to other malignancies with a complex phenotype, the field will likely move towards more individualized therapy. In particular knowledge about the aforementioned disease driving genes will allow to devise more effective combination therapies that inhibit the expansion of malignant cells at multiple levels.

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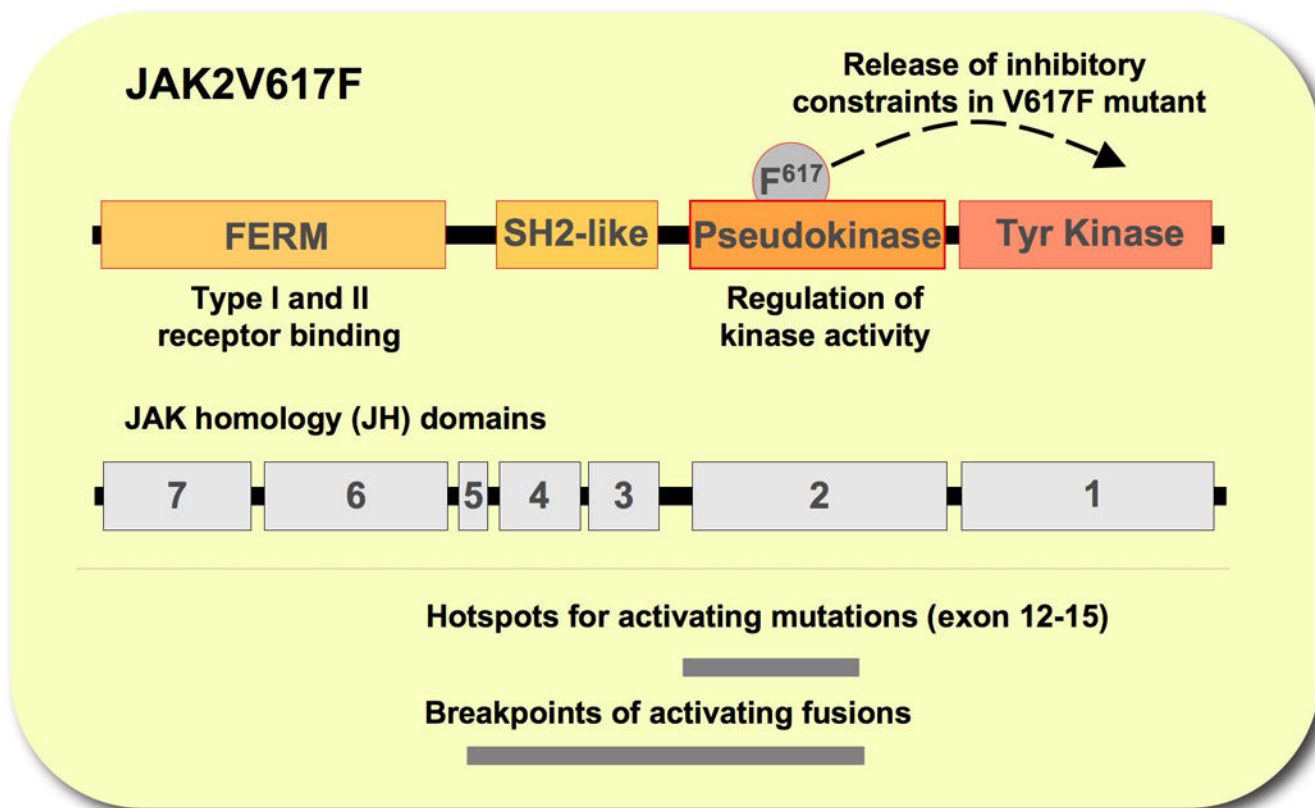
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### Article highlights

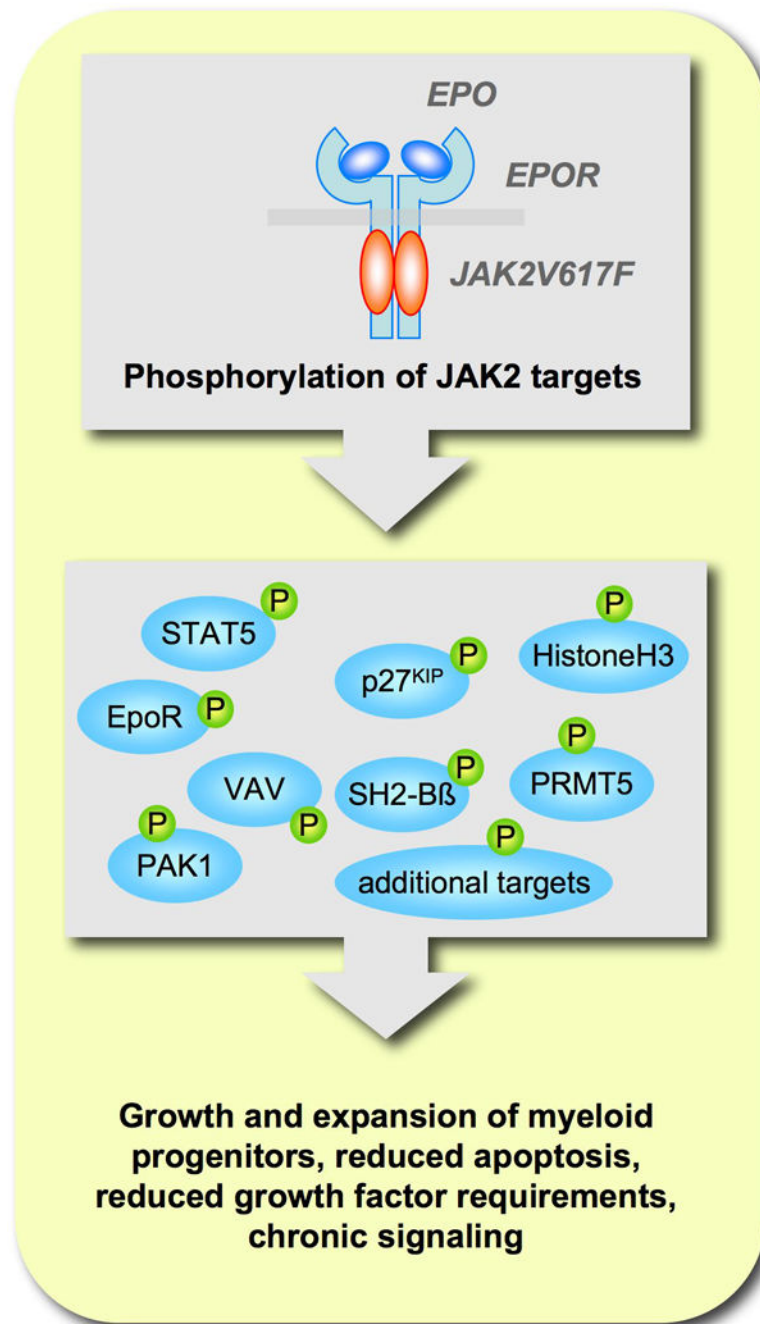
- Myeloproliferative neoplasms (MPNs) are frequently associated with the activating V617F mutation in JAK2. Dysregulation of the JAK2 pathway can also be found in other myeloid as well as non-myeloid malignancies.
- Small molecule JAK2 kinase inhibitors are currently in clinical trials for the treatment of MPNs.
- Secondary resistance to JAK2 inhibitors may be caused by mutations in the kinase domain. Mutational analysis of this domain should therefore be considered for patients that fail to respond to these drugs.
- Inhibition of HSP90 (heat shock protein 90) deacetylation and HSP90 chaperone function may cooperate with JAK2 kinase inhibition.
- Activation of the JAK2 pathway leads to increased carbon metabolism. JAK2V617F dependent and independent metabolic reprogramming hints at novel targets for drug development.



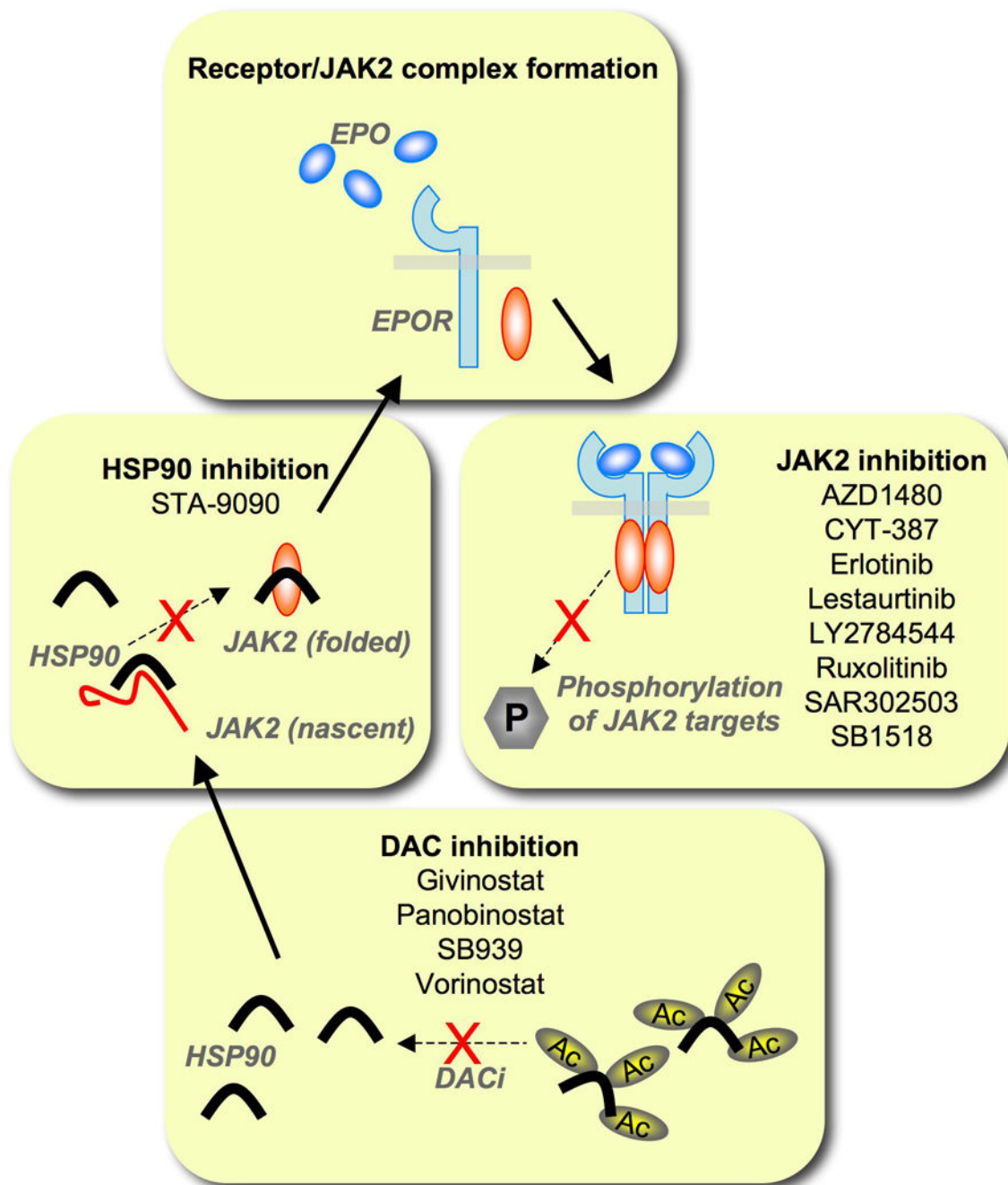
**Figure 1. Schematic structure of JAK2**

Represented are domains within JAK2, including the FERM (4.1 protein, ezrin, radixin, moesin) domain, SH2 (Scr homology 2) like domain, the pseudokinase domain and the kinase domain (top), the JAK homology (JH) domains (middle) as well as regions that include hotspots for activating mutations and breakpoints for activating fusions (bottom).





**Figure 2. Cytoplasmic and nuclear targets of JAK2**  
 Activation of the JAK2/receptor complex leads to direct phosphorylation and functional regulation of cytoplasmic and nuclear proteins.



**Figure 3. Schematic diagram of strategies targeting the JAK2 pathway in MPNs**  
Functional expression of JAK2 can be blocked at several levels. Deacetylase inhibitors (DACi) increase the acetylation and thus inhibit the chaperone function of HSP90 (heat shock protein 90). Inhibition of HSP90 leads to a reduction in levels of matured JAK2. Specific tyrosine kinase inhibitors prevent activation of downstream signaling cascades. Indicated are drugs that are currently in clinical trials for the treatment of MPNs. (see also <http://clinicaltrials.gov>)

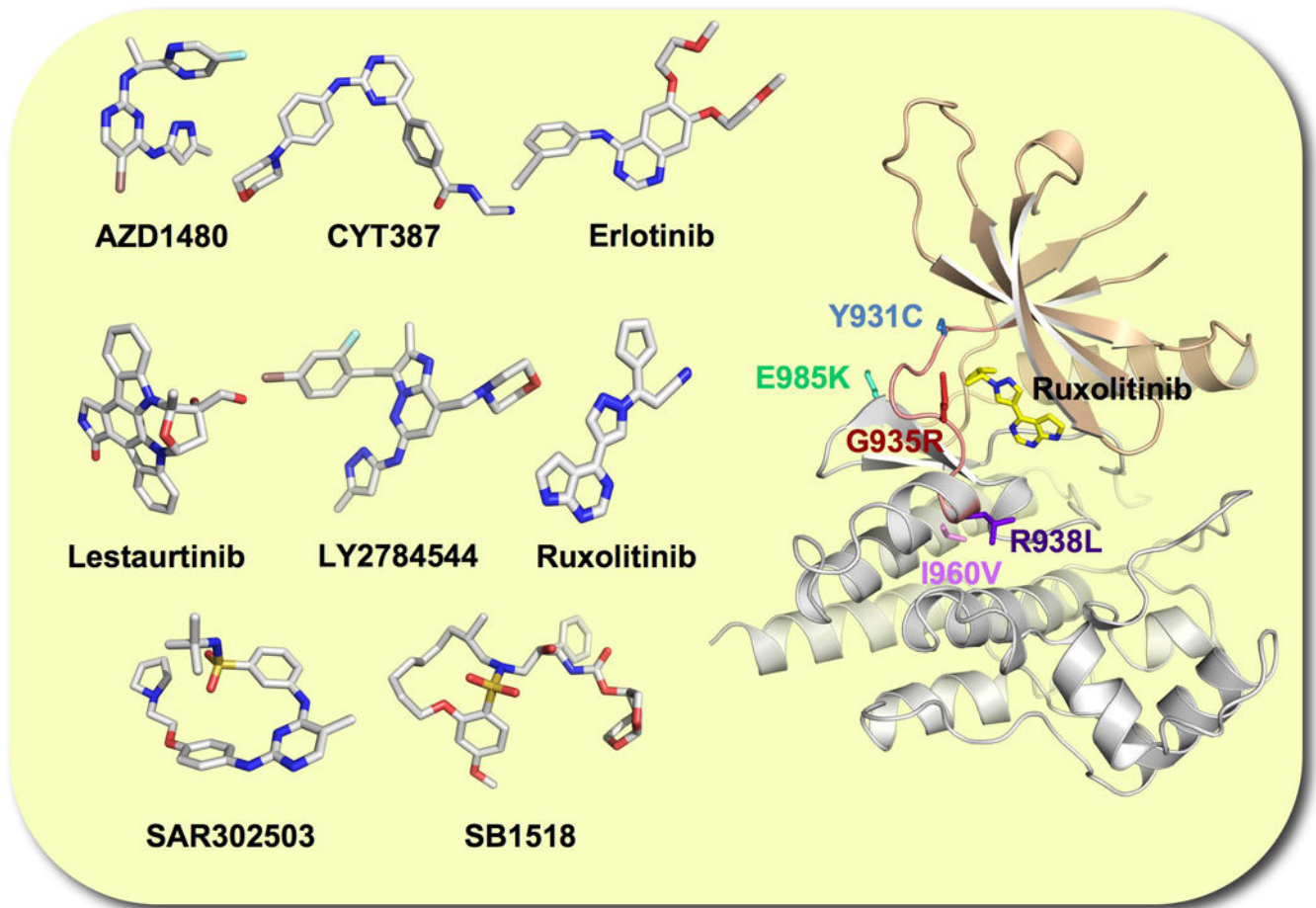
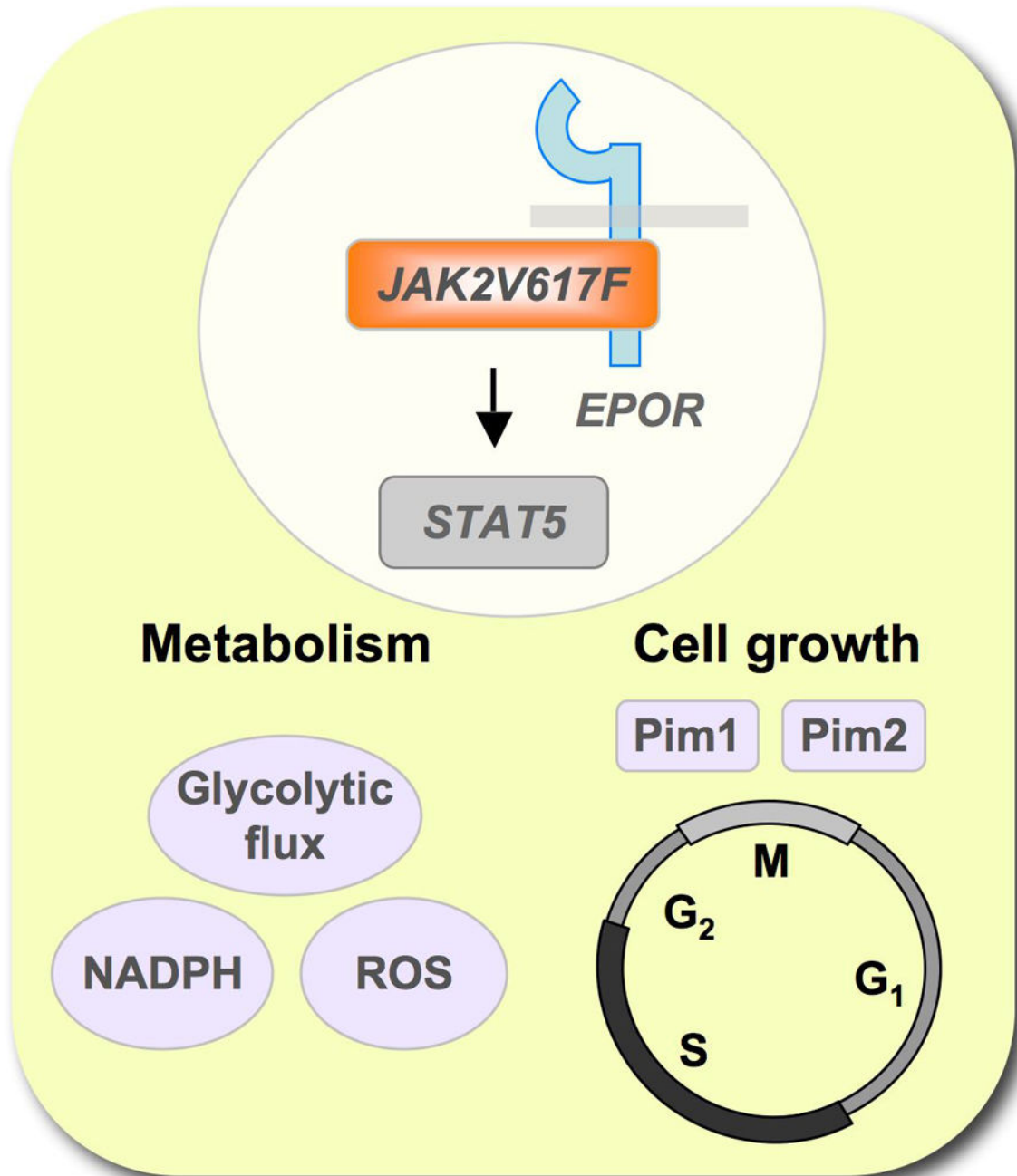


Figure 4. JAK2 tyrosine kinase inhibitors currently in clinical trials for the treatment of MPNs and tertiary structure of the JAK2 kinase domain containing drug resistant mutations



**Figure 5. The JAK2V617F/STAT5 pathway is a key regulator of growth and metabolism**  
 Signaling through JAK2V617F requires a functional interaction of the kinase with a cytokine receptor, such as the erythropoietin receptor (EPOR). Activation of the JAK2 target STAT5 is sufficient for increased cell growth (in part through Pim1 and Pim2) as well as increased metabolism (indicated by increased glycolytic flux or elevated ROS and NADPH levels).

**Table 1**  
**Inhibitors of JAK2 under clinical evaluation. (see also <http://clinicaltrials.gov>)**

Compound	Company	Additional Targets	Phase of study			
			I	II	III	IV
AZD1480	Astra-Zeneca	JAK1, JAK3				
CYT387	YM Biosciences	JAK1, TYK2, JNK1, CDK2				
Erlotinib	OsiPharma	EGFR				
Lestauritinib (CEP-701)	Cephalon	FLT3, TtkA/B/C				currently suspended
LY2784544	Eli-Lilly					
Ruxolitinib (INCB018424)	Novartis (Incyte)	JAK1				**
SAR302503 (TG101348)	Sanofi-Aventis (TargeGen)	FLT3, RET				
SBI518	S*Bio	FLT3				
XL019	Exelixis					early termination

\*\* Ruxolitinib was approved by the FDA (U.S. Food and Drug Administration) for the treatment of intermediate or high-risk myelofibrosis in November 2011.