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A Structure-Function Perspective of Jak2 Mutations and Implications for Alternate Drug Design Strategies: The Road not Taken

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Abstract

Jak2 is a non-receptor tyrosine kinase that is involved in the control of cellular growth and proliferation. Due to its significant role in hematopoiesis, Jak2 is a frequent target for mutations in cancer, especially myeloid leukemia, lymphoid leukemia and the myeloproliferative neoplasms (MPN). These mutations are common amongst different populations all over the world and there is a great deal of effort to develop therapeutic drugs for the affected patients. Jak2 mutations, whether they are point, deletion, or gene fusion, most commonly result in constitutive kinase activation. Here, we explore the structure-function relation of various Jak2 mutations identified in cancer and understand how they disrupt Jak2 regulation. Current Jak2 inhibitors target the highly conserved active site in the kinase domain and therefore, these inhibitors may lack specificity. Based on our knowledge regarding structure-function correlations as they pertain to regulation of Jak2 kinase activity, an alternative approach for specific Jak2 targeting could be via allosteric inhibitor design. Successful reports of allosteric inhibitors developed against other kinases provide precedent for the development of Jak2 allosteric inhibitors. Here, we suggest plausible target sites in the Jak2 structure for allosteric inhibition. Such targets include the type II inhibitor pocket and substrate binding site in the kinase domain, the kinase-pseudokinase domain interface, SH2-JH2 linker region and the FERM domain. Thus, future Jak2 inhibitors that target these sites via allosteric mechanisms may provide alternative therapeutic strategies to existing ATP competitive inhibitors.

Keywords

allosteric inhibitors; Jak2 tyrosine kinase; mutations; MPN; structure-function

Introduction

Janus kinases (Jaks) are non-receptor tyrosine kinases, which play an important role in cytokine receptor signaling. The Jak family consists of four members; Jak1, Jak2, Jak3 and

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Tyk2. Jak1, Jak2 and Tyk2 are expressed ubiquitously, but Jak3 expression is restricted to myeloid and lymphoid tissues. Different cytokines activate different subsets of Jaks. One of the downstream substrates of the Jaks are the Signal Transducers and Activators of Transcription (STATs) and Jak-STAT signaling has been implied in the regulation of cellular growth and proliferation.

Jak-STAT signaling is highly regulated and any change in this controlled process can affect normal physiology. For example, Jak1 knockout mice die perinatally due to defects in signaling through a subset of cytokine receptors [1]. Jak2 has a non-redundant role in erythropoiesis, as the Jak2 knockout mice die embryonically at day 12.5 due to lack of definitive erythropoiesis [2, 3]. Jak3 knockout mice are viable, but have defects in lymphoid development and also present with Severe Combined ImmunoDeficiency (SCID) [4, 5, 6]. Mutations in Jak3 have also been seen in patients with autosomal SCID [7, 8]. Tyk2 knockout mice are viable, but exhibit defects in interferon and IL-12 signaling [9, 10]. Inhibition of Jak mediated signal transduction has been observed in diseases associated with the Human Papilloma Virus (HPV), Human cytomegalovirus (HCMV), *Leishmania donovani* and *Ehrlichia chaffeensis*. Jak inhibition has also been associated with tumor-related immunosuppression caused by the reduction of T-cell proliferation [11].

On the contrary, there has been mounting evidence for the occurrence of Jak mutations that cause constitutive activation in cancer. Specifically, Jak2 mutations have been identified in Acute Lymphoid Leukemia (ALL), Chronic Myelogenous Leukemia (CML), Myeloproliferative Neoplasia (MPN), lymphomas and myelomas. Constitutively active Jak2 has also been implicated in solid cancers of the breast, prostrate, head, and neck. While there are a wide variety of Jak2 mutations present in these disorders, it is important to understand the mechanism by which they lead to constitutive activation of the protein. As such, knowledge of the structure-function correlation for the various mutations will be useful in improvising the drug design strategies for better patient outcomes.

Current Jak2 inhibitors target the active site of the kinase domain and most of them are ATP-competitive. A major drawback in this design strategy has been in achieving specificity for Jak2 among the different Jak family members and also specificity for mutant Jak2 over the native form. However, such selectivity can be achieved by using allosteric inhibitors that are not ATP-competitive and bind to sites remote to the active site of the kinase. This approach has been successful in targeting kinases such as BCR-ABL, B-Raf, MEK, and Akt [12]. The focus of this review will be to analyze the structure-function aspects of the different Jak2 mutations and their implication in the relatively unexplored area of allosteric drug design for Jak2.

Jak2 Regulation

The protein structures of all four Jak family members share seven conserved Jak Homology (JH) domains encoding four major domains (Table 1). The C-terminal JH1 domain is a highly conserved kinase domain which contains the activation loop, primary phosphorylation sites (Y1007 and Y1008 in Jak2), and the ATP binding site (K882 in Jak2). JH2 is the pseudo kinase domain, which has similar structural properties to the kinase

domain, but lacks catalytic activity. The pseudokinase domain is a unique structural feature of the Jak family members. Adjacent to the JH2 domain is the SH2 (Src Homology 2)-like domain, whose function is not yet fully understood. At the N-terminus of the protein is the FERM domain (F for 4.1 protein, E for ezrin, R for radixin and M for moesin), which facilitates interactions between Jak2 and a number of cell surface receptors [13].

When there is no ligand bound to the receptor, Jak2 remains associated with the receptor in an inactive state. Once ligand binds, Jak2 is activated and it phosphorylates tyrosine residues on the intra-cellular tail of the receptor. Downstream effectors such as the STATs bind to the phosphorylated receptors and are subsequently phosphorylated by Jak2. Phosphorylated STATs dimerize and translocate into the nucleus where they activate the transcription of effector genes resulting in cell proliferation and differentiation [14]. While this paradigm of Jak2 activation is widely accepted, one key limitation of this model is the paucity of structural data in support of it.

Strict regulatory control of Jak2 signaling is important under two circumstances. First, in the absence of ligand, Jak2 kinase activity must be maintained at a basal level, which is characterized by an absence of the activation loop phosphorylation and low phospho-transferase activity. Second, following activation, the Jak2-mediated signaling processes must be stopped via appropriate spatial and temporal negative feedback mechanisms. Collectively, these two levels of control are achieved through both *cis* and *trans* mechanisms. At the *cis* level, the regulation is achieved by the allosteric interaction between various Jak domains and by the phosphorylation/dephosphorylation of some of the 49 different tyrosine residues that are distributed throughout the Jak2 protein. Phosphorylation of Y1007 in the activation loop is the initiating and also an essential event for Jak2 activation [15]. Another level of *cis* regulation is achieved by the autoinhibition of the pseudokinase domain (JH2) over the kinase domain (JH1). As such, the JH2 domain suppresses the basal kinase activity of Jak2 in the absence of cytokine stimulation. The JH2 domain inhibits the kinase activity non-competitively by decreasing the maximum velocity (V_{max}) of enzyme catalysis without changing its substrate affinity (K_m) [16]. Ligand binding to the receptor causes conformational changes in the receptor/Jak2 complex, which relieves the autoinhibition and allows for subsequent Jak2 activation.

Post Jak2 activation, trans regulation occurs via negative feedback loops. The Jak-STAT signaling pathway stimulates the expression of proteins involved in the negative feedback regulation, thus terminating the proliferative signals induced by the ligand. Suppressor of Cytokine Signaling (SOCS) is a major regulator in this feedback loop. SOCS proteins that are expressed in response to Jak-STAT signaling, bind directly to active Jak2 via the SH2 domain and inhibit it. Alternately, SOCS binding also facilitates UE3 ligase mediated proteasomal degradation of Jak2. Concurrent with the role of SOCS in Jak2 negative regulation, mutations in the SOCS1 gene have been identified in the classical Hodgkin Lymphoma (cHL). Other *trans* regulators include phosphatases such as SHP1 and SHP2. They inactivate Jak2 through the dephosphorylation of Tyr 1007. Additionally, Lnk, an SH2 (B3) adaptor protein, was identified as an important negative regulator of Jak2 in hematopoietic cells [17]. Adipocyte fatty acid binding protein (AFABP/aP2), which serves as a fatty acid sensor for Jak2, was also recently identified as another negative regulator of

Jak2 [18]. According to this report, when fatty acid levels are high in the cell as in the case of obesity, the AFABP/aP2 binds to and attenuates Jak2 kinase activity.

Jak2 mutations in Myeloproliferative Neoplasms (MPNs)

Deregulation of Jak2 kinase activity is a common event in various types of cancer, especially in hematological malignancies such as the BCR-ABL negative myeloproliferative neoplasms (MPNs). These are a class of stem cell derived hematological disorders include Polycythemia Vera (PV), Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF). They are clinically characterized by the presence of increased red blood cell, platelet and granulocyte counts along with bone marrow fibrosis, respectively [19]. MPN patients also bear a risk of leukemic transformation in the long term. William Dameshek first identified MPNs in 1951, but the molecular mechanism for the dysfunctional hematopoiesis in these patients remained unknown for over 50 years. In 2005, several research groups independently reported a Jak2 V to F substitution at amino acid 617 in a large percentage of MPN patients [20, 21, 22, 23, 24]. The V617F mutation occurs somatically in hematopoietic stem cells and leads to cytokine independent, constitutive Jak-STAT signaling. Following the identification of Jak2-V617F in MPN, there have been several reports of its occurrence in other hematological disorders including leukemia, lymphoma and myeloma (Table 1). Apart from the V617F mutation that occurs in exon 14, Jak2 mutations have also been found in exons 12 - 15 in patients presenting with various types of hematological disorders. These mutations deregulate the Jak-STAT signaling pathway and skew the proliferation and differentiation of hematopoietic stem cells during hematopoiesis. However, the mechanism(s) by which these different Jak2 mutations confer constitutive activation and understanding how any differences can be exploited for drug development have not been examined.

The Jak2-V617F Era

The identification of the Jak2-V617F mutation in 2005 stimulated research in various directions leading to approximately 700–800 publications solely regarding Jak2-V617F over the past 6 years. This mutation is found in at least 95% of PV patients and about 50% of all ET and PMF cases [19]. Investigations from a clinical point of view have focused on studying the occurrence of the Jak2-V617F mutation in other hematological disorders apart from MPNs, the development of diagnostic tools that can be used to screen for the V617F mutation in patients, and in the identification of novel Jak2 mutations in MPN patients. Microarray studies have also been conducted using patient samples in order to identify changes in the gene expression pattern induced by Jak2-V617F [25]. Furthermore, the gene expression pattern of Jak2-V617F-positive ET patients has been found to be different from that of Jak2-V617F-negative patients [26].

One challenging question that arose as a consequence of the discovery of V617F was, how can one mutation, that is, the Jak2-V617F lead to three different disorders; namely, PV, ET, and PMF? To address this issue, several cases have been presented [27]. Since the Jak2 V617F is found to be homozygous in PV patients and heterozygous in ET patients, it is argued that gene dosage may be a determining factor. Mouse models developed for MPN

also support this case [28]. Another possibility is the presence of additional mutations in genes apart from Jak2, which in combination with V617F may determine the specific pathology. Further, germline genetic variations may also be responsible for the similar, yet distinct phenotypes observed clinically among the Jak2-V617F positive patients. For example, in 2009, three independent groups reported a predisposition allele called the “46/1” haplotype which explained the higher occurrence of the Jak2-V617F mutation among first-degree relatives of MPN patients [29, 30, 31].

Another line of current research includes the understanding of the molecular mechanism for the constitutive activation of Jak2-V617F. The point mutation at Val 617 occurs in the auto-inhibitory pseudokinase domain (JH2) of Jak2 and therefore allows the kinase to evade negative *cis* regulation (Figure 1). Kinetic studies of Jak2-V617F have shown that although its V_{max} is similar to that of Jak2-WT, it has a lower K_m and hence an increased affinity towards substrates leading to hyperactivation [32]. Interestingly, an earlier report had shown there was no difference in the enzyme kinetics of Jak2-V617F when compared to Jak2-WT [33]. Nevertheless, *in vitro* assays reported by several groups have demonstrated that Jak2-V617F is hyperkinetic [23, 34, 35] and that the FERM and SH2 domains are required for its function [36, 37]. These reports have shown that the FERM domain is important for the association of Jak2-V617F with the erythropoietin receptor (EpoR), while the SH2-like domain is required for cross-phosphorylation and aggregation in the receptor complexes at the membrane. This in turn implies that the constitutive activation of Jak2-V617F is mainly at the level of relieving the autoinhibition of the pseudokinase domain over the kinase domain. The V617F mutation by itself does not confer independence from the functions of other Jak2 domains. However, in order to obtain an understanding as to how a point mutation causes constitutive activation, more information at the level of protein structure is needed. Since a full length Jak2 crystal structure does not exist, homology models and molecular dynamic simulations have been useful in predicting the mechanism of these mutations [34, 38, 39]. In particular, the creation of full-length homology models and the initial descriptions of novel structure-function relationships of the different domains by Kroemer and colleagues has been the starting point in this area of research [40, 41].

The homology model of Jak2 JH1 and JH2 domains indicated that the activation loop in the kinase domain and V617 in the pseudokinase domain could be involved in an autoinhibitory interaction [40]. Recently, using molecular dynamic simulations and mutagenesis studies *in vitro*, our group along with that of Lee *et al.* and Dusa *et al.*, reported that the V617F mutation in the pseudokinase domain (JH2) of Jak2 interacts with the neighboring F595, which in turn reduces the JH1-JH2 autoinhibitory interaction at the JH1/JH2 interface and leads to constitutive activation [34, 35, 38]. However, at the *trans* level of regulation, several negative feedback regulators such as the SOCS and Lnk are still activated. Thus, one wonders how does the Jak2-V617F mutant protein escape the inhibitory effect of these *trans* regulators? Jak2-V617F seems to evade this negative regulation by hyper methylating the SOCS3 promoter thereby making it insensitive to STAT binding or hyper phosphorylating the SOCS3 protein thus making it more sensitive to degradation [42, 43]. Additionally, an HDAC inhibitor, in combination with a Jak2 inhibitor, has been shown to reduce Jak2-V617F signaling *in vitro* [44]. Thus, gene suppression through epigenetic modifications

could also be a possible explanation for the resistance of Jak2 V617F against the cellular regulators of signaling.

Identification of Jak2-V617F and availability of the crystal structure for Jak2 kinase domain has also opened the doors to the development of small molecule inhibitors for Jak2. Several mouse models for MPNs that have been created as either transgenic or knock-in models using the V617F allele [28, 45, 46] have been very useful in testing the broad range of Jak2 inhibitors that are being developed for use in the MPN patients. Apart from V617F, additional mutations in the exon 14 region that cause constitutive activation of Jak2 in human diseases include C618R and D620E in PV, E627E and C616Y in unclassified MPN, L611S in ALL, ΔIREED in Down syndrome, K607N in AML, and R683G in Down Syndrome-ALL (Table 1).

Jak2 Exon 12 mutations

In 2007, Scott *et al.* reported the occurrence of exon 12 mutations in Jak2-V617F negative MPN patients [47]. There are at least 20 different exon 12 mutations that have been reported to date, which include single or multiple point, insertion, deletion and duplication mutations (Table 1). These mutations are found almost exclusively in PV.

The exon 12 mutations are located structurally in the linker region located between the SH2 domain and pseudokinase domain (Figure 1). Functionally, this linker has been implicated in relaying the engagement of cytokines to the extracellular portion of the receptor with the relief of JH2 mediated autoinhibition. Therefore, it is thought that exon 12 mutations lead to constitutive activation of Jak2 by altering the interaction between JH1 and JH2 domains similar to that of Jak2-V617F. Molecular dynamics simulation studies conducted for the various Jak2 exon 12-15 mutations show that in most of the clinically occurring mutations, the JH1-JH2 interface is open indicating disruption of JH2-mediated autoinhibition and subsequent constitutive activation [39].

The 46/1 Jak2 haplotype has been found to predispose patients to both Jak2-V617F and exon 12 mutation positive MPNs [29, 48]. On the other hand, Jak2-V617F positive PV patients have a distinct clinical phenotype from that of PV patients carrying the exon 12 mutations. While patients with exon 12 mutations have isolated erythrocytosis, Jak2-V617F positive patients present a trilineage pattern of erythrocytosis, leukocytosis and thrombocytosis. PV patients with Jak2 exon 12 mutations have higher hemoglobin levels, lower erythropoietin levels, lower platelet counts, and lower leukocyte counts than Jak2-V617F positive PV patients [49]. Additionally, mitotic recombination leading to homozygosity is more likely to occur in Jak2-V617F-positive PV patients than those that carry the exon 12 mutations. This indicates that even though both Jak2-V617F and exon 12 mutations are constitutively active, their downstream signaling pathways are different, which eventually leads to variation in their pathophysiology and clinical phenotypes. Accordingly, Zou *et al.* reported that the catalytic activity, downstream signaling, ability to bind the erythropoietin receptor and the transforming properties of Jak2-V617F are significantly higher when compared to the exon 12 mutant, Jak2-K539L [50]. Contrary to this report however, Scott *et al.* reported that exon 12 mutants including Jak2-K539L have increased

Jak2 signaling when compared to Jak2-V617F [47]. Interestingly, there are no apparent differences in the assay conditions used by these two groups.

While Jak2-V617F and exon 12 mutations are predominant in MPN patients, Ma *et al.* reported that Jak2 mutations also occur in exons 13 and 15 [51] (Table 1). This indicates that the pseudokinase domain serves as a hotspot for mutations that lead to constitutive Jak2 activation. Based on the antisymmetrical interface between the kinase and pseudokinase domains, most of the Jak2 mutations identified to date occur in the N-lobe of the pseudokinase domain, which participates in the autoinhibition of the C-lobe of the kinase domain.

Jak2 chromosomal translocations

The first evidence for Jak2 constitutive activation in human cancers came in 1997 when the TEL-Jak2 fusion gene was identified among leukemia patients [52, 53]. The chromosomal translocation occurred between ETV6/TEL located on 12p13 chromosome and Jak2 on 9p24, resulting in a fusion gene that contains the N-terminal dimerization domain of TEL and the C-terminal kinase domain of Jak2. The chimeric protein exhibits constitutive kinase activation mediated by increased homodimerization. Since then, at least 14 different chromosomal translocations involving the Jak2 gene have been identified in different hematological malignancies, including both myeloid and lymphoid disorders (Table 2).

Chromosomal translocations between the B-cell Receptor (BCR) and Jak2 genes have been reported in atypical Chronic Myeloid Leukemia (aCML) and Acute Myeloid Leukemia (AML) [54, 55]. The human autoantigen pericentriolar material 1 (PCM1) gene has also been implicated as a fusion partner of Jak2 in atypical CML, AML, Chronic Eosinophilic Leukemia (CEL), PMF, unclassifiable Myelodysplastic/Myeloproliferative neoplasm (MDS/MPN), precursor B- Acute Lymphoblastic Leukemia (B-ALL) and T-cell lymphoma [56, 57, 58, 59]. Nebral *et al.* reported an ALL fusion of Jak2 with the N-terminus of the Paired box protein 5 (PAX5), a master regulator of B-cell development [60, 61]. The DNA binding domains along with the nuclear localization signal of PAX5 are fused with the Jak2 kinase domain. Therefore, the chimeric PAX5-Jak2 is located in the nucleus and is capable of binding to the wild type PAX5 targets. Interestingly, the DNA binding domain of PAX5 has not been shown to mediate dimerization and the kinase activity of the PAX5-Jak2 has not been examined. Therefore, the effect of the fusion partner on Jak2 activity cannot be predicted. Based on the recent findings of histone phosphorylation by Jak2 in the nucleus [62], it is possible that the PAX5-Jak2 affects gene expression in ALL by altering the status of histone phosphorylation. This would be interesting to determine as other PAX5 fusions such as PAX5-TEL and PAX5-ELN have been shown to act as transcriptional repressors via a dominant negative mechanism on wild type PAX5 [63]. Fusion of the Single strand DNA binding protein 2 (SSBP2) with Jak2 has been reported in precursor B-ALL [64]. Recently, a novel SEC31A-Jak2 fusion was identified in classical Hodgkin Lymphoma (cHL) and shown to constitutively activate the Jak-STAT signaling pathway [65]. Interestingly, in this case the constitutive activation of Jak2 did not depend on the WD40 repeats or the proline rich domain in the SEC31A fusion partner, which facilitates protein-protein interactions. The role of the required domain of the SEC31A partner has not yet been identified. Apart from

the Jak2 fusion partners described above, NF-E2, RUNX1 (AML1) and RPN1 have also been identified as putative partners involved in chromosomal translocation with Jak2 [66, 67]. Six additional chromosomal translocations involving Jak2 have been reported, but the fusion partners have not been identified [68, 69]. The functional and molecular characterization of these chimeric proteins may reveal their role in the tumorigenesis of the respective cancers.

The occurrence of Jak2 fusion genes in hematological malignancies is relatively rare when compared to that of the point mutations. While point mutations disrupt auto regulation and lead to ligand hypersensitivity in MPNs, they are generally not sufficient to cause a more deleterious phenotype such as leukemia. However, the clinical phenotypes of Jak2 fusion genes are very aggressive and have rapid progression to blast phase [70]. Further, in most cases of Jak2 fusion, there is a common pattern of an N-terminal dimerization domain being fused with the C-terminal Jak2 kinase domain. Yet, the different fusion genes confer different clinical phenotypes. This may be ascribed to the variability in the site of Jak2 fusion (Figure 2). Given the role of the different JH domains on Jak2 kinase activity, the variation in the clinical phenotypes observed might correlate with the Jak2 domains included in the chromosomal translocation. For example, in the case of the ETV6/TEL-Jak2 fusion found in ALL and T-cell lymphoblastic leukemia, a partial JH2 domain is present along with the entire JH1 domain. However, in the case of ETV6/TEL-Jak2 fusion present in AML, complete JH1 and JH2 domains are present. Further, the N-terminus domains from the different partner genes may influence Jak2 kinase activity by variant mechanisms of regulation. Also, the fusion mutations may occur in different types of somatic cells. Hence, the observed phenotype may vary based on the level of kinase activity of the chimeric protein and also the nature of the cells in which they occur.

Lack of Mutations in the Jak2 Kinase domain

In the case of kinases such as B-Raf, RET, FLT3, KIT and EGFR, the kinase domain serves to be a hotspot for somatic mutations that cause constitutive activation in cancers. Such mutations generally occur in the ATP binding region, catalytic region, activation loop and P-loop of the kinase domain. These mutations cause constitutive activation by destabilizing the inactive conformation of the kinase. However, in Jak2 only a single substitution mutation, T875N, in the kinase domain has been shown to cause MPN in murine models [71]. The Jak2-T875N mutation occurs in the N-terminal lobe of the kinase domain and is predicted to cause constitutive activation by altering the surface properties of the domain and by disrupting the autoinhibition of the JH2 domain over the JH1 domain. Further, a few other kinase domain mutations have been identified in ch-B-ALL (Table 1). This overall rare incidence of kinase domain mutations indicates a rather tight control of Jak2 kinase activity by other *cis* acting JAK homology domains, which are difficult to disrupt by mutations in the kinase domain itself. Keeping this in mind, it is important to understand that current Jak2 small inhibitors target the Jak2 kinase domain, which does not carry these gain-of-function mutations. Therefore, inhibiting Jak2 kinase activity via the selective targeting of JAK regulatory domains may serve to improve the therapeutic index and specificity of the drugs.

Need for Alternative Approaches to ATP mimetic Jak2 Inhibitors

Current Jak2 kinase inhibitors are categorized as either class I or class II drugs. Class I drugs were developed specifically for Jak2 based on available protein structural information. Class II drugs are those that were developed against other kinases, but were found to have beneficial off-target effects on Jak2. Based on the currently available results from Jak2 inhibitor clinical trials, the benefits of these drugs include improvement of splenomegaly, pruritus, erythrocytosis, leukocytosis and thrombocytosis [72, 73]. Though these drugs have positive effects in terms of reducing symptomologies associated with the MPN phenotype, they are unable to hinder the deleterious fibrosis or significantly reduce the mutant-allelic burden in the bone marrow. Also, toxicity, in the forms of anemia, nausea, vomiting and diarrhea, has been observed in these patients.

The current design of Jak2 selective inhibitors is based on structural information available from homology models or crystal structures for the wild type Jak2 kinase domain. Most of these inhibitors target the ATP-binding pocket in the kinase domain and hence are ATP-competitive. ATP-competitive inhibitors bind tightly to the kinase domain in its active conformation and prevent ATP binding or hydrolysis, thereby reducing kinase activity. The kinase domains of proteins from different kinase families have distinct conformations in their inactive state. However, upon activation, they assume a very similar active state conformation [74]. Targeting the active kinase conformation may provide increased sensitivity towards constitutively active mutant Jak2. On the other hand, this strategy may also result in non-specific effects through action on Jak2-WT and other important kinases that are active in the cell. Accordingly, results from the clinical trials reveal that treatment with current generation ATP-competitive Jak2 inhibitors results in myelosuppression and anemia. This could be a possible effect of these drugs on wild type Jak2, which plays an important role in normal hematopoiesis. Although it would be premature to judge the general success or failure of existing ATP-competitive drugs, the results clearly indicate the need for specific targeting of mutant forms of Jak2. Such specific ablation of Jak2 mutant activity would, in theory, reduce the mutant allelic burden and hence improve the therapeutic window. Apart from the issue of selectivity, development of drug resistance is also higher with the use of ATP competitive inhibitors. Using *in vitro* assays, it was shown that mutation of residues Y931, Y958 and P960 in the hinge region of the Jak2 kinase domain confer constitutive activation along with resistance to ATP-competitive Jak2 inhibitors, including INCB018424 [75]. These findings indicate the possibility of such secondary mutations occurring in patients during prolonged treatment with ATP-competitive inhibitors. Therefore, in order to gain specificity and avoid drug resistance, alternative strategies such as allosteric inhibitor design, should be considered.

Current Allosteric Inhibitors for Kinases in Cancer

Since the role of kinases in cancer has become more obvious in the recent years, kinases have become attractive targets for inhibition. An important part of drug design against kinases includes the selection of the drug binding site that would achieve high target specificity. Additionally, the nature of interactions between the inhibitor and the target atoms at the binding site would determine its binding affinity and potency. Therefore, the active

site, where the phosphate from ATP is catalytically transferred to the substrate, would be an ideal targeting site for kinases. However, the catalytic activity of kinases can also be moderated allosterically from sites that are remote to the active site. Binding of a drug to the allosteric site can therefore moderate the catalytic activity indirectly through a network of residues that connects to the active site, without directly competing with the ATP.

There are four general types of kinase inhibitors based on their binding site and mode of inhibition: ATP-competitive inhibitors (type I), inhibitors that bind to the inactive kinase conformation (type II), allosteric inhibitors, and covalent inhibitors [76]. Of these, type II and allosteric inhibitors provide high kinase selectivity. Selective targeting of Jak2 is important because of its critical role in hematopoiesis, for which the allosteric inhibitors may be suitable. The term “allostery” means “different shape”. Allosteric inhibitors are non-ATP competitive and inhibit kinase activity by binding to a site that is remote to the active site. This approach can help achieve target specificity and increased drug efficacy. There are several examples in the literature for such non-ATP competitive inhibitors that are being currently tested for treatment in various cancers. A review of these drugs may provide perspective for a similar design against Jak2 in hematological malignancies.

BCR-ABL

Despite having good efficacy, the first generation inhibitors for BCR-ABL, such as imatinib, induced secondary mutations in the kinase that resulted in drug resistance. These mutations mostly occur in the P-loop and alter the inactive conformation of the BCR-ABL kinase domain, to which the imatinib binds preferentially. Consequently, more potent second-generation inhibitors, such as dasatinib, nilotinib and bosutinib, were developed [77]. Both the first and second generation of BCR-ABL inhibitors bind to the active site of the kinase domain. While imatinib has been shown to be non-ATP competitive, it still binds to the inactive kinase near the active site. A BCR-ABL T315I mutation, which disrupts the gatekeeper residue of the kinase domain, is resistant to both first and second-generation inhibitors. Henceforth, several alternative approaches, such as the development of non-ATP competitive inhibitors, are being pursued to combat this resistance mutation. Current allosteric inhibitors for BCR-ABL target three regions that are remote to the active site:

1. *Myristoyl binding cleft:* Since myristoylation has been known to stabilize ABL in its inactive conformation, the binding of inhibitors to this pocket in the ABL kinase domain are predicted to have the same effect. GNF-2 is a lead compound that binds to this cleft and allosterically inhibits BCR-ABL kinase activity (Table 3). GNF-2 has a 4,6-disubstituted pyrimidine core structure, which is essential for its activity and it binds to the cylindrical myristate-binding cavity of the Abl kinase domain in a trans conformation. The 4-trifluoromethoxyaniline group interacts at the base of the pocket and is essential for activity. However, substitutions at the pyrimidine 6-positions can be tolerated since they are exposed to the solvent [78]. The selectivity of these compounds for BCR-ABL is exceptional and they also have significant *in vivo* efficacy when used in combination with imatinib or nilotinib [79]. Therefore, GNF-2 may have

good clinical efficacy, reduced drug resistance, and decreased toxicity due to its high selectivity.

2. *Switch pocket inhibitors:* DCC-2036 is an inhibitor that binds to a switch control pocket in the BCR-ABL kinase domain and locks it in the inactive conformation [80]. During the active state, R386 in the Abl1 kinase domain stabilizes the phosphorylated Y393 in the activation loop. However, in the inactive state R386 forms a salt bridge with E282 under the αC-helix. Therefore, DCC-2036 was designed with a tetrahydro-isoquinoline ring that hydrogen bonds to both E282 and R386 in order to target the inactive Abl1. Further, DCC-2036 also has a carboxamide-substituted pyridine ring, which hydrogen bonds with the backbone of the ATP hinge residue M318 as a second docking site in order to have increased binding energy and potency. In preclinical studies, this drug has shown good oral bioavailability, limited off-target effects, and excellent safety. It is currently being evaluated in phase I/II clinical trials for the treatment of imatinib-refractory CML.

3. *Substrate-competitive inhibitors:* ON012380 is an inhibitor that binds to the substrate-binding site of the ABL kinase domain and inhibits it at least ten-fold more potently than imatinib [81]. ON012380 was designed to have a structure that is unrelated to ATP or any other purine or pyrmidine nucleosides such that it blocks the substrate binding site rather than the ATP binding site. This inhibitor has good efficacy and reduced toxicity in murine models. However, the activity of ON012380 in clinical trials has not been tested.

Similar to BCR-ABL, allosteric inhibitors are also being developed for other kinases including Akt and various MAP kinases such as BRAF and MEK [82, 83, 84, 85]. Another leading example of a drug that does not target the kinase domain is the rapamycin derivative, temsirolimus. It is an FDA approved anti-cancer drug that binds to the FKBP12/Rapamycin Binding (FRB) domain of the mTOR kinase and inhibits its function [86].

Thus, allosteric inhibitors have proven useful for improved selectivity and decreased toxicity resulting in improved clinical efficacy. These drugs also serve as good alternatives to ATP-competitive inhibitors that can sometimes lead to drug resistance. Therefore, lessons learnt from these examples may guide the development of Jak2 allosteric inhibitors which can act as suitable alternatives to the existing class of ATP mimetic inhibitors.

Allosteric Inhibitors for Jak2

While there are a number of ATP-competitive inhibitors for Jak2, to date, there are only two known non-ATP competitive inhibitors for this target.

LS104—LS104 is a hydroxystyryl-acrylonitrile compound, which is an analog of a non-specific Jak2 inhibitor, AG490. It has increased affinity and specificity for Jak2 when

compared to AG490. LS104 has an elongated linker between the nitrile and 3,4-dihydroxyphenyl groups. It inhibits the growth of leukemic cell lines and primary cultures obtained from leukemia patients [87] (Table 3). It has been shown to inhibit BCR-ABL, Jak2, and FLT3, but does not have any effect on Src family kinases [88]. LS104 inhibits the Jak2 kinase activity, signaling distal to Jak2, and selectively induces apoptosis in Jak2-V617F transformed cells, *in vitro.* The combination of LS104 with ATP-competitive inhibitors induced apoptosis in Jak2-V617F transformed cells, synergistically. It prevents cytokine-independent colony growth of bone marrow cells derived from MPN patients, *ex vivo*. LS104 is predicted to be a substrate competitive inhibitor. However, the crystal structure of LS104 with Jak2 is not available to confirm its binding mode. Additionally, its clinical efficacy is yet to be determined.

ON044580—ON044580 is an α-benzoyl styryl benzyl sulfide, which is shown to inhibit the growth of leukemic cell lines and Jak2-V617F transformed cells [89]. Similar to ON012380, ON044580 was also designed to have a structure that is unrelated to ATP or any other purine or pyrimidine nucleosides such that it would not be ATP-competitive and still possess kinase inhibitory activity. ON044580 inhibits both Jak2 and BCR-ABL in a non-ATP competitive manner. It is predicted to be substrate-competitive inhibitor. Both BCR-ABL and Jak2 share a common substrate, STAT5, which in turn supports the substrate inhibitor hypothesis. ON044580 also inhibits the imatinib-resistant BCR-ABL-T315I mutant with higher sensitivity. Moreover, it was observed that ON044580 leads to the degradation of BCR-ABL, Jak2 and STAT5 possibly by destabilizing their multi-protein complex [90]. Interestingly, the Jak2 pseudokinase domain was found to be required for complete kinase inhibition by ON044580. This further suggests that ON044580 could be binding to Jak2 in its inactive conformation and that the pseudokinase domain is required to maintain the inactive form. No crystal structure information is available regarding the interaction of ON044580 with Jak2 or BCR-ABL so its exact binding mode is not known. This drug also effectively inhibits the growth and induces apoptosis in *ex vivo* cultures of bone marrow cells from patients in different stages of leukemia and monosomy 7 myelodysplastic syndrome [89]. Jak2 signaling is constitutively active in both these conditions and therefore, it is proposed that ON044580 could be used to treat both MDS and MPN.

Collectively, LS104 and ON044580 are two known allosteric inhibitors for Jak2, which effectively down regulate Jak2-dependent, transformed cell growth. Further studies are required to determine their binding interactions with Jak2, selectivity for Jak2 mutant over wild type, relative toxicity, and drug resistance. This information will be useful to perform a comparative analysis with their ATP-competitive counter parts and identify the benefits of allosteric inhibitor development for Jak2.

Possible targets on the Jak2 surface for Allosteric Inhibition

Jak2 is a multi-domain tyrosine kinase, which is made up of 1132 amino acids, weighs approximately 130 kDa and occupies a volume of about 158,000 cubic angstroms. Regulation of Jak2 activity can be achieved using different levels of coordination among its domains. This aspect will be useful in identifying allosteric pockets that could be targeted for Jak2 inhibition. During the development of ATP-competitive Jak2 inhibitors for instance,

the target pocket is usually defined as that surrounding the ATP-binding pocket. However, in order to develop allosteric inhibitors for Jak2, it would be necessary to identify druggable pockets on the Jak2 surface that are remote to the active site. Exploring the vast surface area of Jak2 to identify such pockets may seem like a daunting task. However, this task is feasible when the recent advancements in molecular docking are combined with available Jak2 structure-function relationship data. The contribution of structural biology to the development of inhibitors, specifically for kinases, is notable and has been discussed in detail elsewhere [86]. Based on the available structural information, possible areas for allosteric targeting in Jak2 are discussed below.

Jak2 kinase domain

The ATP-binding site in the kinase domain is currently being targeted for ATP-competitive inhibitors that bind to the active conformation of the kinase. However, targeting the pockets in the inactive kinase conformation may provide more Jak2 selectivity. In the inactive conformation, when the catalytic loop is in the DFG-out conformation, it blocks ATP binding and this in turn may result in distinct pockets adjacent to the ATP binding site (Figure 3). Specifically, the type II inhibitor pocket formed between the C-helix and the activation segment could be a possible target. A classic example for this is Gleevec, which preferentially binds to and stabilizes the inactive conformation of BCR-ABL [91]. The crystal structure of the inactive form of Jak2 is not available and this information may be useful in identifying such pockets. Also, the Jak2 kinase domain has a unique insertion loop, which is not conserved in other kinases. This feature should be considered during the design of Jak2 allosteric inhibitors. Additionally, considering the case of LS104, targeting the substrate-binding site of Jak2 may also serve as an effective strategy for inhibition. There is also precedent for a peptide inhibitor, Tkip, which inhibits Jak2 by binding to the activation loop in its autophosphorylated state similar to that of SOCS binding [92]. Thus, targeting regions apart from the ATP binding site may also result in effective Jak2 inhibition through an allosteric mechanism.

Autoinhibitory Interface between the Kinase and Pseudokinase Domains

Among the non-receptor tyrosine kinases, Jak2 has a unique architecture of having a pseudokinase domain located adjacent to the kinase domain. The JH2 domain is classified to be a pseudokinase due to the lack of tyrosine kinase activity and the absence of a conserved HRD motif, which is replaced by HGN [93, 94]. Saharinen and Silvennoinen first reported the role of the JH2 domain being an autoinhibitory domain over the JH1 kinase domain [95, 96]. They also showed that residues 758-807 in the JH2 domain are important for the autoinhibition, while residues 619-757 enhance this inhibition. Therefore, Jak2 activation in response to ligand binding involves the relief of the kinase domain from the autoinhibition of JH2, possibly via the phosphorylation of its activation loop. The molecular mechanism for the constitutive activation of Jak2-V617F involves the disruption of inhibitory interactions at this interface [34, 35, 38]. The autoinhibitory regulation of Jak2 makes it an attractive drug target since it involves conformational changes as a part of its functional cycle [97]. Therapeutically successful inhibitors that stabilize the auto-inhibited fold of proteins have been identified for other targets including BCR-ABL (imatinib) and N-WASP (wiskostatin).

Therefore, targeting Jak2 at its JH1-JH2 interface may stabilize the auto-inhibited form of the protein.

SH2-linker - JH2 interface

Function of the SH2-like domain located adjacent to the pseudokinase domain has not been well characterized. However, the linker connecting the SH2 domain to the pseudokinase domain has been implicated in Jak2 activation (Figure 3). Specifically, Zhao *et al.* reported that upon ligand binding, the SH2-pseudokinase linker flexes the hinge between the JH1 and JH2, thus leading to Jak2 activation [98]. Accordingly, several Jak2 exon 12 mutations that lead to constitutive Jak2 activation occur in the SH2-JH2 linker region. It is possible that these mutations are stabilizing the linker region in the 'ON' mode that is normally associated with Jak2 activation. However, this needs to be demonstrated experimentally. It is interesting to note that in another tyrosine kinase, ZAP-70, the linker between the SH2 and kinase domains auto-inhibits kinase activity by reducing the flexibility of the hinge region [99]. Targeting the pockets that might control conformational changes induced by the SH2-JH2 linker will be useful in regulating Jak2 kinase activity. Further, targeting different allosteric sites based on the location of the mutations may also provide mutant specific inhibition, as in the case of exon 14 vs. exon 12.

FERM domain

The Jak2 FERM domain has been known to be important for Jak-receptor association. Zhou *et al.* has shown that the Jak3 FERM domain physically interacts with the kinase domain and regulates both Jak3 receptor association and kinase activity [100]. Interestingly, in the case of Jak2, it was found that the FERM domain negatively regulates Jak2 wild type, but positively regulates Jak2-V617F kinase activity [32]. Further, phosphorylation of tyrosines in the Jak2 FERM domain has been reported to have both positive and negative regulatory effects on Jak2 kinase activity. Mutation of tyrosines 114, 119 and 317 in the FERM domain or deletion of the FERM domain itself has been shown to increase the kinase activity of Jak2-WT [36, 101, 102]. Recently, a potential activation mutation in the FERM domain, R340Q, was identified in Jak2-V617F negative MPN patients [103]. There is precedent for negative regulation through FERM domain in Focal Adhesion Kinase (FAK). The FAK kinase domain is auto-inhibited by the FERM domain via sterical blocking of the catalytic cleft, which is very similar to the predicted interaction between the JH1and JH2 domains in the JAKs [104].

Overall, Jak2 FERM domain seems to be regulating both Jak2 receptor association and kinase activity. Thus, targeting pockets in the FERM domain may help modulate Jak2 kinase activity. In this case, available information on the actual mode of FERM domain interactions with the receptor should be considered while assessing the pockets for targeting.

Identification of Druggable Allosteric Pockets using Virtual Screening

While the above-mentioned surfaces can be used as targets, identifying a druggable pocket within those surfaces that can bind a high affinity compound involves a different approach. Identification of lead compounds for targets using structure based virtual screening has

become popular in the past decade and it has proved very useful in drug discovery. Its high throughput nature allows for the screening of a large number of compounds in a fast and inexpensive manner, which is not feasible experimentally. Virtual screening involves the molecular docking of various inhibitors to a defined pocket on the target and ranking the ligands based on parameters such as binding energy, geometrical complementarity, and bonding interactions. The scoring function used to rank the compounds varies based on the docking program used, such as a force field-based function in DOCK, an empirical function in Glide and a shape based function in LigandFit [105]. Ranking of compounds using current docking programs is very crude and therefore a large number of compounds have to be tested biologically using high throughput screening methods in order to identify a possible lead compound. This limitation arises due to unreliable scoring methods, which sometimes fail to account for the conformational flexibility of the protein and hence do not reflect the nature of ligand-protein interactions accurately.

Due to a high degree of conservation among kinase structures, even in the absence of crystal structures for certain kinases, homology models are being used for virtual screening of inhibitors. For example, before the crystal structure of the Jak2 kinase domain was solved in 2006 [106], this method was employed for the development of ATP-competitive Jak2 inhibitors, In addition, given the vast amount of Jak2 surface available for targeting, the homology model can also be adopted for Jak2 allosteric inhibitor design. Identification of allosteric binding sites can be tricky and use of molecular dynamics may be required. Since allosteric sites are linked to remote functional sites indirectly, they can be identified using cooperative coupling methods. This may require a combination of approaches that are based on both sequence, as in the Statistical Coupling Analysis (SCA) and protein structure, as in the COREX algorithm [107]. This virtual screening process can in turn be guided by the available biochemical data on Jak2 structure-function relationships. Further, Haan *et al.,* has suggested a chemical genetics based approach to achieve more specificity while targeting Jak kinases [108].

Collectively, using allosteric inhibitor design as an alternative approach to ATP-competitive inhibitors for Jak2 may result in more effective inhibitors that are specific to mutant Jak2 and also prevent drug resistant mutations. Such allosteric inhibitor design can be guided by the existing information on Jak2 structure-function, though a crystal structure of full length Jak2 will certainly prove more useful.

Conclusion

The convergence of the identification of Jak2-V617F in MPNs and the recent developments in kinase targeting has catalyzed efforts on targeting Jak2 for the treatment of MPN. Many ATP-competitive Jak2 inhibitors that exhibited good pre-clinical results are now being tested in clinical trials. The most important ones will be those that can reduce the mutant allelic burden in the bone marrow. While these inhibitors may come to fruition, an alternative approach is to target Jak2 via allosteric design. Though the active conformation of protein kinases is highly conserved, ATP-competitive inhibitors can still be specific. The extended region of the drug that interacts outside the active site may provide specificity and significant effort has been expended to achieve such specificity using structure-activity

relationship (SAR) studies of lead compounds. Still, non-specific, off-target effects have been unavoidable in several cases. While such non-specificity has proved toxic during some treatments, it has also been deemed favorable in the combinatorial targeting of multiple kinases involved in tumorigenesis [109]. For Jak2 targeting, non-specific effects with other Jak kinases or even on wild type Jak2 should be avoided due to its critical role in hematopoiesis. Therefore, in cases like Jak2 where selectivity is required, alternative approaches such as allosteric inhibitor design may be useful. Since allosteric inhibitor design utilizes the principles of autoinhibition adopted by nature and applies it to synthetic inhibitors, its efficiency could be greater. Using a combination of the available structural information and improved molecular docking methodologies, druggable allosteric pockets on Jak2 surface and high affinity drugs for the target pockets can be identified. We have made an attempt to point out the possible surfaces for Jak2 allosteric inhibition in this review. However, a systematic dissection of the Jak2 surface using computational approaches will be required to identify druggable allosteric sites. Identification of the Jak2 allosteric sites would require the use of cooperative coupling methods and analysis of the geometrical and physiochemical properties of multiple pockets on the Jak2 surface in various conformations as identified using molecular dynamics simulations. Further, the predicted allosteric sites can be cross-referenced to available biological data from the Jak2 structure-function studies for validation. It is our hope that future research in the area of Jak2 drug development would focus on identifying and targeting the allosteric sites on Jak2 surface, in order to improve the specificity during inhibition. While no judgments can be made on the current ATP-competitive inhibitors for Jak2, allosteric inhibitor design may simply provide an alternative approach that could lead to selective and more effective inhibitors for MPN treatment. Moreover, a combination of allosteric and ATP-competitive inhibitors could prove more effective than the individual inhibitors alone.

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Abbreviations

Jak	Janus Kinase
JH	Janus Kinase Homology
STAT	Signal Transducers and Activators of Transcription
FERM	4.1 protein/ezrin/radixin/moesin
SH2	Src Homology 2
MPN	Myeloproliferative Neoplasms
PV	Polycythemia vera
ET	Essential thrombocythemia
PMF	Primary Myelofibrosis

SCID	Severe Combined Immunodeficiency
HPV	Human Papilloma Virus
HCMV	Human cytomegalovirus
ALL	Acute Lymphoid Leukemia
DS	Down Syndrome
CML	Chronic Myelogenous Leukemia
SOCS	Suppressor of Cytokine Signaling
cHL	classical Hodgkin Lymphoma
BCR	B-cell Receptor

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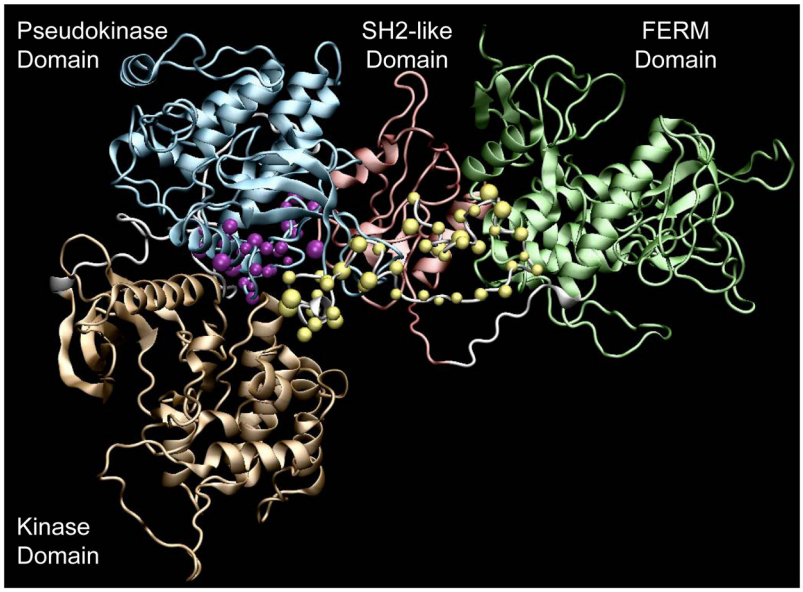


FIGURE 1. The Jak2 Protein Structure and Hotspots for Jak2 Mutations

Cartoon representation of the full length Jak2 homology model prepared using VMD 1.8.6. showing the kinase domain (tan), pseudokinase domain (blue), SH2-like domain (red) and the FERM domain (green). Also shown are the regions where most of the known Jak2 mutations occur; exon 14 (purple spheres) and exon 12 (yellow spheres).

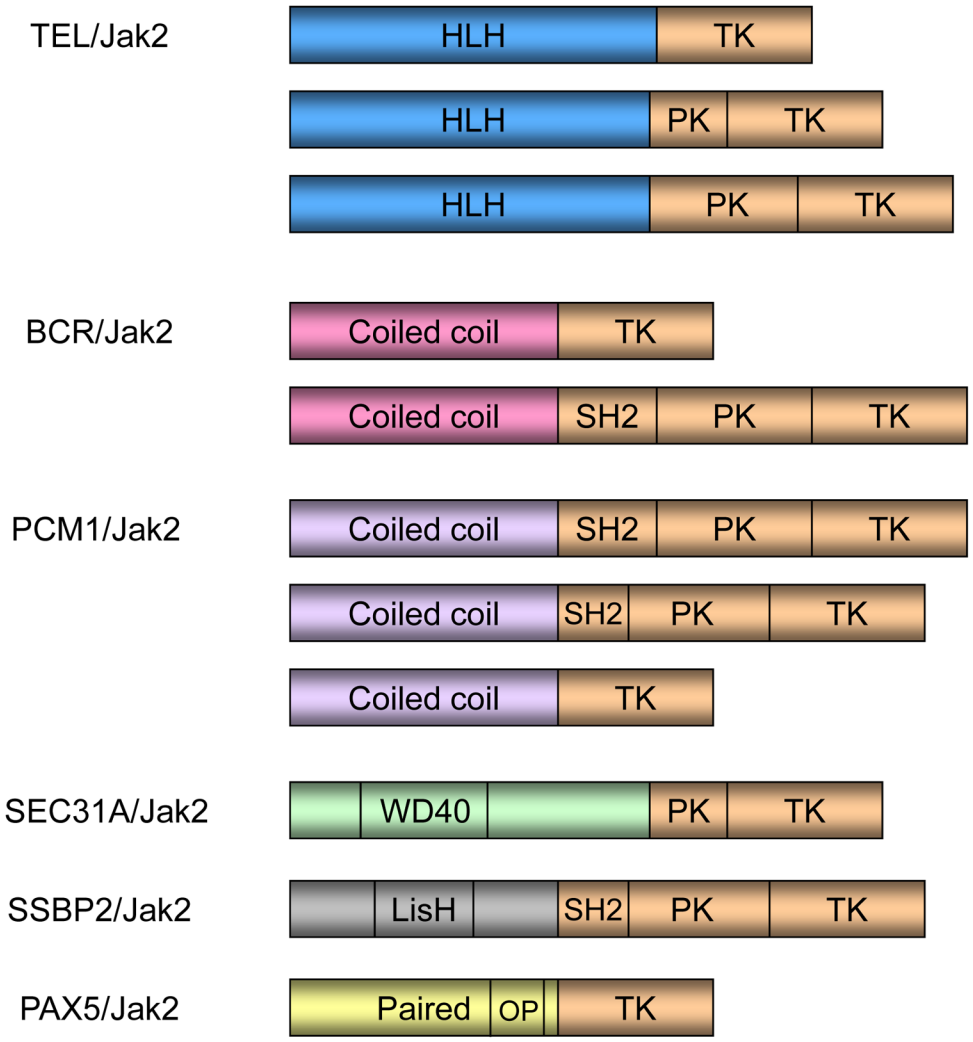


FIGURE 2. Jak2 Chromosomal Translocations

N-terminus domains from the different fusion partners are fused with the C-terminus of Jak2 including as a minimum the kinase domain (tan). OP-Octapeptide, WD40 - beta transducin repeat motifs terminating in W-D dipeptide, LisH - LIS1 homology domain, TK - Tyrosine kinase, PK - pseudo kinase, SH2 - Src Homology 2, HLH - Helix Loop Helix.

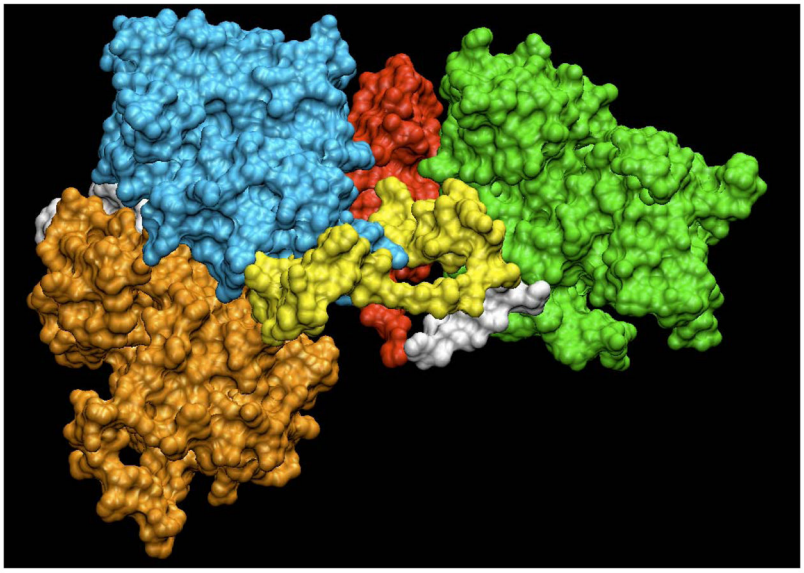


FIGURE 3. Possible Targets for Jak2 Allosteric Inhibition

Surface representation of a full length Jak2 homology model prepared using VMD 1.8.6. The kinase domain is shown in orange, pseudokinase domain in cyan, SH2-JH2 linker in yellow, SH2-like domain in red and the FERM domain in green. Other linker regions between the domains are shown in white.

TABLE 1

Jak2 Mutations that occur in various hematalogical disorders

EXON LOCATION (Amino acids encoded)	MUTATION	DISEASE	REFERENCE
Kinase domain (849-1123)			
Exon 20 (858–920)	R867Q	Ch-B-ALL	[110]
	D873N	Ch-B-ALL	[110]
	T875N	AMKL cell line	[71]
Exon 21 (921 – 962)	P933R	Ch-B-ALL	[110]
Pseudokinase domain (545-806)			
Exon 16 (665 to 710)	I682F		[110]
	R683G	DS-B-ALL	[110,111, 112, 113]
	R683S	DS-B-ALL	[110,111, 112, 113]
	RQIns683		[114]
	I682-D686 (IREED) del	DS-B-ALL	[51]
Exon 15 (623 to 664)	L624P	MPNs	[115]
	E627E	MPNs	[51]
	I645V	MPNs	[51]
Exon 14 (593–622)	H606Q	MPNs	[116]
	K607N	AML	[51]
	H608Y	MPNs	[117]
	L611S	Ch-B-ALL	[20–24, 116, 118, 119–122]
	V617F	MPNs, CMML, JMML, RARS, AML, MDS	[51]
	V617I	MPNs	[115, 123]
	D620E+V617F	MPNs	[124]
	C616Y+V617F	MPNs	[51, 125]
	C618R+V617F	MPNs	[126]
	Exon 14 del	MPNs	[51]
Exon 13 (548–592)	F557L	MPNs	[51]
	V567A	MPNs	[51]
	L579F	MPNs	[51]
	H587N	MPNs	[51]
	S591L	MPNs	[51]
	R564L, R564Q	MPNs	[51]
	G571S, G571R	MPNs	[51]
SH2-JH2 Linker (501-544)			
Exon 12 (501–547)	T514M	MPNs	[51]
	N533Y	MPNs	[51]
	F537I	MPNs	[127]
	K539L	MPNs	[47]

EXON LOCATION (Amino acids encoded)	MUTATION	DISEASE	REFERENCE
	L545V	MPNs	[51]
	F547V	MPNs	[128]
	F547L	MPNs	[51]
	H538QK539L	MPNs	[47, 128, 129]
	H538-K539del	MPNs	[51, 129]
	E543del	MPNs	[128]
	N542-E543del	MPNs	[47, 128, 129]
	E543-D544del	MPNs	[128, 129, 130]
	D544-L545del	MPNs	[129]
	F537-K539delinsL	MPNs	[47, 128, 129]
	H538-K539delinsL	MPNs	[128, 131]
	R541-E543delinsK	MPNs	[131, 132]
	F537-I546dup10+F547L	MPNs	[49, 128]
	547insLI540-F547	MPNs	[128]
	I540-E543delinsMK	MPNs	[128, 132]
	H538DK539LI540S	MPNs	[128, 129]
	I540-N542delinsS	MPNs	[128]
	F537-F547dup	MPNs	[128]
	V536-F547dup	MPNs	[128, 129]
	V536-I546dup11	MPNs	[49, 128]
	I540-D544delinsMK	MPNs	[51]
	N542-D544delinsN	MPNs	[51]
FERM domain (37-380)			
Exon 8 (313–352)	R340Q	MPNs	[103]

[a]Abbreviations used: Ch-B-ALL - childhood B-cell precursor acute lymphoblastic leukemia, DS- down syndrome, AML - acute myeloid leukemia, AMKL - acute megakaryoblastic leukemia, CMML - chronic myelomonocytic leukemia, JMML - juvenile myelomonocytic leukemia, MPNs - myeloproliferative neoplasms, MDS - myelodysplastic syndrome, RARS - refractory anemia with ringed sideroblasts.

TABLE 2

Jak2 Chromosomal Translocations

TRANSLOCATION	FUSION PARTNER	DISEASE	REFERENCE
t(9;12)(p24;p13)	TEL-Jak2	T-ALL, precursor B-ALL, atypical CML, MDS	[52,53,66]
t(9;22)(p24;q11.2), t(9;22)(p24;q11)	BCR-Jak2	atypical CML, AML	[54,55]
t(8;9)(p22;p24)	PCM1-Jak2	atypical CML, precursor B-ALL, CEL, AML, T-cell lymphoma, PMF, MDS/MPN	[56–59]
del(9)(p13:p24)	PAX5[b]	ALL	[60–61]
t(5;9)(p14.1;p24.1)	SSBP2	precursor B-ALL	[64]
t(4;9)(q21;p24)	SEC31A-Jak2	classical Hodgkin Lymphoma (cHL)	[65]
t(9;12)(p24;q13)	NFE2[c]	MDS	[66]
add (9) (p24) and del (21)(q11.2)	AML1 (RUNX1)[c]	MDS	[66]
t(3;9)(q21;p24)	RPN1 [c]	CIMF	[67]
t(9;22)(p24;q11.2)	Unknown	B-ALL	[68]
t(2;9)(p21;p24)	Unknown	PMF	[69]
t(8;9)(q22;p24)	Unknown	PMF	[69]
t(9;17)(p24;q23)	Unknown	PV	[69]
t(4;9)(q25;p24)	Unknown	PMF	[69]
t(8;9)(q13;p24)	Unknown	DLBCL	[69]

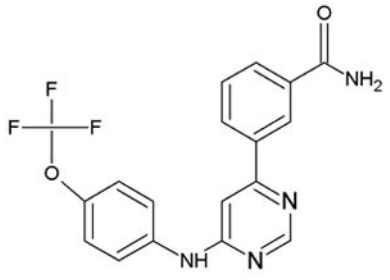
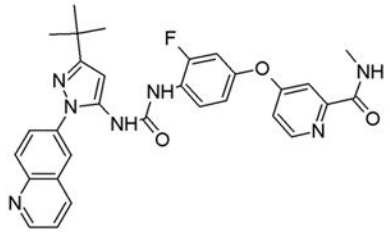
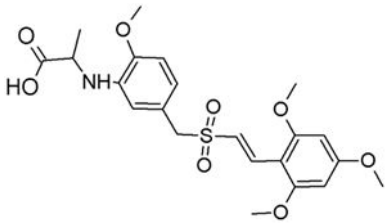
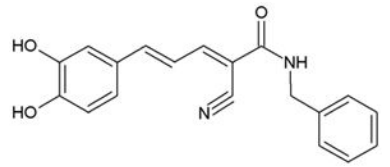
[a]Abbreviations: AML1 - acute myeloid leukemia 1, BCR - B-cell receptor, CML - chronic myeloid leukemia, CEL - chronic eosinophilic leukemia, cHL - classic hodgkin's lymphoma, CIMF - chronic idiopathic myelofibrosis, DLBCL - diffuse large B-cell lymphoma, ETV6 - Ets variant gene 6, NFE2 - nuclear factor erythroid-derived 2, PAX5 - paired box gene 5, PCM1 - Human autoantigen pericentriolar material 1, PMF - primary myelofibrosis, PV - polycythemia vera, RPN1 - ribophorin 1, SSBP2 - single stranded DNA binding protein 2, T-ALL - T cell ALL.

[b]PAX5-Jak2 is not constitutively active.

[c]Not fully characterized

TABLE 3

Current allosteric inhibitors for BCR-ABL and Jak2 that target sites other than the ATP-binding site

NAME	STRUCTURE	TARGET KINASES	IC_{50} (nM)	PREDICTED MECHANISM OF INHIBITION	REF.
GNF-2		BCR-ABL	138	Targets the myristoyl binding cleft	[79]
DCC-2036		BCR-ABL	5.8	Targets the switch control pocket	[80]
ON012380		BCR-ABL	10	Substrate-competitive	[81]
LS104		Jak2, FLT3	1500 (Jak2), 4000 (FLT3)	Substrate-competitive	[87,88]

NAME	STRUCTURE	TARGET KINASES	IC_{50} (nM)	PREDICTED MECHANISM OF INHIBITION	REF.
ON044580		BCR-ABL, Jak2	3000 (BCR- ABL), 4000 (Jak2)	Substrate-competitive	[89]