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Review

Structural Regulation and Therapeutic Perspectives of JAK2 Kinase

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Abstract

Janus kinase 2 (JAK2) occupies a central position in cytokine signaling and plays essential roles in hematopoiesis, immune regulation, and cancer. Although recent advances in structural biology, cryo-EM, receptor modeling, and biophysical analysis have substantially expanded current views of JAK2 function, key mechanistic questions remain regarding how receptor geometry, JH2-mediated autoinhibition, and disease-associated mutations are structurally integrated. In this review, we discuss the multidomain organization of JAK2 and examine how the FERM–SH2 module, the pseudokinase domain (JH2), and the catalytic kinase domain (JH1) cooperate to govern receptor specificity, allosteric control, and cytokine-induced activation. We further analyze how pathogenic mutations rewire this regulatory system by weakening autoinhibitory contacts, altering linker-mediated communication, or stabilizing active dimeric conformations. Finally, we assess current and emerging therapeutic strategies, from ATP-competitive inhibitors to macrocyclic and JH2-selective allosteric modulators, with emphasis on how structural insight can guide next-generation drug design. These advances support a more integrated view of JAK2 regulation and define new opportunities for selective therapeutic intervention.

Keywords: JAK2; allosteric regulation; structure-based drug design; JAK inhibitors

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1. Introduction

Reversible protein phosphorylation, first characterized in the pioneering work of Fischer and Krebs in the 1950s, is now recognized as one of the fundamental biochemical mechanisms governing cellular regulation. Their discovery, honored with the 1992 Nobel Prize in Physiology or Medicine, opened a field that has since expanded to encompass virtually all aspects of cell biology. Phosphorylation controls essential processes including metabolism, transcription, apoptosis, cytoskeletal organization, and cell cycle progression. More than two-thirds of human proteins carry at least one phosphorylated residue, making phosphorylation the most prevalent post-translational modification in eukaryotes [1,2]. These reactions are catalyzed by a structurally diverse superfamily of protein kinases,

which includes more than 500 human members and at least 20 kinase-like families across metazoans, prokaryotes, and plants [3,4]. Dysregulated kinase signaling is a hallmark of numerous oncogenic processes, and kinase inhibitors now represent nearly one quarter of all drug discovery pipelines. To date, 85 small-molecule inhibitors have been approved by the U.S. Food and Drug Administration (FDA) targeting central nodes such as: Epidermal Growth Factor Receptor (EGFR), Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2), Vascular Endothelial Growth Factor Receptor (VEGFR), v-Raf Murine Sarcoma Viral Oncogene Homolog B1 (B-Raf), Platelet-Derived Growth Factor Receptor (PDGFR), Abelson Tyrosine Kinase (Abl), Proto-oncogene tyrosine-protein kinase Src (Src), mechanistic Target of Rapamycin (mTOR), and members of the Janus kinase (JAK) family [5].

Among these, the Janus kinase (JAK) family has emerged as a critical signaling hub at the intersection of cytokine responses, immune regulation, and cancer. Discovered in the early 1990s in parallel with studies on STAT transcription factors [6–11], the JAK family comprises four non-receptor tyrosine kinases: JAK1, JAK2, JAK3, and TYK2. Each is approximately 1100 residues long and shares a conserved modular architecture [12]. JAK1, JAK2, and TYK2 are broadly expressed in most tissues except mature erythrocytes, whereas JAK3 expression is restricted to hematopoietic lineages [13]. JAK2 is unique among these enzymes, playing a dual role as an essential mediator of normal hematopoiesis and a central driver of malignant transformation.

Constitutive activation of JAK2 is a defining indicator of myeloproliferative neoplasms (MPNs). The JAK2-V617F mutation, present in more than 95% of polycythemia vera and approximately 50–60% of essential thrombocythemia and primary myelofibrosis cases, destabilizes the autoinhibited conformation of the kinase and promotes cytokine-independent signaling [14–16]. Additionally, chromosomal rearrangements such as PCM1–JAK2, BCR–JAK2, and ETV6–JAK2 generate oncogenic fusions that drive leukemias through aberrant STAT3/STAT5 activation [17–20]. Beyond hematologic malignancies, JAK2 contributes to tumorigenesis by promoting inflammation-driven proliferation, modulating cytokine networks in the tumor microenvironment, and enabling immune evasion via persistent STAT3/STAT5 activation in solid cancers [21–23]. Although ATP-competitive JAK inhibitors, particularly ruxolitinib and fedratinib, have transformed the management of MPNs, their disease-modifying activity is limited, and resistance frequently emerges [24–26]. These shortcomings reflect an incomplete understanding of the complex structural and regulatory logic governing JAK2 activation.

JAK proteins are organized into seven domains known as JAK Homology (JH) domains, which are assembled in four major functional domains [27] (Figure 1a): JH5–JH7: the Four-point-one, Ezrin, Radixin, Moesin (FERM) domain; JH3–JH4: the Src homology 2 domain (SH2-like); JH2: the pseudokinase; and JH1: the functional protein tyrosine kinase domain at the C-terminal region (Figure 1a). JH1 catalyzes the phosphorylation reaction, and JH2 modulates JH1's catalytic activity. Their relation and function are described in Table 1.

Table 1. JAK homology domains (JH1–JH7) and their structural and functional assignments.

JH Region	Functional Name	Function
JH1	Kinase domain	Catalyzes the phosphorylation reaction
JH2	Pseudokinase domain	Modulates JH1's catalytic activity
JH3–JH4	SH2-like domain	Regulates kinase activity, receptor stability, and intramolecular interactions
JH5–JH7	FERM domain	Mediates receptor binding and membrane association

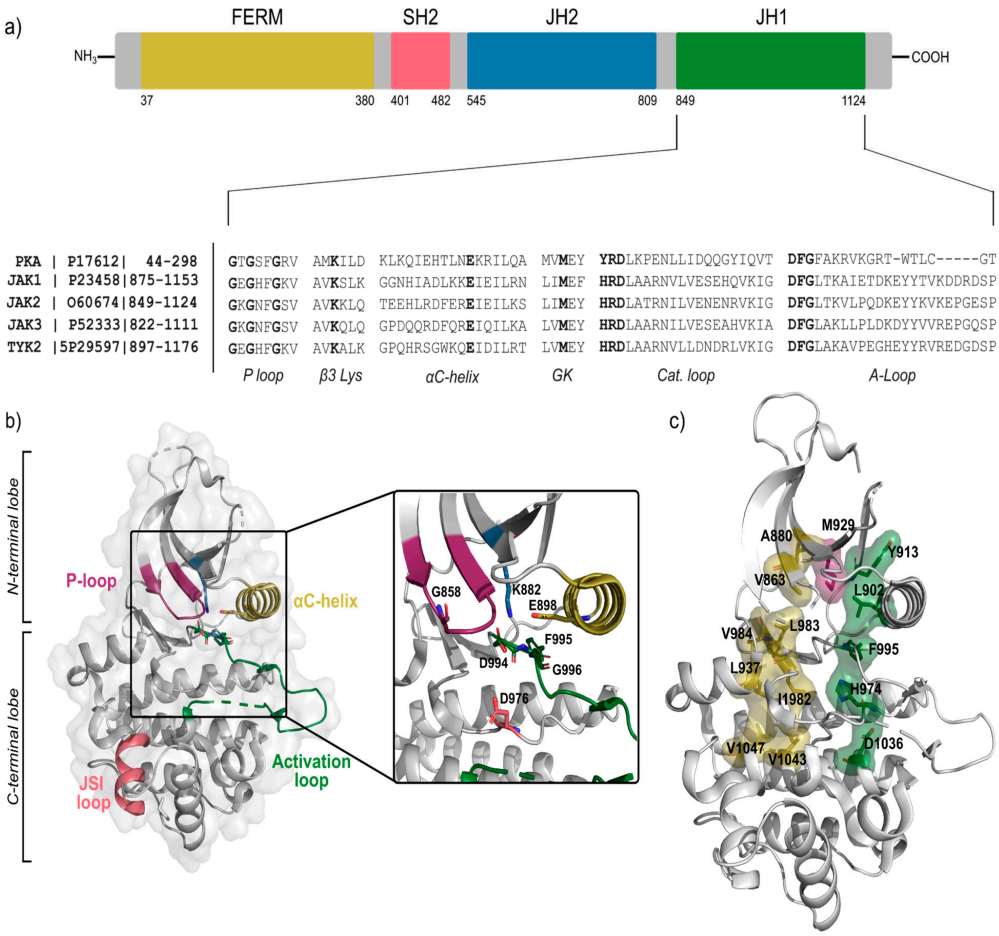


Figure 1. JAK domain organization: (**a**) Schematic JAK domain organization and the conservation of main features of the kinase domain among the PKA and JAK family; (**b**) Crystal structure of JAK2 JH1 domain highlighting the main kinase motifs (PDB ID: 6WTO); (**c**) Regulatory (green) and catalytic (yellow) spines of JAK2, and gatekeeper M929 residue (magenta) (PDB ID: 4C61).

Despite extensive biochemical and clinical research, major questions remain regarding how the FERM–SH2 module engages cytokine receptors, how the pseudokinase JH2 domain enforces autoinhibition, how conformational transitions enable activation, and how oncogenic mutations disrupt this architecture. While the recent cryo-EM structure of the analogous JAK1–receptor complex [28] provides a critical blueprint for the full domain organization, the definitive, active structure of the JAK2–receptor complex, particularly in the context of the V617F mutation, remains to be elucidated. Existing reviews primarily address pathway biology or clinical therapies. In contrast, fewer explore the structural and biophysical mechanisms underlying JAK2 regulation, particularly in the context of cancer-associated mutations and emerging therapeutic strategies.

In this review, we integrate recent structural, mechanistic, and biophysical insights into JAK2 regulation with a particular emphasis on principles relevant to cancer biology and therapeutic targeting. We first outline the modular architecture of JAK family proteins, emphasizing domain organization, conserved motifs, and the interplay among functional modules. We then analyze how receptor binding, domain–domain communication, and allosteric regulation govern JAK2 activation, and how cancer-associated mutations disrupt these mechanisms. In subsequent sections, we examine current ATP-competitive inhibitors, the structural basis of resistance, and emerging strategies, including allosteric modulators and protein–protein interactions, that may enable next-generation therapeutics. Finally, we provide a focused section summarizing the practical aspects of JAK inhibitor use in clinical oncology, including daily management considerations, therapeutic monitoring, and

treatment limitations. Together, these perspectives aim to provide a unified mechanistic and translational framework to guide both fundamental understanding and drug discovery efforts centered on JAK2 in cancer.

2. Domain Architecture and Regulatory Mechanisms of JAK2

Members of the JAK family comprise approximately 1150 amino acids, corresponding to a molecular weight of about 130 kDa, and the four functional domains encompass seven homologous regions (JH1–JH7). The FERM domain, formed by the JH7–JH4 regions (residues 37–380), is followed by the SH2-like domain, which includes the JH4 and JH3 regions. Subsequently, the pseudokinase domain (ΨK), corresponding to the JH2 region, precedes the C-terminal kinase domain, composed of the JH1 region [29,30]. Together, these domains form a multidomain unit in which receptor binding, interdomain communication, and catalytic output are tightly coupled. In the following subsections, each functional domain is described in detail.

2.1. Kinase Domain (JH1)

The kinase domain (JH1) constitutes the catalytic core of JAK2 and is responsible for ATP binding, substrate phosphorylation, and signal propagation. As shown in Figure 1b, JH1 adopts the canonical bilobal architecture characteristic of protein tyrosine kinases, consisting of a smaller N-terminal lobe involved primarily in ATP coordination and a larger C-terminal lobe that mediates substrate recognition and catalysis [31]. The hinge region, characterized by residues ^{930}EYLPYGS^{936}, anchors the adenine ring of ATP via hydrogen bonds. The gatekeeper residue M929 (Figure 1c) regulates access to a hydrophobic pocket adjacent to the ATP-binding site, a feature exploited by many selective inhibitors [31].

The glycine-rich loop (P-loop) stabilizes the ATP triphosphate moiety; within this loop, G858 plays a key role in positioning the ATP phosphate group for proper catalysis. A conserved lysine residue (K882) in the β3 strand interacts electrostatically with the α- and β-phosphates of ATP [32]. K882 also forms a salt bridge with E898 on the αC-helix, an interaction essential for maintaining the kinase's active conformation. When the JAK2 αC-helix adopts an outward orientation, it disrupts this interaction and misaligns the catalytic residues, leading to an inactive enzyme. Thus, the movement of the αC-helix between "in" and "out" positions serves as a mechanical switch that couples ATP binding to catalysis [33,34].

The catalytic loop contains the conserved HRD motif, where the aspartate residue (D976) acts as a general base during phosphoryl transfer. Downstream of this region, the activation loop, harboring the ^{994}DFG^{996} motif, is critical for coordinating the divalent metal ion required for catalysis [35]. The orientation of this DFG motif is another structural element that defines the kinase's functional state: in the active "DFG-in" conformation, D994 coordinates the magnesium ion. It facilitates phosphoryl transfer, whereas in the inactive "DFG-out" conformation, F995 flips inward, occluding the ATP-binding pocket and disrupting catalysis (Figure 2a) [36,37]. In the active DFG-in conformation, the activation loop (residues 994–1023) is stabilized by a network of β-sheets and arginine residues (R971 and R975), which position the phosphorylated Y1007 for catalysis. A distinctive structural feature of JAK family kinases is the JAK-specific insertion (JSI), an additional helical segment within the C-lobe, which is thought to participate in autophosphorylation and intramolecular regulation [38].

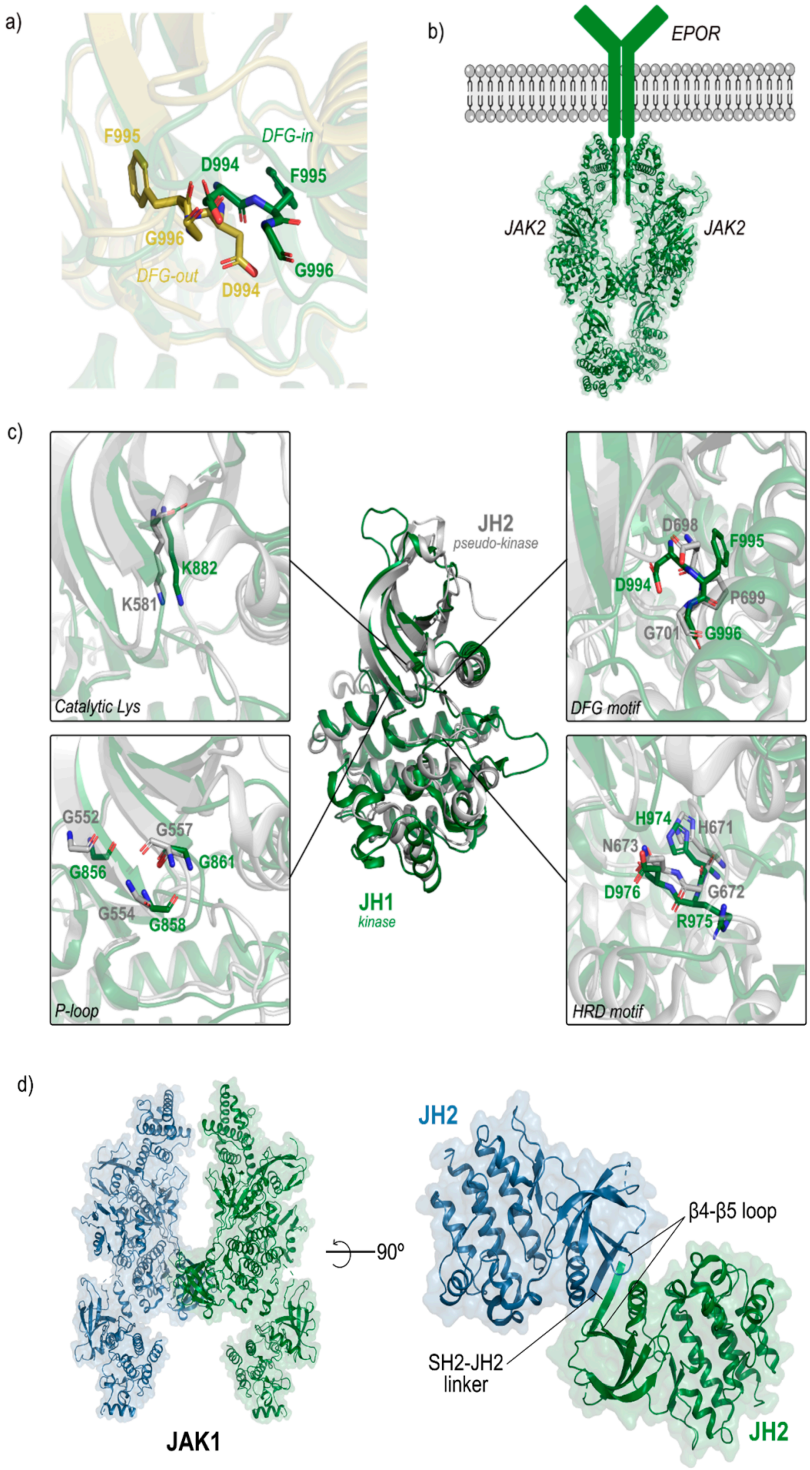


Figure 2. Structural features of the JAK kinase (JH1) and pseudokinase (JH2) domains: (**a**) Structural comparison of the JH1 activation loop in its active DFG-in conformation (green) (PDB ID: 6WTO) and inactive DFG-out conformation (gold) (PDB ID: 7TEU). (**b**) Model of cytokine-dependent JAK2 activation. Dimerization of the erythropoietin receptor (EPOR) brings two JAK2 molecules into proximity, enabling trans-phosphorylation of the activation loop, particularly Y1007, which is essential for reorganization of the activation loop, alignment of the HRD and DFG motifs, and restoration of the K882–E898 electrostatic bridge that supports ATP coordination (PDB ID: 7T6F). (**c**) Structural superposition of the active JH1 kinase domain (green) with the JH2 pseudokinase domain (gray) (PDB ID: 4FVQ), highlighting conserved elements and domain-specific divergences. Insets illustrate key motifs: Catalytic lysine, DFG motif, HRD motif, and P-loop. (**d**) Organization of the JAK1 pseudokinase domain (JH2, blue) and its interface with JH1 (green) (PDB ID: 8EWY). The β4–β5 loop and the SH2–JH2 linker form regulatory contacts that stabilize autoinhibited conformations and participate in allosteric control of JAK activation.

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Phosphorylation within the activation loop of JAK2 is a key regulatory event for kinase activation. In particular, the conserved ^{1006}EYY^{1008} motif contains two tyrosine residues (Y1007 and Y1008) that are phosphorylated. Among these, Y1007 is functionally dominant, being essential for catalytic activity, while Y1008 contributes to stabilization of the active conformation. Dual phosphorylation of this EYY motif promotes structural rearrangements of the activation loop, enabling proper alignment of catalytic elements and transition from an autoinhibited to an active kinase state, thereby supporting efficient signal transduction [39–41]. When expressed in insect or mammalian cells, JAK2 undergoes extensive autophosphorylation. Substitution of Y1007 by phenylalanine abolishes kinase activity both in vitro and in cells, mimicking the effect of the catalytically inactive K882E mutant. In contrast, mutation of the adjacent Y1008 residue has no significant impact on catalysis or signaling. Structural and biochemical analyses indicate that phosphorylation of Y1007 reorganizes the activation loop, displacing it from the catalytic core to allow proper alignment of the HRD and DFG motifs and to restore the electrostatic K882–E898 bridge required for ATP coordination [42,43].

The dynamic interplay between the N-lobe and C-lobe is structurally supported by two hydrophobic networks, the regulatory (R-) spine and the catalytic (C-) spine. These spines connect conserved residues across the kinase lobes, stabilizing the active conformation (Figure 1c) [44,45]. The R-spine assembles upon activation and includes residues from the αC-helix, the HRD motif, the DFG motif, and Y913 from the β4 strand. The C-spine, on the other hand, forms around the adenine ring of ATP, which serves as a structural element to complete the hydrophobic core. Assembly of the R-spine defines activation, while completion of the C-spine primes the enzyme for catalysis. Disruption of these spines, either by mutation or inhibitor binding, renders the kinase inactive, highlighting their central role in JAK2 regulation [46,47].

2.2. Pseudokinase Domain (JH2)

The pseudokinase domain (JH2) closely mirrors the overall fold of a conventional kinase, adopting the characteristic bilobal architecture with a smaller N-lobe, rich in β-sheets and containing the αC-helix and P-loop, and a larger C-lobe composed mainly of α-helices and harboring the catalytic motifs (Figure 2c) [48,49]. In functional kinase domains such as JH1, the aspartate in the HRD motif acts as a catalytic base, abstracting a proton from the substrate's hydroxyl group. At the same time, the arginine stabilizes the negative charge that develops on the phosphate groups of ATP during catalysis. In JH2, however, substitution of the HRD motif by HGN removes the catalytic aspartate and the arginine, abolishing catalytic activity and breaking the communication between the catalytic loop, the P-loop, and the αC-helix [49,50].

In JH1, the DFG motif is optimally positioned to bind Mg^{2+} and support catalysis, whereas in JH2, the DFG is replaced by DLG. Replacement of phenylalanine by leucine diminishes hydrophobic packing and destabilizes the loop, rendering the aspartate more solvent-exposed and weakening coordination of the metal ion. Structural studies of JAK1, JAK2, and TYK2 JH2 show that ATP binds in a noncanonical manner involving only one Mg^{2+} ion coordinated by D678 [51–53]. JAK2 JH2 demonstrates low-level kinase activity (~10% of JH1 activity) and can auto-phosphorylate at S523 and Y570, which act as negative regulatory sites for both JH1 and JH2. These phosphorylation events fine-tune JAK2's activation state but are not conserved among other JAK family members. ATP binding in JH2 does not primarily serve a catalytic purpose but instead contributes to structural stability and regulation of interdomain communication, supporting its function as an allosteric modulator of JH1 activity [49,54].

Mechanistically, the JH2 pseudokinase domain acts as a negative regulator of the adjacent JH1 kinase domain, maintaining low basal activity until cytokine stimulation. Biochemical studies indicate that JH2 attenuates JH1 catalytic efficiency, in part by reducing its affinity for ATP. Although JAK2 contains numerous tyrosine residues, many of which are structurally conserved across the JAK family, only a subset undergoes regulatory phosphorylation. Among these, trans-phosphorylation at Y570 has been identified as a key negative regulatory event [55]. Serine phosphorylation at S523 represses JAK2 activity in response to growth hormone, epidermal growth factor, and leptin through a pathway independent of Y570 [56].

Recent cryo-EM, crystallographic, and molecular dynamics studies in JAK1 showed that JH2 also participates in dimer interfaces that drive activation and are exploited by oncogenic mutations, with structural rearrangements propagating through the FERM–SH2 and kinase domains (Figure 2d) [51]. AlphaFold Multimer models of full-length cytokine-receptor–JAK2 complexes support a similar mechanism in which cytokine-induced receptor dimerization brings JH2 domains into proximity, enabling a JH2–JH2 dimer that stabilizes active JAK2 dimers and facilitates JH1 trans-phosphorylation [57]. These findings establish JH2 as a versatile regulatory module integrating allosteric inhibition, ATP-dependent stabilization, and phosphorylation-dependent feedback.

2.3. SH2-Like Domain (JH3–JH4)

The Src Homology 2 (SH2) domain is classically defined as a phosphotyrosine (pTyr)-binding module composed of a central antiparallel β-sheet flanked by two α-helices. Canonical SH2 domains bind pTyr through a pocket formed by loops adjacent to the αB helix, anchored by a highly conserved arginine residue that coordinates the phosphate group [58]. Although all JAKs (JAK1, JAK2, JAK3, and TYK2) contain an SH2-like domain, it is now well established that these domains no longer function as conventional pTyr-binding modules [29,59].

Early mutational data already suggested that JAK-family SH2 domains deviate from classical SH2 biology. Mutation of the conserved arginine in the JAK1 SH2 domain produced no defect in JAK/STAT signaling [59], implying that pTyr binding is dispensable. Structural analyses later revealed the physical basis for this divergence. Superposition of the JAK2 SH2 domain with a prototypical SH2 domain from Lck demonstrated that the canonical pTyr pocket in JAK2 is structurally blocked [60]. Although JAK2 preserves the signature arginine (Arg426), the pocket is occluded by Phe436, a bulky hydrophobic residue that occupies the site where the phenyl ring of pTyr normally binds. In canonical SH2 domains, this position is typically occupied by a small polar or hydrophobic residue (e.g., serine or alanine), thereby permitting access to pTyr. The presence of a bulky residue at this position is a conserved feature across JAKs, effectively preventing classical pTyr recognition [60] (Figure 3a).

Rather than binding phosphorylated ligands, the JAK2 SH2 domain functions primarily as a constitutive receptor-binding module. This repurposing is evident in the remodeling of what would correspond to the "secondary site" of canonical SH2 domains. In classical SH2 proteins, this secondary site provides specificity for residues C-terminal to the pTyr. In JAK2, the analogous region forms a deep hydrophobic groove that specifically accommodates the Box2 motif of cytokine receptors [29,58]. Structural analysis reveals that the SH2-EF loop and a β-hairpin formed by strands βG1 and βG2 create a large hydrophobic slot that is pre-formed even in the apo structure, indicating that receptor binding does not require large conformational rearrangements [29]. This configuration makes the Box2 motif the main anchoring element for JAK2 recruitment to its cognate receptors.

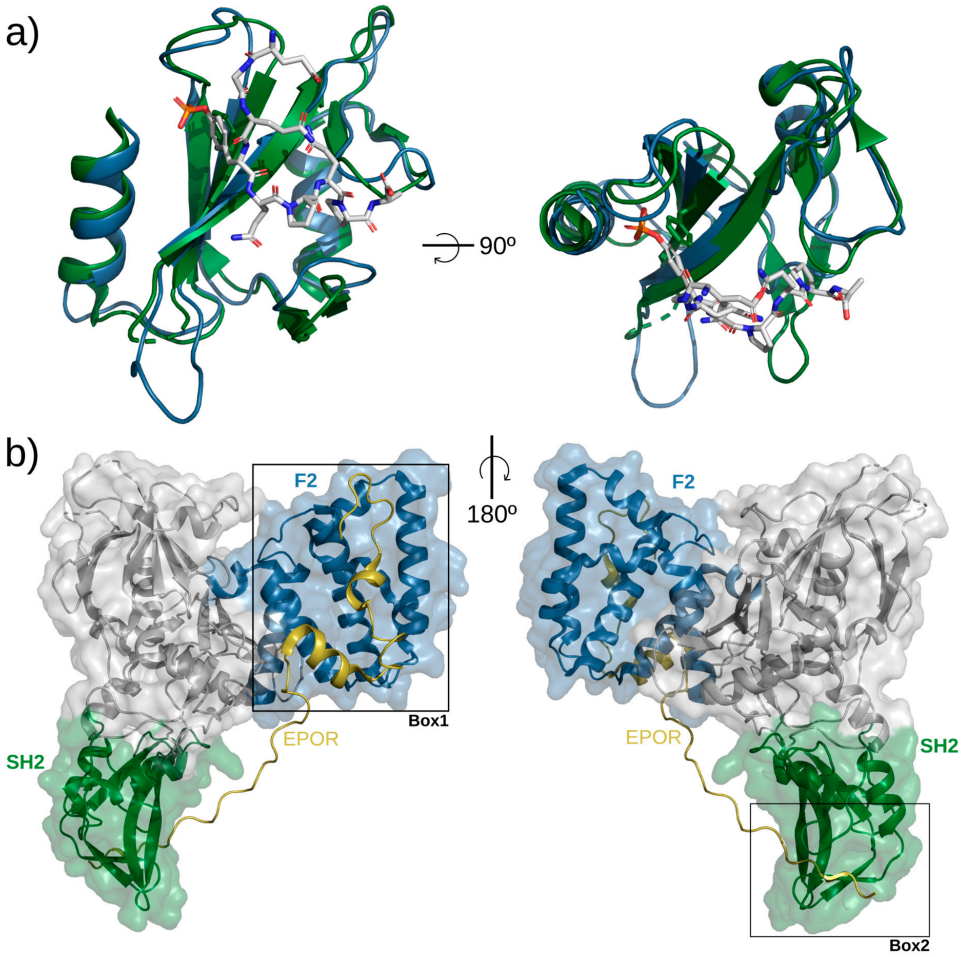


Figure 3. (**a**) Structural superposition of the JAK2 SH2 domain (green) (PDB ID: 4Z32) with the SH2 domain of lck (PDB ID: 1LCK), Abl (magenta) (PDB ID: 2ABL). The Phe436 side chain in JAK2 is highlighted to illustrate the steric occlusion of the canonical phosphotyrosine-binding pocket. (**b**) Crystal structure of the JAK2 FERM–SH2 module bound to the Box1 and Box2 regions of the erythropoietin receptor (EPOR) (PDB ID: 6E2Q). Box1 engages the F2 region on the FERM domain (blue), whereas Box2 interacts with the SH2 domain (green). The FERM domain, F2 region, SH2 domain, and EPOR Box1–Box2 peptide (residues 866–885) are shown in gray, blue, green, and gold, respectively.

Across the JAK family, the canonical pTyr pocket has been adapted to recognize a glutamate residue of the receptor [29]. JAK2 follows this pattern: the conserved arginine remains present, but due to occlusion by Phe436, the site can no longer coordinate a phosphotyrosine. Instead, it appears specialized for stabilizing the receptor interface in conjunction with the dominant hydrophobic Box2 (Figure 3b).

Although structurally divergent from classical SH2 modules, the JAK2 SH2 domain remains indispensable for kinase function. It stabilizes the pseudokinase–kinase core, secures receptor binding, and ensures proper assembly of the signaling complex. Consistent with this role, genetic disruption of the SH2 domain is embryonically lethal in JAK2 and similarly essential in other JAKs, underscoring the domain's non-redundant contribution to cytokine signaling [30,61,62].

2.4. FERM Domain (JH5–JH7)

The JAK2 FERM–SH2 module recognizes two conserved receptor elements, Box1 and Box2. Box1 is a proline-rich sequence, whereas Box2 typically contains hydrophobic residues flanked by acidic amino acids (Figure 3b). Although the precise sequences of these

motifs vary among cytokine receptors, their overall architecture is essential for productive JAK2 recruitment and subsequent signaling across diverse cytokine systems [29,63].

Beyond the interaction with Box1 and Box2, the FERM domain also plays a central role in regulating the C-terminal kinase domain. Structural and biochemical studies indicate that, in the absence of receptor engagement, the FERM domain helps maintain JAK2 in an autoinhibited conformation [29]. Accordingly, deletions or point mutations within the FERM domain frequently impair kinase activation by disrupting the allosteric control between the N-terminal module and the catalytic domain, as well as by preventing correct receptor association [64].

JAK2 specificity for homodimeric cytokine receptors, such as the erythropoietin receptor (EPOR) and the thrombopoietin receptor (MPL), is largely encoded within the N-terminal FERM domain [63] (Figure 3b). Specific amino acid residues create a surface that is geometrically and chemically compatible with the Box1/Box2 regions of these receptors but incompatible with motifs from receptors that pair with other JAKs, such as those using the common γ-chain (γc). Among these residues, Y119 plays a dual structural and regulatory role. Upon ligand activation, Y119 undergoes autophosphorylation, promoting partial dissociation of JAK2 from the receptor and contributing to signal attenuation. Mutation of this site illustrates its importance: Y119E disrupts receptor binding and abrogates kinase activation, whereas Y119F stabilizes the receptor–JAK2 complex and prolongs downstream signaling [29,65]. Additional residues, such as E127 and L144, help sculpt a binding interface optimized for the Box1/Box2 region of EPOR and MPL, reinforcing JAK2's high receptor specificity [29,66].

A second regulatory site within the FERM domain, Y221, also contributes to signal control. Phosphorylation at Y221 modulates receptor association and provides an additional layer of regulation, emphasizing that efficient signaling requires coordinated input from multiple JAK2 domains [55]. Through these combined functions of receptor docking, autoinhibition, and phosphorylation-dependent regulation, the FERM domain acts as an essential hub for JAK2 localization, stability, and activation.

3. Principles of Signaling and Mechanism of Deregulation

3.1. JAK-STAT Signaling Pathways

The JAK–STAT pathway is activated by a diverse group of extracellular ligands that engage members of the Class I and Class II cytokine receptor superfamilies. These ligands include hematopoietic growth factors such as erythropoietin (EPO), thrombopoietin (TPO), granulocyte colony-stimulating factor (G-CSF), prolactin (PRL), and growth hormone (GH), as well as classical immunomodulatory cytokines, including interleukins (IL-2, IL-6, IL-12, and IL-23) and interferons (IFN-α/β, IFN-γ, and IFN-λ). Although growth factors and cytokines are often classified according to their predominant physiological functions, both groups signal through cytokine receptor family members and rely on JAK-dependent mechanisms to transmit extracellular cues into intracellular responses.

Based on their structural organization, these receptors are divided into two major classes (Figure 4a,b). Class I cytokine receptors include receptors for interleukins, hematopoietic growth factors, prolactin, and growth hormone, whereas Class II cytokine receptors comprise interferon receptors and members of the IL-10 receptor family. Signaling specificity arises from the composition of receptor subunits and their associated JAK kinases. Homodimeric receptors, such as the EpoR, TpoR, growth hormone receptor (GHR), and prolactin receptor (PRLR), primarily utilize JAK2, whereas heteromeric receptor complexes typically recruit combinations of JAK1, JAK3, and TYK2. Together, these receptor–kinase pairings generate the diverse signaling outputs characteristic of the JAK–STAT pathway [67].

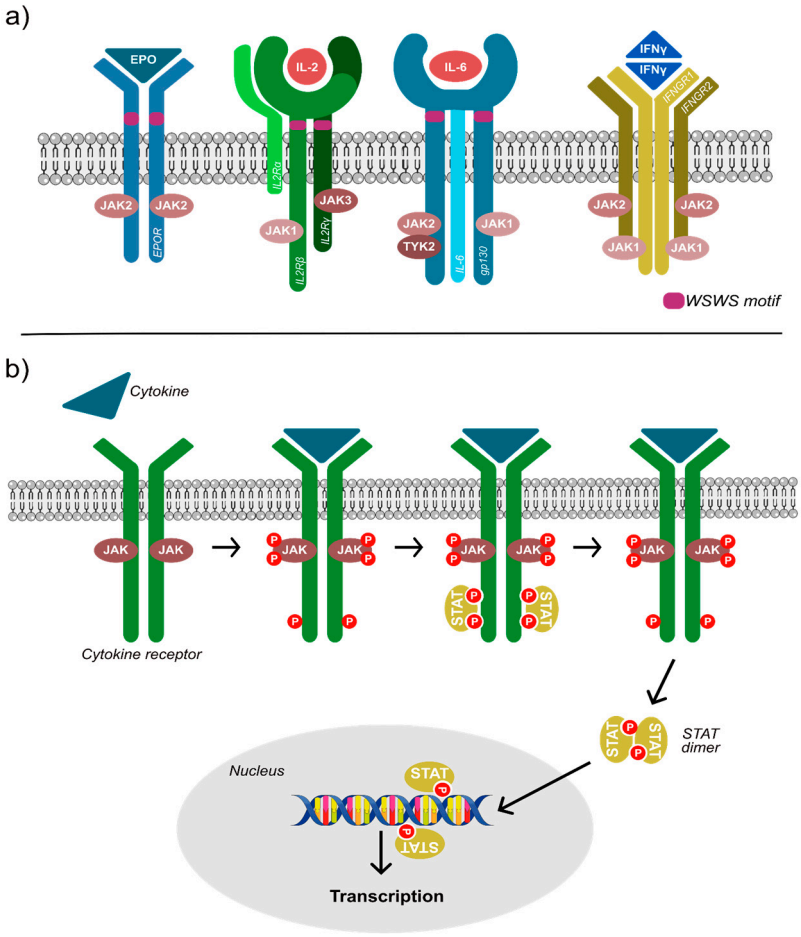


Figure 4. Schematic representation of the JAK–STAT signaling pathway, illustrated by the JAK1/JAK3-mediated γc receptor signaling. (**a**) The simplified representation of receptor chains is shown (EPOR: erythropoietin receptor, IL2R: interleukin-2 receptor, IFNGR: interferon-γ receptor). (**b**) Upon cytokine binding, receptor subunits dimerize, enabling transphosphorylation of JAK1 and JAK3. STAT5 then associates with the receptor complex and is phosphorylated by activated JAKs. Phosphorylated STAT5 molecules dimerize, translocate to the nucleus, and activate the transcription of target genes [11].

Among the four mammalian JAKs, JAK2 is the central mediator of hematopoietic and growth-related cytokine responses. Over fifty cytokines signal through JAK–STAT cascades, including hematopoietic growth factors such as (EPO), thrombopoietin (TPO), granulocyte colony-stimulating factor (G-CSF), and growth hormone (GH), as well as immunomodulatory cytokines like interleukins (IL-2, IL-6, IL-12, IL-23) and interferons (IFN-α/β, IFN-γ, IFN-λ). These ligands are organized into two structural classes based on receptor architecture (Figure 4a,b): Class I cytokines, encompassing interleukins, hematopoietins, and growth hormones; and Class II cytokines, including interferons and the IL-10 family. In all cases, signal specificity is determined by the pairing of receptor chains and their associated JAKs. For comprehensive discussions of cytokine and growth-factor diversity within the JAK–STAT system, see the detailed reviews by Morris et al. [67].

Cytokines initiate signaling by promoting dimerization of their single-pass receptors, thereby aligning the intracellular regions containing Box1 and Box2 motifs. These short, conserved segments constitute the bipartite docking platform for JAKs: Box1, a proline-rich stretch, and Box2, an adjacent hydrophobic element, together form the composite interface that secures the FERM–SH2 domains of JAK2 to the receptor cytoplasmic tail [28]. This anchoring ensures that ligand-induced receptor dimerization aligns the two JAK2 molecules for trans-phosphorylation of the JH1 tyrosine kinase domains, relieving autoinhibition and

initiating catalytic activity. Active JAK2 then phosphorylates discrete tyrosines in the receptor tail, generating SH2-binding sites for downstream effectors, most prominently the STAT5s.

JAK2 homodimerizes to signal in receptors such as EpoR, TpoR, GHR, and PRLR. The EpoR–JAK2 complex could serve as a model for cytokine signaling. Quantitative single-molecule and FRET studies reveal that EpoR exists as monomers under basal conditions and dimerizes only upon EPO engagement, which positions the bound JAK2 molecules for reciprocal JH1 phosphorylation [51]. Within this framework, Box1/Box2-mediated anchoring is essential for maintaining receptor–kinase alignment and ensuring efficient signal transduction.

Following receptor phosphorylation, STAT5 is recruited via its SH2 domain to specific phosphotyrosine motifs on EpoR (Figure 4a). Activated JAK2 phosphorylates STAT5 on a critical C-terminal tyrosine (Y694 in STAT5A, Y699 in STAT5B), enabling reciprocal SH2–pTyr-mediated dimerization (Figure 4b). The dimer exposes nuclear-localization sequences and translocates to the nucleus, where it binds γ-activated sequence (GAS) motifs in the promoters and enhancers of responsive genes. Co-activators such as CBP/p300, together with erythroid transcription factors GATA1 and KLF1, integrate STAT5 output into the erythroid gene-regulatory network [68–70].

These sequential events, from receptor dimerization to nuclear transcription, form a highly ordered cascade. The EpoR–JAK2 axis exemplifies how extracellular ligand binding is translated into temporally and spatially precise transcriptional responses through Box1/Box2-mediated receptor anchoring, JH1 trans-activation, and STAT relay. The fidelity of this architecture relies on the transient nature of JH1 dimerization and on secondary phosphorylation sites within JAK2 (Y813, Y868, Y966, Y972), which modulate adaptor recruitment and catalytic amplitude. The phosphorylation of Y813, for instance, recruits the adaptor SH2-Bβ, thereby enhancing JAK2 kinase activity and amplifying STAT5 signaling [71]. Similarly, Y966 interacts with the protein p70, though its biological function remains unclear, and mass spectrometry has identified Y868 and Y972 as sites whose mutations impair catalytic efficiency [72,73].

JAK2 signaling is self-limiting through powerful negative feedback loops. Suppressors of Cytokine Signaling (SOCS): proteins like SOCS1 and SOCS3 inhibit JAK2 through direct binding to its activation loop, substrate mimicry via a Kinase Inhibitory Region (KIR), and by targeting JAK2 for proteasomal degradation [74,75]. Protein Tyrosine Phosphatases (PTPs): Enzymes such as PTP1B and SHP1 directly dephosphorylate JAK2 and its associated receptors, terminating the activation signal [76,77].

In addition to receptor activation and STAT recruitment, substrate recognition represents an important determinant of signaling specificity. Although JAK2 phosphorylates a diverse set of substrates, including cytokine receptors, STAT proteins, and regulatory adaptors, a strict phosphorylation consensus sequence has not been established. Unlike many serine/threonine kinases that recognize well-defined linear motifs, JAK2 substrate selection appears to depend on a combination of local sequence preferences, structural context, and protein–protein interactions within receptor-associated signaling complexes [78]. Studies employing peptide libraries and phosphoproteomic approaches have suggested enrichment of acidic and hydrophobic residues surrounding target tyrosines, but these sequence features alone do not fully account for substrate selectivity [79]. Instead, substrate recruitment through receptor scaffolds and spatial organization within cytokine receptor complexes likely play dominant roles in determining phosphorylation efficiency and signaling output [67,80].

Signal initiation is a meticulous process: receptor engagement triggers dimerization and structural reorganization; the receptor's tail provides the platform; and JAK2 serves as the catalytic engine. This elegant division of labor highlights the receptor's central role,

not simply as a ligand-binding module, but as a molecular director that enables precise, context-dependent JAK2 signaling in hematopoiesis and immune regulation. Disruption of any of these checkpoints, whether through mutation, receptor overexpression, or defective feedback, can convert a physiological signal into a self-sustaining oncogenic cascade, as discussed in the next section.

3.2. Basis of Pathogenic Activation

In physiological conditions, JAK kinases function within a finely balanced allosteric network that ensures cytokine-dependent activation while suppressing basal signaling. In resting states, the pseudokinase domain (JH2) enforces autoinhibition of the catalytic JH1 domain through a network of inter-domain interactions and controlled ATP binding, acting as a molecular rheostat [67,81]. Ligand engagement promotes receptor dimerization, aligning the Box1/Box2-anchored JAK2 molecules for trans-phosphorylation and catalytic activation [51,82]. However, pathogenic mutations can modify these regulatory checkpoints, driving cytokine-independent activation and persistent downstream signaling.

This pathogenic activation arises through three interconnected structural disturbances. First, loss of autoinhibition, often caused by disruption of the JH2–JH1 interface or the SH2–JH2 linker, destabilizes the inactive conformation. Second, stabilization of the active dimer, exemplified by the canonical JAK2 V617F mutation, locks JH2 domains into a configuration that mimics cytokine-induced receptor dimerization and promotes unrestrained trans-activation [51,82]. Third, perturbation of allosteric coupling between the FERM–SH2 and JH2 modules alters receptor specificity and biases downstream STAT selection. Collectively, these mechanisms lead to constitutive phosphorylation of receptor tails and persistent activation of STAT5 and STAT3, reprogramming gene-expression networks that sustain proliferation, survival, and aberrant differentiation characteristic of myeloproliferative neoplasms and lymphoid leukemias [83].

Building on this framework, the following subsections examine: (i) the structural basis of physiological autoinhibition and its allosteric release, (ii) the mechanistic classes of oncogenic JAK2 mutations, and (iii) their downstream rewiring of STAT signaling and feedback regulation. Together, these principles explain how discrete molecular lesions transform a precisely regulated cytokine pathway into a self-sustaining pathogenic circuit, establishing the mechanistic foundation for disease-specific JAK2 vulnerabilities addressed in subsequent sections.

3.2.1. Physiological Autoinhibition and Allosteric Control

Under basal conditions, JAK2 adopts an autoinhibited conformation that prevents spontaneous kinase activation and maintains tight dependence on cytokine stimulation. This state is enforced by an intricate network of intramolecular interactions between the pseudokinase (JH2) and kinase (JH1) domains, which restrain ATP turnover and limit catalytic access to substrate tyrosines. High-resolution structural and biophysical studies have revealed that the JH2 domain folds against JH1 through a cluster of polar and hydrophobic contacts that position the αC-helix of JH2 as an autoinhibitory brace, locking the activation loop of JH1 in an inactive orientation [67,81].

A defining feature of this regulation is the non-canonical ATP-binding pocket in JH2, which acts as a molecular rheostat. Occupancy of this site stabilizes the inactive JH2 conformation and dampens spontaneous kinase activity by modulating local electrostatic interactions and Mg^{2+} coordination. Mutational or pharmacological disruption of this site decreases the stability of the autoinhibited ensemble and primes JH1 for activation, highlighting its allosteric role in setting the activation threshold [81].

In the cytokine-bound receptor complex, conformational signals transmitted through the FERM–SH2–JH2–JH1 axis overcome JH2-mediated autoinhibition. Ligand binding induces receptor dimerization and rearrangement of the Box1/Box2-associated FERM–SH2 module, repositioning the attached JAK2 molecules so that the two JH1 catalytic lobes approach in a trans-phosphorylation-competent geometry [28] (Figure 3b). Structural and cryo-EM data support a two-step activation model: first, a "closed" JH2–JH1 configuration in the resting state; second, an "extended" active dimer in which inter-JH1 phosphorylation relieves autoinhibition and stabilizes the open conformation [28,51,82].

This hierarchical allosteric control enables JAK2 to respond with remarkable fidelity to external cues: small conformational shifts within JH2 are amplified through interdomain communication, yielding a binary on/off catalytic switch. Such modular control also explains how distinct receptors impose unique activation geometries by shaping the spatial relationship between the FERM–SH2 anchor and the kinase core, thereby coupling structural organization to signaling specificity. Any mutation or external perturbation that weakens these autoinhibitory contacts, distorts ATP sensing, or misaligns the receptor interface can therefore tilt the equilibrium toward a constitutively active state, setting the stage for pathogenic activation.

3.2.2. Structural Mechanisms of Pathogenic Activation

Pathogenic activation of JAK2 results from discrete structural perturbations that either release the intrinsic autoinhibition described above or stabilize conformations that mimic cytokine-bound receptor complexes. Crystallographic, cryo-EM, and computational models collectively reveal that oncogenic JAK2 mutations exploit the same molecular surfaces that regulate physiological activation, transforming transient allosteric transitions into constitutive signaling states [51,82].

A unifying concept emerging from these studies is that gain-of-function (GOF) mutations cluster within three structural hotspots: the SH2–JH2 linker, the pseudokinase (JH2) core, and the JH2–JH1 interface. Each region contributes uniquely to maintaining the inactive conformation, and disruption of any one can trigger constitutive activation.

1. JH2–JH1 interface destabilization: mutations such as R683S/G in exon 16 weaken the electrostatic and hydrophobic clamp between JH2 and JH1, uncoupling the autoinhibitory brace that restricts the catalytic cleft. This loss of interdomain restraint allows the kinase lobes to adopt an open, phosphorylation-competent geometry even in the absence of receptor engagement [51]. These mutations are commonly observed in lymphoid malignancies, where they drive hyperactivation of STAT1 and STAT3 signaling [76].
2. SH2–JH2 linker perturbation: Mutations within exon 12, typified by K539L, alter the helical hinge connecting the SH2 and JH2 domains. This linker functions as a flexible tether transmitting receptor-derived mechanical signals to JH2. Its destabilization promotes partial opening of the JH2–JH1 assembly and increases basal catalytic turnover [51]. Such mutants show modest ligand-independent signaling but heightened responsiveness to low cytokine concentrations, consistent with the polycythemia vera phenotype.
3. JH2 core activation and dimer stabilization: The archetypal V617F mutation, located on the αC-helix of JH2, introduces an aromatic residue that forms π–π stacking across JH2 dimers (Figure 5), stabilizing a configuration equivalent to cytokine-induced receptor dimerization [82]. This conformational lock enforces persistent juxtaposition of JH1 domains, enabling unrestrained trans-phosphorylation and constitutive activation of STAT5. Cryo-EM reconstructions and molecular simulations confirm that the V617F-

driven JH2–JH2 interface mimics the spatial orientation observed in ligand-bound EpoR–JAK2 complexes [28].

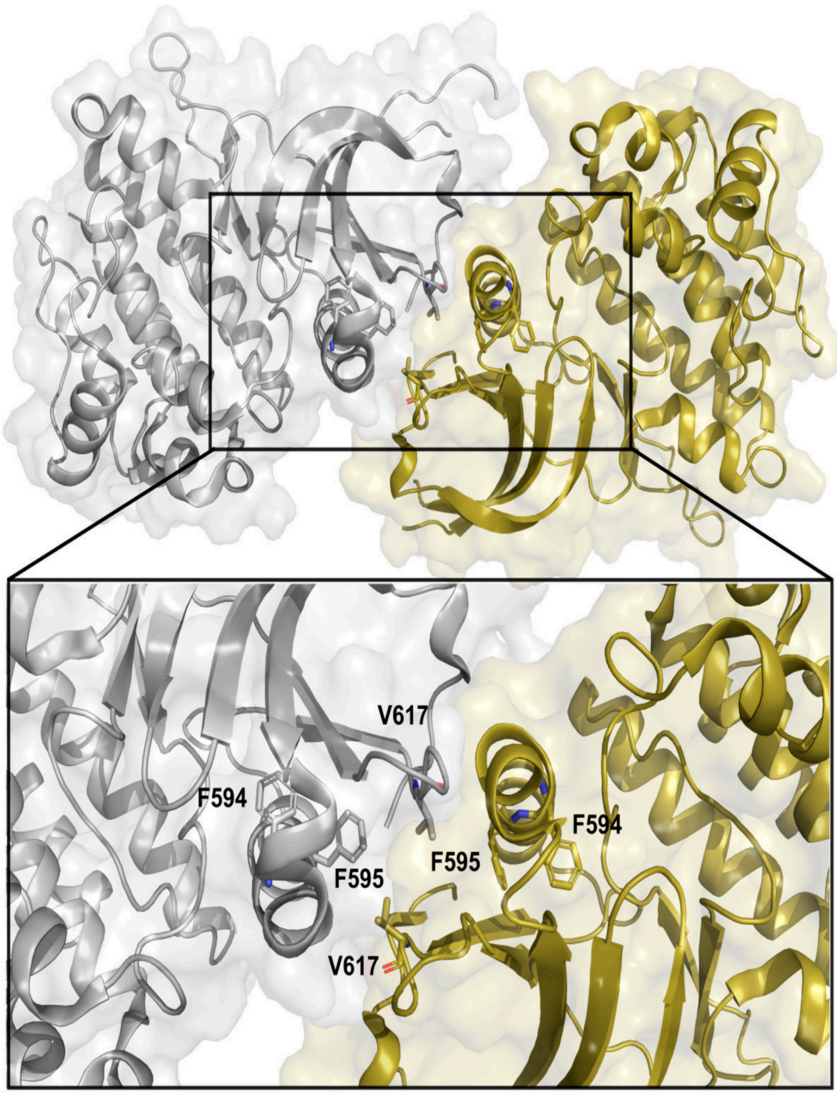


Figure 5. Structural representation of the JAK2 pseudokinase domain (JH2; PDB ID: 8C08) shown as a dimer, with the two protomers displayed in light gray and gold, respectively. The inset focuses on the V617F substitution and the adjacent aromatic residues F594 and F595, represented as stick models colored by their parent protomer (gray or gold), with heteroatoms highlighted in standard colors (blue for nitrogen, red for oxygen). These aromatic contacts form a stabilizing cluster within JH2, reducing its autoinhibitory flexibility and favoring constitutive activation of the adjacent JH1 kinase domain.

These mechanisms exemplify a common pathogenic theme: oncogenic JAK2 mutations convert a dynamic, reversible signaling module into a structurally locked active dimer. In the wild-type enzyme, JH2 oscillates between inhibitory and permissive states under the control of receptor geometry and cytokine occupancy. Mutations such as V617F, K539L, and R683S shift this equilibrium toward the open conformation, decoupling JAK2 from receptor-mediated signaling and leading to chronic activation of downstream STATs. This constitutive phosphorylation promotes cytokine-independent growth and alters receptor specificity by favoring engagement of EpoR and TpoR even in the absence of ligand, thus rewiring hematopoietic signaling circuits [77,83].

3.2.3. Pathway Bias, Functional Divergence, and Functional Consequences

Although pathogenic JAK2 mutations converge on constitutive kinase activation, they do not produce identical signaling outputs. The biological consequences of JAK2 activation depend on how individual mutations reshape receptor coupling, STAT utilization, feedback regulation, and lineage-specific transcriptional programs. Interface-disrupting mutants favor STAT1/3 phosphorylation and inflammatory gene expression, whereas core-stabilizing variants, such as V617F, preferentially drive STAT5-centric transcriptional programs that promote proliferation and erythroid differentiation [83]. Moreover, differences in receptor compatibility amplify this divergence: V617F and exon 12 mutants signal most efficiently through EpoR and TpoR, whereas R683S/G variants often aberrantly associate with IL-7R or CRLF2 complexes, altering lineage outcomes [81].

The constitutive activation of JAK2 introduced by pathogenic mutations reconfigures intracellular signaling networks, decoupling them from cytokine availability and receptor regulation. At the biochemical level, persistent JH1 activity results in sustained phosphorylation of receptor cytoplasmic tails and chronic activation of STAT transcription factors, particularly STAT5 and STAT3. This unrestrained flux overrides the transient, pulsatile dynamics characteristic of physiological cytokine signaling, transforming what should be a regulated developmental cue into a stable transcriptional program [51].

Oncogenic JAK2 variants exhibit distinct STAT-activation spectra that mirror their structural class. V617F and exon 12 mutants maintain the geometry compatible with EpoR and TpoR, resulting in persistent phosphorylation of STAT5, which dimerizes, translocates to the nucleus, and drives expression of pro-survival and proliferative genes such as BCL2L1 (Bcl-xL), CISH, PIM1, SOCS2, and CCND2 [84]. In contrast, mutations such as R683S/G and F595A bias the catalytic core toward STAT1/STAT3 activation, yielding an inflammatory and anti-apoptotic transcriptional profile enriched for IRF1, SOCS3, CXCL10, and IL6 [81].

Pathogenic activation also broadens receptor promiscuity. Under normal conditions, JAK2 associates primarily with homodimeric receptors such as EpoR, TpoR, GHR, and PRLR, whose Box1/Box2 motifs align the kinase for precise trans-phosphorylation [28]. Mutant JAK2 molecules, however, can aberrantly engage heteromeric receptors such as IL-3Rβ, IL-7R, and CRLF2, often in the absence of cytokine ligands. This receptor cross-activation reshapes lineage-commitment programs, permitting erythroid-specific mutants like V617F to activate myeloid or lymphoid signaling circuits, further expanding the pathological repertoire of JAK2 [81].

Physiological JAK-STAT signaling is self-limiting: phosphorylated STATs induce the expression of suppressors such as SOCS1, SOCS3, and CIS, which bind the receptor–JAK complex to terminate signaling. In JAK2 mutants, persistent phosphorylation of these inhibitors paradoxically inactivates them or causes their proteasomal degradation, abolishing feedback restraint. The resulting feedback collapse amplifies signal amplitude and duration, perpetuating a feed-forward loop of STAT5 activation and anti-apoptotic gene expression [84]. At the cellular level, these rewirings yield hallmark morphological and metabolic phenotypes. Persistent STAT5 signaling promotes erythroid proliferation, enhanced heme biosynthesis, and cytoskeletal remodeling conducive to reticulocyte enucleation, while attenuated apoptosis ensures progenitor survival. Concurrently, aberrant STAT3/STAT1 activation reprograms metabolic flux toward glycolysis and oxidative stress tolerance, favoring malignant transformation [81,83].

These findings underscore that pathogenic JAK2 mutations are not functionally equivalent. Each reshapes the signaling landscape by establishing a distinct conformational equilibrium that influences STAT engagement, receptor preference, and the modulation of feedback regulators such as SOCS and PIAS. This mutation-specific bias defines the

molecular basis for the phenotypic diversity of JAK2-driven diseases, from erythrocytosis to lymphoid leukemia. Table 2 summarizes representative oncogenic JAK2 mutations, their structural locations, proposed mechanisms of activation, predominant signaling outputs, and associated disease phenotypes.

Table 2. Representative activating and disease-associated JAK2 variants and their biological consequences.

Mutation	Structural Location	Mechanism of Activation	Predominant Signaling Output	Associated Diseases	References
V617	JH2 αC-helix (exon 14)	Stabilization of JH2 dimer interface and disruption of autoinhibitory control	Strong STAT5 activation; erythroid and megakaryocytic proliferation	Essential thrombocythemia, Polycythemia vera, Primary myelofibrosis	[51,84]
Exon 12 mutation (e.g., K539L, N542-E543 del)	SH2-JH2 Linker	Destabilization of JH2-mediated autoinhibition	Predominantly STAT5 signaling	JAK2 V617F-negative polycythemia vera; idiopathic erythrocytosis-like presentations	[85–87]
R683S/G	JH2–JH1 interface	Disruption of inhibitory interdomain interactions	STAT1/STAT3 bias; cytokine-independent growth	B-cell acute lymphoblastic leukemia, especially Down syndrome-associated ALL and CRLF2-rearranged/Ph-like ALL	[88]
F595 substitutions	JH2 αC-helix region	Altered pseudokinase regulatory network	Variable STAT activation depending on substitution	Experimental and disease-associated activating variants	[89]
M535I	Exon 12/SH2–JH2 region	Destabilization of autoinhibitory conformation	Enhanced JAK–STAT signaling	Acute megakaryoblastic leukemia, particularly pediatric non-Down syndrome AMKL	[90]

4. Pharmacological Strategies for JAK2 Inhibition

4.1. Classification of Protein Kinase Inhibitors

Kinase inhibitors are classified systematically by mechanism of action and binding model [5,44]. Type I inhibitors bind to the hydrophobic ATP-binding pocket of kinases in their active conformation, in which the DFG motif adopts an inward orientation (DFG-in). Type I½ inhibitors interact with both the ATP-binding site and adjacent regions of the inactive enzyme conformation, where the aspartate residue of the DFG motif still faces the ATP-binding site. Type II inhibitors occupy a pocket adjacent to the ATP-binding site in kinases stabilized in an inactive (DFG-out) conformation. The greater conformational flexibility associated with the DFG-out state allows for greater structural diversity among kinases, often resulting in higher selectivity of Type II inhibitors compared to Type I. Type III inhibitors bind to a site adjacent to the ATP-binding site. In contrast, Type IV inhibitors bind away from it. Type V inhibitors are bivalent molecules that reversibly interact with both the ATP-binding site and an adjacent allosteric region. Finally, Type VI inhibitors bind covalently to a nucleophilic residue, typically a cysteine, on the target kinase.

The development of several selective JAK inhibitors over the past decade underscores the central importance of this kinase family in contemporary drug discovery. Recently, Perner et al. (2025) [91] provided an overview of approved JAK inhibitors, their primary therapeutic applications, and late-stage clinical trials for selective compounds. Ruxolitinib, approved by the FDA in 2011 for the treatment of myelofibrosis, was the first-in-class JAK inhibitor [92]. It acts as a Type I inhibitor, binding to the ATP-binding site of JAK in its active conformation. Subsequently, in 2012, tofacitinib, another Type I inhibitor, received approval for the treatment of autoimmune diseases [93]. Currently, in addition to these two drugs, other JAK inhibitors are in clinical use, including baricitinib (JAK1 and JAK2) [94], upadacitinib (JAK1) [95], and fedratinib (JAK2) [96].

Table 3 provides an overview of approved late-stage clinical candidates targeting the Jak family, encompassing inhibitors with distinct selectivity profiles and mechanisms of action.

These inhibitors illustrate the evolution of JAK inhibition from first-generation Type I ATP-competitive compounds such as Ruxolitinib, fedratinib, and baricitinib to compounds that exploit allosteric or covalent binding mechanisms, including deucravacitinib and ritlecitinib [96]. The table highlights their respective kinase targets, inhibition classes, regulatory status, and therapeutic indications, reflecting the broad clinical applicability of JAK inhibition across myeloproliferative neoplasms, autoimmune, and inflammatory disorders.

Table 3. Overview of clinically approved JAK2 inhibitors and advanced clinical candidates, including indications and selectivity profiles. Adapted from Perner et al., 2025 [91].

Inhibitor	Main Target/Selectivity	Binding Class/Type	Regulatory Status	Approved Usage	Selected Advanced Trials/Non-Approved Uses	References
Ruxolitinib	JAK1/JAK2	Type I; ATP-competitive JAK inhibitor	FDA and EMA approved	Oral: intermediate/high-risk myelofibrosis; polycythemia vera after inadequate response/intolerance to hydroxyurea; steroid-refractory or steroid-dependent acute and chronic GVHD (age restrictions vary by label/region). Topical formulation has separate dermatologic approvals in some regions.	Investigational or additional contexts include alopecia areata, atopic dermatitis, psoriasis, T-ALL, essential thrombocythaemia, COVID-19, HLH, pancreatic cancer and vitiligo; these should not be presented as universal oral systemic approvals.	[91,97]
Momelotinib	JAK1/JAK2 plus ACVR1 activity	Type I; ATP-competitive	FDA and EMA approved	Adults with intermediate/high-risk myelofibrosis, including primary MF, post-PV MF and post-ET MF, with anemia or moderate-to-severe anemia depending on jurisdiction; indicated for disease-related splenomegaly/symptoms.	Other MPN/solid-tumor entries should be listed only if directly supported by a current trial registry search.	[98,99]
Fedratinib	JAK2-selective; also inhibits FLT3	Type I; ATP-competitive	FDA and EMA approved	Adults with primary MF, post-PV MF or post-ET MF with disease-related splenomegaly/symptoms; used in JAK-inhibitor-naive patients or after ruxolitinib according to regional wording.	No additional indication should be listed without current registry confirmation.	[100]
Pacritinib	JAK2/IRAK1/ACVR1; also reported FLT3 activity	Type I; ATP-competitive	FDA approved; not EMA approved	Adults with intermediate- or high-risk primary or secondary MF with platelet count below 50×10^9/L.	Continued clinical investigation in cytopenic MF settings; not an EMA-approved medicine.	[101–104]
Tofacitinib	JAK1/JAK2/JAK3; functional JAK1/JAK3 predominance	Type I; ATP-competitive	FDA and EMA approved	Rheumatoid arthritis, psoriatic arthritis, ulcerative colitis, ankylosing spondylitis and polyarticular-course juvenile idiopathic arthritis/juvenile idiopathic arthritis indications, with wording and age restrictions varying by region.	Alopecia areata, psoriasis, Takayasu arteritis and other immune-mediated conditions should be listed as investigational/off-label unless label-confirmed for the target region.	[105]

Table 3. *Cont.*

Inhibitor	Main Target/Selectivity	Binding Class/Type	Regulatory Status	Approved Usage	Selected Advanced Trials/Non-Approved Uses	References
Baricitinib	JAK1/JAK2	Type I; ATP-competitive	FDA and EMA approved	FDA: rheumatoid arthritis, severe alopecia areata in adults and COVID-19 in selected hospitalized patients. EMA: rheumatoid arthritis, atopic dermatitis, alopecia areata and selected pediatric/inflammatory indications depending on current product information.	Juvenile idiopathic arthritis, systemic lupus erythematosus and other indications require region-specific confirmation.	[105,106]
Peficitinib	Pan-JAK; JAK3 > JAK1/JAK2/ TYK2 reported	Type I; ATP-competitive	Not FDA or EMA approved; approved in Japan, Korea and Taiwan	Rheumatoid arthritis in approved Asian jurisdictions.	Not an FDA/EMA advanced clinical candidate unless supported by an active current trial.	[107,108]
Upadacitinib	JAK1-selective over JAK2/JAK3/TYK2	ATP-competitive JAK inhibitor; type not usually stated in labels	FDA and EMA approved	Rheumatoid arthritis, psoriatic arthritis, axial spondyloarthritis/ankylosing spondylitis, atopic dermatitis, ulcerative colitis, Crohn's disease, polyarticular-course JIA and giant cell arteritis, with label-specific restrictions.	Additional vasculitis/immune indications may be under study; AD, UC and Crohn's disease should not be left only in the clinical-trials column because they are approved indications.	[109,110]
Filgotinib	JAK1-selective over JAK2	Type I/ATP-competitive JAK inhibitor	EMA approved; not FDA approved	EU/EMA: moderate-to-severe active rheumatoid arthritis and moderate-to-severe active ulcerative colitis in adults.	Crohn's disease and psoriatic arthritis should be kept as investigational/non-approved unless region-specific approval is documented.	[110]
Deucravacitinib	TYK2; allosteric pseudokinase/ JH2 binding	Type IV; allosteric TYK2 inhibitor	FDA and EMA approved for plaque psoriasis; FDA also approved active psoriatic arthritis in adults	FDA: moderate-to-severe plaque psoriasis in adults; active psoriatic arthritis in adults. EMA: moderate-to-severe plaque psoriasis in adults eligible for systemic therapy.	PsA should no longer be listed as merely Phase 3 for the US. Other TYK2 development areas require current trial confirmation.	[111]
Ritlecitinib	Covalent JAK3 and TEC-family kinase inhibitor	Type VI/covalent irreversible inhibitor	FDA and EMA approved	Severe alopecia areata in adults and adolescents 12 years and older.	Other autoimmune indications are investigational unless label-confirmed.	[112]
Izencitinib	Gut-selective pan-JAK: JAK1/JAK2/ JAK3/TYK2	ATP-competitive; formal binding class not consistently specified	Not approved; development status uncertain/discontinued after Phase 2/ 2b-3 programs	No approved indication.	Crohn's disease Phase 2 study and ulcerative colitis Phase 2b/3 program were completed/terminated; UC Phase 2b did not meet the primary endpoint. Do not list as active Phase 3 Crohn's disease candidate without fresh registry evidence.	[113]

The table was adapted and updated from Perner et al. (2025) [91] using official regulatory sources, including U.S. Food and Drug Administration (FDA) prescribing information, European Medicines Agency (EMA) European Public Assessment Reports/Product Information, and ClinicalTrials.gov records when applicable. Approved indications may vary according to jurisdiction; therefore, FDA and EMA approvals were reported separately when relevant. Investigational indications refer to selected clinical trial settings and should not be interpreted as approved uses. Abbreviations: FDA, U.S. Food and Drug Administration; EMA, European Medicines Agency; EPAR, European Public Assessment Report; JAK, Janus kinase; TYK2, tyrosine kinase 2; FLT3, FMS-like tyrosine kinase 3; GVHD, graft-versus-host disease; RA, rheumatoid arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; UC, ulcerative colitis; CD, Crohn's disease; MF, myelofibrosis; PV, polycythemia vera; ET, essential thrombocythemia; AA, alopecia areata; PsO, psoriasis; AD, atopic dermatitis; GCA, giant cell arteritis; pJIA, polyarticular juvenile idiopathic arthritis.

The following section focuses on the structural and mechanistic basis of JAK2 inhibition, examining how clinically approved compounds engage the kinase domain and how structure–activity relationship (SAR) studies have informed the rational design of improved JAK2-selective inhibitors.

4.2. JAK2-Approved Inhibitors

The JAK2 inhibitors listed in the table above, including Ruxolitinib, Momelotinib, Fedratinib, Pacritinib, Tofacitinib, Baricitinib, Peficitinib, Upadacitinib, and Filgotinib, share a conserved binding mechanism centered on the ATP-binding pocket of the JAK kinase domain (JH1). Despite differences in selectivity across the JAK family (JAK1, JAK2, JAK3, TYK2), these molecules are all classified as Type I ATP-competitive inhibitors (Figure 6).

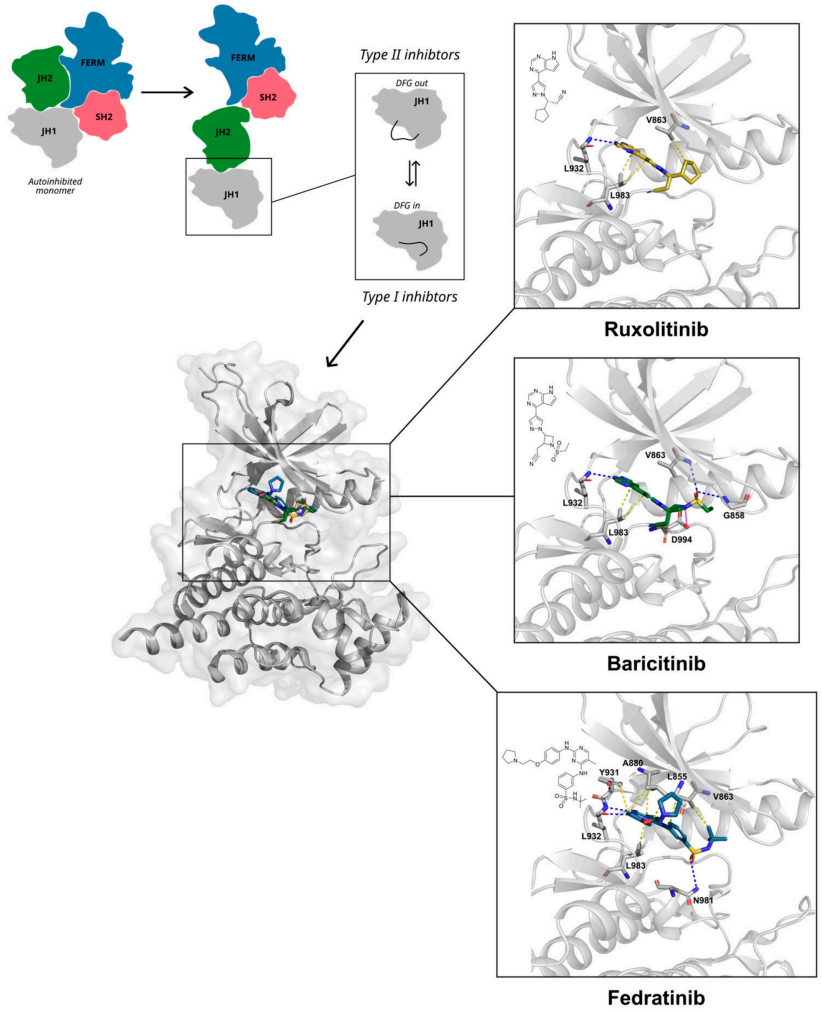


Figure 6. Structural basis of JAK2 pseudokinase domain regulation and the effects of pathogenic mutations. The illustration depicts the proposed transition from the autoinhibited to the active JAK2 conformation, in which the pseudokinase domain (JH2) restrains the catalytic kinase domain (JH1) through interdomain interactions. Structural rearrangements within JH2 relieve this inhibition and promote JH1 activation. The figure also shows the co-crystal structures of the JAK2 kinase domain (green cartoon) bound to ruxolitinib (red sticks), fedratinib (blue sticks), and baricitinib (green sticks), corresponding to PDB entries 6VGL, 6VNE, and 6VN8, respectively. Close-up views of the ATP-binding site highlight the interactions formed by ATP and the selected inhibitors, with hydrogen bonds and polar contacts represented as dashed lines. The V617F-associated regulatory region is located adjacent to the JH2 αC-helix, where pathogenic mutations disrupt the allosteric network responsible for maintaining autoinhibition, thereby promoting constitutive JAK2 activation. Protein residues within 4 Å of the ligand are shown as sticks.

Structurally, they act as adenine mimetics: their heteroaromatic scaffolds (usually pyrrolopyrimidine cores) occupy the same site as ATP's adenine ring, forming two key hydrogen bonds with the hinge residues of JAK2 (typically L932 and E930). These interactions anchor the inhibitor within the catalytic cleft. The remainder of each molecule extends into adjacent hydrophobic subpockets (such as the gatekeeper and back-pocket regions), forming van der Waals, π–π stacking, and dipolar contacts that enhance affinity and subtype selectivity [43,114].

The study by Davis et al. (2021) provides a seminal contribution to the structural biology of JAK2 by reporting the first co-crystal structures of its kinase domain (KD) bound to the clinically approved inhibitors ruxolitinib and fedratinib, as well as the rheumatoid arthritis drug baricitinib and a series of novel derivatives [35] (Figure 7). This breakthrough was enabled by a novel methodology for producing high-quality JAK2 protein from mammalian cells, overcoming historical challenges associated with the instability of recombinant JAK2.

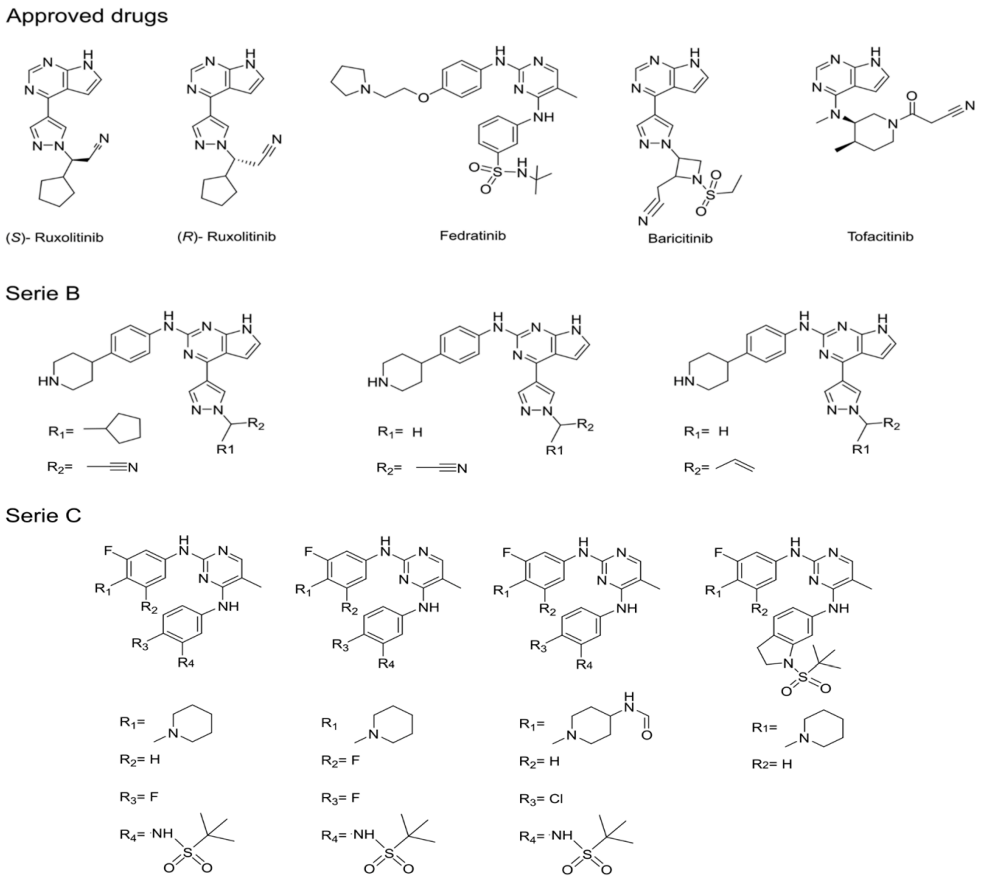


Figure 7. A series of JAK2 inhibitors was subjected to SAR studies: Ruxolitinib isomers and FDA-approved derivatives, piperidine–phenylamine analogs of ruxolitinib, and fedratinib and its derived dual JAK2–BRD4 inhibitors.

4.2.1. Binding Mode and Stereoselectivity

Co-crystal structures establish that pyrrolopyrimidine Type I inhibitors bind competitively in the ATP-binding pocket of JAK2, anchored by hydrogen bonds to the hinge residue L932, and further stabilized by hydrophobic contacts along the P-loop (L855/G856) and DFG motif (D994) [35]. In the resolved JAK2–ruxolitinib complex, only the (R)-enantiomer adopts a productive pose: the (S)-isomer requires ~180° rotation around the chiral center, weakening hinge interactions and pocket complementarity, consistent with the >10-fold potency difference ((R) $IC_{50} \approx 0.4$ nM vs. (S) ≈ 5 nM). Crystal soaking with a racemic ligand yields exclusive (R)-occupancy, demonstrating absolute stereochemical selectivity in the

ATP pocket [36]. The JAK2–baricitinib complex overlays closely with that of (R)-ruxolitinib, preserving hinge H-bond geometry and pocket packing, which accounts for their similar biochemical inhibition profiles [35]. Thus, the ATP-site binding geometry of JAK2 imposes a strict stereochemical requirement for optimal affinity in pyrrolopyrimidine inhibitors. At the same time, baricitinib circumvents this constraint through an achiral scaffold that preserves the hinge-binding topology and hydrophobic pocket occupancy. This stereochemical requirement is generalizable: analogous aniline–pyrrolopyrimidine derivatives exhibit the same enantioselective binding behavior, with (R)-isomers consistently achieving sub-nanomolar to picomolar inhibitory potency [35].

4.2.2. Series B (Piperidine–Aniline Analogs of Ruxolitinib)

Piperidine–aniline derivatives (series B) achieve sub-nanomolar potency (IC_{50} < 0.26 nM) by introducing an additional hinge H-bond from the aniline N–H to L932, strengthening the anchoring interaction beyond the pyrrolopyrimidine contacts with E930–L932. However, this added polarity reduces cell permeability, leading to weaker cellular efficacy despite excellent enzymatic inhibition. This illustrates a recurring structure–pharmacokinetic trade-off: more H-bonds lead to greater in vitro potency and reduced permeability in cells. This structural mimicry explains the comparable biochemical potency and similar inhibitory profiles in cellular systems driven by constitutively active JAK2 [35].

4.2.3. Fedratinib and Series C Analogs

Fedratinib and its series C analogs also bind at the ATP site but lack the pyrrolo moiety and therefore form only a single hinge H-bond with L932. This shift correlates with moderately weaker potency (IC_{50} ≈ 0.75–11.4 nM) relative to ruxolitinib. Subtle conformational changes in the aniline–sulfonamide vector can reposition the ligand relative to the DFG region (D994), modulating inhibition. Notably, series C also inhibits BRD4, and the suppression of JAK2-driven UKE-1 cell growth is attributed to combined JAK2 + BRD4 inhibition, demonstrating the functional relevance of polypharmacology. The work by Davis et al. [35] fundamentally advances our understanding of how small molecules inhibit JAK2. The detailed structural data confirm the binding poses of first-generation drugs such as ruxolitinib and fedratinib, resolving prior uncertainties. Explain the molecular basis for stereoselectivity, which is crucial for designing future chiral inhibitors. Reveal strategies for enhancing potency, such as the addition of hinge-binding aniline groups. Provide a robust platform for SAR by demonstrating excellent correlation between thermal shift (DSF) data and enzymatic inhibition. These insights provide a critical foundation for the structure-guided design of next-generation JAK2 inhibitors with improved selectivity, potency, and therapeutic profiles for the treatment of myeloproliferative neoplasms and autoimmune diseases.

4.3. Emerging Strategies for Next-Generation JAK2 Inhibition

First-generation JAK inhibitors (e.g., ruxolitinib and fedratinib) bind the active "DFG-in" conformation of the JH1 domain and effectively suppress downstream STAT signaling. However, their clinical benefit in MPNs is largely palliative, with limited reduction in mutant allele burden and frequent dose-limiting cytopenias, reflecting incomplete pathway suppression and inhibition of JAK1-dependent immunoregulatory signaling. Moreover, persistent signaling can emerge despite continued drug exposure through epigenetic up-regulation and stabilization of phosphorylated JAK2, leading to heterodimer-dependent pathway reactivation rather than classical kinase mutation-driven resistance [115]. These limitations have motivated the development of new inhibitory strategies that target alternative conformations, domains, or regulatory interactions of JAK2.

Below, we summarize four major mechanistic advances derived from recent structural and SAR-driven medicinal chemistry efforts.

4.3.1. Covalent and Isoform-Selective Inhibitors Targeting Unique Residues

Covalent inhibition enables prolonged target engagement by forming a covalent bond with a reactive residue near the active site. JAK3 is uniquely amenable to this strategy due to the presence of a non-conserved cysteine (C909) proximal to the ATP-binding pocket. Ritlecitinib (PF-06651600) exemplifies a type V covalent inhibitor that selectively modifies C909, achieving >90-fold selectivity over kinases with homologous cysteines, thereby limiting hematopoietic suppression. Ritlecitinib has demonstrated strong efficacy in alopecia areata and vitiligo, confirming that covalent strategies can be clinically viable [116]. Analogous approaches have recently been adapted to JAK1, using reversible covalent warheads to engage nucleophile-tuned pockets adjacent to the hinge, enabling prolonged action with fewer immunologic side effects [117].

Although JAK2 lacks a suitable nucleophilic cysteine for analogous covalent engagement, covalent strategies remain conceptually relevant when redesigned to target allosteric regulatory surfaces rather than the ATP site.

4.3.2. Type II JAK2 Inhibitors: Stabilizing the Inactive "DFG-Out" Conformation

Type II inhibitors bind JAK2 in its inactive conformation, occupying both the ATP-binding pocket and the adjacent allosteric hydrophobic pocket, which becomes accessible only upon DFG-out activation-loop rearrangement. Unlike Type I inhibitors, which suppress signaling without eliminating the mutant clone, Type II inhibitors demonstrate superior suppression of JAK2V617F-driven signaling, including reduction in mutant allele burden in vivo [118]. Examples are: BBT594 (NVP-BBT594) demonstrated that locking JAK2 in an inactive conformation can effectively block activation loop phosphorylation, though limited by off-target liabilities; and CHZ868 (NVP-CHZ868) improved upon BBT594 using structure-guided scaffold refinement and displayed robust efficacy in MPN models, including suppression of splenomegaly and normalization of hematologic parameters [118].

Type II inhibitors may overcome pathway persistence, making them particularly promising in MPNs, where JAK2 is constitutively activated by the V617F mutation or by receptor-mutant signaling. In contrast to autoimmune disease, where broad and reversible inflammatory suppression is desirable, the disease-modifying potential of Type II inhibitors is uniquely suited for clonal myeloproliferation [119,120].

4.3.3. Macrocyclic JAK2 Inhibitors: Conformational Pre-Organization for Selectivity

Macrocyclization has recently emerged as a powerful tool to reduce conformational entropy penalties and improve selectivity toward JAK2 over JAK1/3/TYK2. Structure-guided optimization yielded compound 15au, a macrocyclic JAK2 inhibitor (IC_{50} = 2 nM) with 89.5-fold selectivity over JAK1, 80.5-fold over JAK3, and 51-fold over TYK2, improved metabolic stability and oral exposure, and potent activity in JAK2-V617F-driven MPN models at lower doses than pacritinib or fedratinib [121]. It is important to mention that this compound's selectivity reduces JAK1-linked immunosuppression and JAK3-linked lymphocyte dysfunction, making macrocyclic scaffolds strong candidates for chronic long-term treatment in MF and PV with fewer hematologic toxicities.

The high homology of the ATP-binding site across JAK family members has limited the selectivity of conventional kinase inhibitors, driving the search for alternative strategies that target the unique structural features of JAK2's regulatory machinery. These next-generation approaches include:

1. Targeting the Pseudokinase Domain (JH2): Developing allosteric inhibitors that exploit the lower sequence conservation in JH2 to achieve selectivity, particularly against mutants like V617F.
2. Modulating Protein Interactions: Designing compounds that disrupt JAK2-receptor binding, mimic the inhibitory function of SOCS proteins, or compete with substrate recruitment.
3. Exploiting Allosteric Sites: Discovering Type II inhibitors that stabilize inactive kinase conformations or developing molecules that target the interface between JAK2's regulatory domains.

4.3.4. Targeting the Pseudokinase (JH2) Regulatory Domain

The recognition that JAK2-mediated myeloproliferative neoplasms are not driven solely by excessive catalytic activity, but rather by a structural failure in the pseudokinase domain (JH2), has fundamentally shifted drug discovery efforts. The V617F mutation, which is present in approximately 95% of patients with polycythemia vera and in a substantial fraction of those with essential thrombocythemia and myelofibrosis, resides not in the catalytic kinase domain, but in JH2. Early biochemical and structural studies demonstrated that JH2 is not merely a catalytically impaired relic; rather, it functions as a regulatory domain, maintaining JAK2 in an autoinhibited conformation in the absence of ligand-induced receptor dimerization. The V617F substitution stabilizes a hydrophobic network surrounding the αC-helix of JH2, shifting the conformational equilibrium toward a partially active state, even in the absence of cytokine signaling. This produces a phenotype of ligand-independent STAT activation, clonal expansion of erythroid progenitors, and cytokine hypersensitivity, the core biological features of JAK2-driven MPNs.

The JH2 pseudokinase domain is the master regulator of JAK2 autoinhibition, catalytic tuning, and dimerization geometry. Unlike the highly conserved ATP cleft, the JH2 surface exhibits greater structural divergence, enabling isoform-specific inhibition. The landmark success of BMS-986165 (deucravacitinib), which binds an allosteric pocket in the TYK2 JH2 domain rather than the kinase cleft, validated the pseudokinase as a clinically druggable regulatory switch [122].

Emerging efforts now focus on JAK2-JH2-selective inhibitors, macrocyclic scaffolds, and peptides that disrupt JH2-JH1 intramolecular contacts or the JH2-mediated receptor dimer geometry. Macrocyclic compounds have successfully captured JH2 surface cavities, yielding sub-nanomolar selectivity for JAK2 over JAK1 and TYK2 in biochemical assays [121]. These next-generation scaffolds offer a path to therapeutic precision, suppressing mutant JAK2 signaling while preserving cytokine-regulated hematopoiesis.

Using crystallographic models of JH2 and medicinal-chemistry iterations centered on the hinge-binding core, the authors demonstrated that JH2 can indeed form well-defined ligand interactions, including hydrogen bonding with L579 and E592 in the hinge region. Importantly, binding of these ligands increased the thermal stability of JH2 in vitro, with stronger stabilization observed for the V617F mutant than for the wild-type domain, indicating mutation-biased affinity and a key advantage over ATP-competitive JH1 inhibitors. In cellular models, these compounds selectively reduced STAT5 phosphorylation and inhibited EPO-independent colony formation in patient-derived hematopoietic cultures, while preserving normal erythroid differentiation in wild-type systems. These findings provided the first experimental demonstration that small molecules can allosterically reverse the pathogenic conformation of JH2 [48,123].

Building on this concept, subsequent studies focused on improving cell permeability, metabolic stability, and interactions with the hydrophobic regulatory surface adjacent to the αC-helix, which is directly altered by the V617F mutation. Using structure-guided scaffold optimization, researchers developed cell-permeable JH2-directed modulators [123] that

rebalance the JH2–JH1 regulatory interface. These compounds do not act as classical kinase inhibitors; rather, they reduce the likelihood that JAK2 adopts the active conformation by stabilizing the autoinhibited pre-activation state. In contrast to ATP-competitive inhibitors, they do not significantly suppress EPO-induced JAK2 activation in wild-type hematopoiesis. This functional discrimination between inhibition of mutant signaling and preservation of physiological cytokine responses represents a therapeutic profile unmatched by existing JAK inhibitors.

The implication of these discoveries is profound. Current type I and type II JAK2 inhibitors improve symptoms but do not eliminate the malignant clone, in part because they target kinase activity rather than the structural mechanism of oncogenic activation. In contrast, JH2-targeted inhibitors directly engage the regulatory defect that causes disease, rather than merely blocking downstream phosphorylation. This means they have the potential to shift the underlying clonal dynamics of MPNs, offering not only disease control but also eventual true disease modification, which remains the primary unmet therapeutic need in this field.

Thus, the emergence of JH2-selective modulators marks a turning point in the pharmacologic approach to JAK2-driven disease. By restoring the natural balance between activation and autoinhibition, rather than forcing suppression of catalytic output, JH2-directed therapies represent a mechanistically rational path toward precise, mutation-biased, and hematopoiesis-preserving treatment of MPNs.

5. A Practical Example: Clinical–Laboratory Features and Management of JAK2-Mutated Myeloproliferative Neoplasms

Chronic BCR-ABL1-negative MPNs are classically associated with activating mutations in the Janus kinase 2 (JAK2) gene. The JAK2 V617F mutation is present in approximately 97% of patients with PV and in about 50–60% of cases of essential thrombocythemia (ET) and primary myelofibrosis (PMF). In addition, JAK2 exon 12 mutations are found in most of the remaining JAK2-positive PV cases. The most recent classifications of myeloid neoplasms, such as the International Consensus Classification (ICC) and the World Health Organization (WHO) update of 2022, emphasize the integration of bone marrow morphology, molecular profiling, and clinical findings as essential components of diagnostic definition and risk-adapted management [124].

Polycythemia vera typically presents with erythrocytosis in the setting of subnormal serum erythropoietin (EPO) levels. It is often accompanied by leukocytosis, thrombocytosis, pruritus, splenomegaly, and an increased risk of thrombotic events. The diagnostic criteria include elevated hemoglobin or hematocrit levels, bone marrow panmyelosis, and the presence of a JAK2 mutation, while low serum EPO represents a minor criterion. Diagnosis requires the presence of all three major criteria or a combination of two major and one minor. However, in certain patients with unequivocal erythrocytosis and JAK2 positivity, bone marrow biopsy may be deferred [124–126]. The principal therapeutic goal in PV is to prevent thrombosis, with a universal target of maintaining hematocrit below 45%. All patients should receive low-dose aspirin, unless contraindicated, and undergo phlebotomy to control hematocrit [126,127]. In high-risk patients, defined as those aged $\geq$60 years or with a history of thrombosis, cytoreduction is indicated. Hydroxyurea has long been the standard therapy, but in recent years, interferon-α, particularly ropeginterferon alfa-2b, has emerged as a preferred first-line option in younger patients and those seeking a disease-modifying approach. Ropeginterferon has demonstrated durable hematologic responses and reductions in JAK2 allele burden, suggesting the potential for molecular remissions [126,127]. For patients intolerant or resistant to hydroxyurea, interferon, or the JAK1/2 inhibitor ruxolitinib can be used [126]. In addition, the hepcidin mimetic rusfertide

has shown promising efficacy in reducing the need for phlebotomies and maintaining hematocrit control in clinical trials, with phase 3 results recently reported [128].

Essential thrombocythemia is defined by sustained thrombocytosis, megakaryocytic proliferation with atypia in the bone marrow, and exclusion of other myeloid neoplasms. Approximately 55% of patients harbor the JAK2 mutation, which significantly influences thrombotic risk. Clinically, arterial events are more common than venous ones, and microvascular disturbances such as erythromelalgia may occur. At very high platelet counts, acquired von Willebrand syndrome can predispose to bleeding complications [124,129]. Risk stratification has been refined by the revised International Prognostic Score for Thrombosis in Essential Thrombocythemia (IPSET-thrombosis), which incorporates age, prior thrombotic history, JAK2 mutation status, and cardiovascular risk factors to classify patients into very low, low, intermediate, and high-risk groups [130]. The cornerstone of therapy is low-dose aspirin for most patients, along with aggressive management of cardiovascular risk factors. Cytoreductive therapy is recommended for high-risk patients and for selected intermediate-risk or symptomatic individuals. Hydroxyurea remains the most widely used agent, while interferon-α is preferred in younger patients and during pregnancy. Anagrelide is an alternative option for patients intolerant of hydroxyurea, although it has a distinct toxicity profile. Routine use of JAK inhibitors is not currently recommended in chronic ET outside clinical trials [131].

Primary myelofibrosis is a heterogeneous disorder characterized by progressive bone marrow fibrosis, constitutional symptoms such as fatigue, night sweats, weight loss, anemia, splenomegaly, and circulating immature myeloid cells. Approximately half of patients carry the JAK2 V617F mutation, while others may harbor CALR or MPL mutations. Diagnosis relies on a combination of clinical, morphologic, and molecular features, as defined by the ICC and WHO classifications [124]. Prognosis is determined using validated scoring systems such as DIPSS-plus, MIPSS70+ v2.0, and GIPSS, which integrate clinical and genetic variables [131]. Therapeutic decisions are challenging because of the heterogeneity of presentation and prognosis. JAK inhibitors are central to symptoms and spleen management. Ruxolitinib remains the most established therapy, while fedratinib has demonstrated efficacy in patients previously treated with ruxolitinib or as upfront therapy in higher-risk disease [131]. Pacritinib has been specifically approved for patients with severe thrombocytopenia (platelet counts $< 50 \times 10^9$/L), and momelotinib, approved in 2023, offers the additional advantage of improving anemia through ACVR1 inhibition [131–133]. For anemia management, erythropoiesis-stimulating agents may be considered in patients with low or normal EPO, while danazol and androgens are used in select cases. Recently, luspatercept has shown encouraging results in improving anemia in myelofibrosis, including in transfusion-dependent patients [9]. The only curative treatment for PMF remains allogeneic hematopoietic cell transplantation, which is reserved for younger, fit patients with intermediate-2 or high-risk disease [131].

An additional consideration across MPNs is the monitoring of JAK2 allele burden. In PV, higher V617F allele burden has been associated with symptoms such as pruritus and splenomegaly, as well as increased thrombotic risk. Interferon therapy is notable for its ability to reduce mutant allele burden, in contrast to hydroxyurea, which primarily controls blood counts without significantly altering clonal architecture. Response evaluation follows European LeukemiaNet (ELN) and International Working Group criteria, incorporating hematologic normalization, symptom relief, and spleen size reduction. Regular monitoring includes complete blood counts, ferritin in PV, LDH levels, and vigilance for thrombotic and hemorrhagic complications [126,127,129,130].

In summary, JAK2 mutations are central to the pathogenesis, diagnosis, and management of the classic BCR-ABL1-negative MPNs. Advances in classification and therapeutic

options over the past five years have reshaped clinical practice, highlighting interferon as a disease-modifying therapy in PV, refining risk stratification in ET, and the expansion of JAK inhibitors in MF with tailored indications for cytopenic subsets. These developments underscore the importance of integrating molecular insights with clinical and laboratory parameters to achieve precision medicine in MPNs.

6. Outlook and Future Directions

Over the past decade, structural, computational, and biochemical advances have transformed our understanding of how JAK2 integrates receptor geometry, allosteric regulation, and catalytic activation into a unified signaling system. Yet, despite this progress, several critical questions remain unresolved. Addressing these gaps will be essential not only for a complete mechanistic understanding of JAK2 regulation but also for the development of next-generation therapeutics that specifically modulate disease-driving conformational states.

Structures of full-length JAK2 in native receptor complexes: Current models of JAK2 activation rely on fragmentary structures, domain-level crystallography, and integrative modeling. High-resolution cryo-EM structures of full-length JAK2 bound to intact cytokine receptors, ideally in both inactive and active states, would provide definitive insight into how receptor geometry dictates the alignment of the FERM–SH2, JH2, and JH1 domains. These structures are essential to understanding how ligand identity, receptor stoichiometry, and extracellular conformational tuning influence intracellular activation.

Molecular basis of physiological and pathogenic JH2–JH2 dimerization: While V617F stabilizes the active JH2–JH2 interface, the geometry and energetic landscape of wild-type JH2 dimerization remain poorly defined. Determining whether wild-type JH2 forms transient dimers, and how receptor pairing constrains this interaction, will clarify how physiological activation differs from pathogenic stabilization.

Quantitative energetics of the JH2–JH1 autoinhibitory interface: The autoinhibitory grip imposed by JH2 is central to JAK2 regulation, yet the energetic architecture underlying this control, including the effects of ATP binding, phosphorylation, and mutations on interface strength, remains largely unexplored. High-resolution dynamics studies (NMR, HDX-MS, cryo-EM with conformational sampling, and long-timescale MD) could reveal how subtle changes propagate through the spine networks of JH1.

Receptor-specific modulation and STAT bias: Pathogenic mutations produce distinct STAT1, STAT3, and STAT5 signaling outcomes, suggesting that receptor pairing, JH2 geometry, and STAT recruitment are coordinated within a precise structural framework. Dissecting how different cytokine receptors impose unique intracellular alignment constraints will be pivotal for understanding lineage-specific outcomes and the mechanistic basis of biased signaling in disease.

Development of JH2-selective allosteric inhibitors: The success of the TYK2 JH2 inhibitor deucravacitinib demonstrates that targeting pseudokinase domains can yield high selectivity and improved therapeutic profiles. JH2-directed small molecules for JAK2 represent one of the most promising directions for disease-modifying therapy in MPNs. Structural mapping of druggable JH2 pockets, combined with ligand-based stabilization of autoinhibitory conformations or disruption of the V617F aromatic cluster, could enable selective suppression of pathogenic activation while preserving physiologic cytokine signaling.

Mechanistically grounded models of disease progression: Current clinical management of MPNs does not incorporate structural or conformational biomarkers. Integrating mutation-specific signaling architecture with transcriptomic, chromatin, and microenvironmental data could provide predictive frameworks for disease evolution, therapeutic response, and malignant transformation.

7. Conclusions

JAK2 has emerged as a paradigmatic example of how multidomain protein kinases integrate receptor organization, allosteric regulation, and catalytic activation into a highly coordinated signaling system. Rather than functioning as an isolated catalytic module, JAK2 operates through dynamic communication among the FERM–SH2, JH2, and JH1 domains, enabling precise control of cytokine-dependent signaling. Pathogenic mutations exploit this regulatory architecture by destabilizing autoinhibition, promoting aberrant dimerization, or altering interdomain communication, thereby generating distinct signaling outputs and disease phenotypes.

Therapeutic strategies must therefore consider the full conformational landscape of JAK2. While first-generation type I inhibitors effectively suppress symptoms, they do not correct the structural lesions driving constitutive activation. Type II inhibitors, macrocyclic scaffolds, and emerging JH2-selective allosteric modulators highlight the potential of state-specific and domain-focused interventions that more precisely intercept the structural mechanisms underlying disease. As structural and biophysical tools continue to evolve, they will enable rational design of inhibitors that restore autoinhibition, disrupt pathogenic dimerization, or selectively modulate mutant-specific conformations.

By unifying domain architecture, receptor geometry, pathogenic activation, and mechanism-based drug design, this review outlines a coherent structural framework for understanding JAK2 regulation. Continued progress in this direction will be essential for the development of next-generation therapies that selectively target mutant JAK2 while preserving physiological cytokine signaling, with profound implications for hematologic disease, immunobiology, and precision pharmacology.

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