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Review

# Deucravacitinib is an allosteric TYK2 protein kinase inhibitor FDA-approved for the treatment of psoriasis

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ABSTRACT

Psoriasis is a heterogeneous, inflammatory, autoimmune skin disease that affects up to 2% of the world's population. There are many treatment modalities including topical medicines, ultraviolet light therapy, monoclonal antibodies, and several oral medications. Cytokines play a central role in the pathogenesis of this disorder including TNF-α, (tumor necrosis factor-α) IL-17A (interleukin-17A), IL-17F, IL-22, and IL-23. Cytokine signaling involves transduction mediated by the JAK-STAT pathway. There are four JAKS (JAK1/2/3 and TYK2) and six STATS (signal transducer and activators of transcription). Janus kinases contain an inactive JH2 domain that is aminoterminal to the active JH1 domain. Under basal conditions, the JH2 domain inhibits the activity of the JH1 domain. Deucravacitinib is an orally effective *N*-trideuteromethyl-pyridazine derivative that targets and stabilizes the TYK2 JH2 domain and thereby blocks TYK2 JH1 activity. Seven other JAK inhibitors, which target the JAK family JH1 domain, are prescribed for the treatment of neoplastic and other inflammatory diseases. The use of deuterium in the trimethylamide decreases the rate of demethylation and slows the production of a metabolite that is active against a variety of targets in addition to TYK2. A second unique aspect in the development of deucravacitinib is the targeting of a pseudokinase domain. Deucravacitinib is rather specific for TYK2 and its toxic effects are much less than those of the other FDA-approved JAK inhibitors. The successful development of deucravacitinib may stimulate the development of additional pseudokinase ligands for the JAK family and for other kinase families as well.

## 1. An overview of psoriasis

Psoriasis is a heterogeneous, inflammatory, autoimmune skin disease that is characterized by erythematous (reddish) sharply demarcated rounded plaques covered by silvery mica-like (micaceous) scales [1]. Patients with this disorder have slowly enlarging plaques which remain more or less unchanged for long periods of time. The most commonly involved areas involve the knees, elbows, gluteal cleft, and scalp. The skin lesions are variably pruritic. Plaque psoriasis develops slowly and runs an indolent course and it rarely remits spontaneously. Psoriasis is one of the most common skin disorders and affects up to 2% of the world's population. Accordingly, the potential market for treatments and drugs is large. Up to 30% of people with psoriasis develop psoriatic arthritis which occurs between the ages of 30 and 50 years.

Psoriasis is associated with many treatment modalities that vary with the severity of the disease [2–4]. These treatments may employ a variety of corticosteroids, both topically, orally, or by injection. Topical steroids such as hydrocortisone are used for the treatment of mild to moderate psoriasis. Stronger medications such as triamcinolone or clobetasol are used for treatment-resistant areas. Long-term use or overuse of strong corticosteroids thins the skin. Over time, topical steroids may lose their effectiveness, a general phenomenon called tachyphylaxis. Topical vitamin D analogues including calcipotriene or calcitrol slow epidermal cell growth and may be used in combination with topical steroids. Topical calcineurin inhibitors such as tacrolimus and pimecrolimis reduce scaling. Owing to the increased risk of skin cancer or lymphoma, they are precluded from long-term use. Calcineurin is a calcium-calmodulin–dependent protein-serine/threonine phosphatase and

*Abbreviations:* AS, activation segment; CML, chronic myelogenous leukemia; CS or C-spine, catalytic spine; CS1, catalytic spine residue 1; CL, catalytic loop; EGFR, epidermal growth factor receptor; GK, gatekeeper; GRL, glycine-rich loop; HΦ or Φ, hydrophobic; IFNλR, interferon lambda receptor; IL, interleukin; JAK1/2/3, Janus kinases 1/2/3; JH, JAK homology; KLIFS-3, kinase-ligand interaction fingerprint and structure residue-3; NK cell, natural killer cell; PDGFR, platelet-derived growth factor receptor; PKA, protein kinase A; ΨK, Pseudokinase; Ro5, Lipinski rule of 5; RS or R-spine, regulatory spine; RS1, regulatory spine residue 1; Sh2, shell residue 2; STAT, signal transducer and activator of transcription; TNF, tumor necrosis factor.

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blockade of this enzyme decreases immune signaling. Topical salicylic acid is a keratolytic agent used for the treatment of numerous dermatologic conditions including psoriasis, dandruff, acne, skin tags, and calluses. Topical coal tar reduces the inflammation, itching, and scaling of psoriasis. This preparation is untidy, however, and stains clothing and bedding.

Light therapy or phototherapy represents a first-line treatment of moderate to severe psoriasis [4,5]. Phototherapy involves exposing the skin to ultraviolet (UV) light on a regular basis under medical supervision. UVB phototherapy is a treatment for skin eruptions using ultraviolet light. UVB refers to type B ultraviolet light with an energy between UVA tanning rays and higher energy UVC rays. UVB rays are the part of the sunlight spectrum that produces sunburn. Carefully controlled, phototherapy is an extremely effective tool for the treatment of a variety of skin diseases. UVB penetrates the skin and slows the growth of skin cells. Light therapy has been a standard treatment since its invention at the Mayo Clinic in the 1920s. It is the recommended procedure for people with moderately severe psoriasis (covering 20% or more of their body) who have not responded to topical medicines.

Several oral and injected medications are used in the treatment of moderate to severe psoriasis (Table 1). Apremilast is an orally bioavailable phosphodiester-4 blocker; its action increases cAMP levels in inflammatory cells and down-regulates the production of pro-inflammatory cytokines. Methotrexate is an orally effective antimetabolite that blocks dihydrofolate reductase activity leading to the conversion of its substrate to tetrahydrofolate. The latter is required for the biosynthesis of purines, thymidylic acid, and certain amino acids; methotrexate retards cell growth. Cyclosporine is a calcineurin blocker that lowers T-cell activity and is given orally or intravenously. Etanercept, which is given subcutaneously (SQ), blocks the action of TNF-α and is used in the treatment of plaque psoriasis, psoriatic arthritis, rheumatoid arthritis, juvenile idiopathic arthritis, and ankylosing spondylitis. Adalimumab (SQ) is a monoclonal antibody (mab) targeting TNF-α. It is given to patients with moderate to severe chronic plaque psoriasis. It is also FDA-approved for the treatment of psoriatic arthritis, rheumatoid arthritis, juvenile idiopathic arthritis, and Crohn disease. Certolizumab (SQ) is prescribed for the treatment of plaque psoriasis, psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, and Crohn disease. Infliximab is given intravenously in a period of two hours or longer and is approved for the treatment of plaque psoriasis, psoriatic arthritis, rheumatoid arthritis, ankylosing spondylitis, ulcerative colitis, and Crohn disease. Golimumab (SQ) is prescribed to patients with psoriatic arthritis, rheumatoid arthritis, and ankylosing spondylitis. TNF-α, which is targeted by these biological agents, is a small protein (17.3 kDa) that participates in the pathogenesis of a variety of inflammatory diseases.

Ustekinumab (SQ) is monoclonal antibody prescribed for the treatment of plaque psoriasis, psoriatic arthritis, and Crohn disease (Table 1). Risankizumab (SQ) targets IL-23 and is given for the treatment of plaque psoriasis and psoriatic arthritis. Additionally, tildrakizumab and guselkumab are each given subcutaneously for the management of plaque psoriasis. Secukinumab (SQ) targets IL-7 and is prescribed for the treatment of plaque psoriasis, psoriatic arthritis, and ankylosing spondylitis. Ixekizumab (SQ) is an approved therapy for plaque psoriasis and psoriatic arthritis. Brodalumab (SQ), which also targets IL-17, is prescribed for the management of moderate to severe plaque psoriasis in adult patients who are candidates for systemic therapy or phototherapy and have failed to respond or have lost response to other systemic therapies. Tofacitinib is an orally effective blocker of JAK1/3 activity that is FDA-approved for the treatment of rheumatoid arthritis, ulcerative colitis, and psoriatic arthritis, but not plaque psoriasis. Upadacitinib is an orally bioavailable JAK1 inhibitor that is prescribed for the treatment of rheumatoid arthritis, atopic dermatitis, ulcerative colitis, and psoriatic arthritis, but not plaque psoriasis. Deucravacitinib is an orally bioavailable medicine that blocks the activity of TYK2 and is FDA-approved for the treatment of plaque psoriasis.

**Table 1**
Treatment options for psoriasis[a].

| Inhibitor class | Examples | Comments |
|---|---|---|
| *Topical medications* | | |
| Corticosteroids | Hydrocortisone | Weak steroid used for mild disease |
| | Triamcinolone | Intermediate strength steroid used for mild disease |
| | Clobetasol | Potent steroid used for moderate disease |
| Vitamin D analogs | Calcipotriene<br>Calcitrol | Used alone or with topical corticosteroids |
| Retinoid | Tazarotene | Decreases inflammation and retards skin cell growth |
| Calcineurin inhibitors | Tacrolimus<br>Pimcrolimus | Used in areas of thin skin such as around the eyes |
| Salicylic acid | | Used in shampoos |
| Coal tar | | Reduces scaling, itching, and inflammation |
| Anthralin | | A tar cream applied for a short time and washed off |
| *Light therapy* | | |
| Light therapy | Sunlight<br>UVB | First-line treatment for moderate to severe psoriasis. Repeated treatments are necessary. |
| | Psoralen plus ultraviolet A | Psoralen is a light-sensitizing medication |
| *Injected medications* | | |
| Corticosteroid | Triamcinolone | Injected into lesions |
| TNF-α inhibitor | Etanercept | TNF receptor fused to constant end of IgG1 that blocks immune system cell signaling mediated by TNF-α |
| | Adalimumab<br>Certolizumab<br>Infliximab<br>Golimumab | Monoclonal antibody that blocks TNF-α |
| Anti-Il-12 and anti-Il-23 | Ustekinumab | Il-12 plays a role in the activity of T-cells and natural killer cells and acts via TYK2 and JAK2; Il-23 is produced by dentritic cells and macrophages that participates in the inflammatory response |
| Anti-Il-23 | Risankizumab<br>Tildrakizumab<br>Guselkumab | Il-23 is produced by dentritic cells and macrophages that participates in the inflammatory response |
| Anti-Il-17 | Secukinumab<br>Ixekizumab<br>Brodalumab | Il-17 plays a role in numerous immune regulatory functions |
| *Orally bioavailable medications* | | |
| Antimetabolite | Methotrexate | Inhibits dihydrofolate reductase and is an immune-system suppressant |
| Calcineurin inhibitor | Cyclosporin | Immunosuppressant lowers T-cell activity; also given intravenously |
| JAK1/3 inhibitor | Tofacitinib | Approved for psoriatic arthritis, not psoriasis per se; binds to the JH1 domains |
| Phosphodiester-4 inhibitor | Apremilast | Increases levels of cAMP in inflammatory cells and down regulates the production of TNF-α, Il-17, and Il-23 |
| Retinoid | Acitretin | Reduces skin cell production |
| TYK2 inhibitor | Deucravacitinib | Binds to the JH2 pseudokinase domain |

[a] Ref. [2]

## 2. Cytokines and JAK/TYK2 signal transduction in psoriasis

Psoriasis results from the complex interactions of genetics, environmental triggers, and immune dysregulation. Cytokines play a central role in immune regulation and cytokine signaling is an intricate process owing to the existence of more than 200 of these factors, many with overlapping activities [1]. Cytokines can be divided into several categories including the interferons (which aid in the defense against cancer and viral diseases), colony-stimulating-factors (CSFs are secreted glycoproteins that bind to receptor proteins on the surfaces of hemopoietic stem cells and activate intracellular signaling pathways that cause the cells to proliferate and differentiate into specific types of blood cell),

interleukins (cytokines that act as chemical signals between white blood cells), chemokines (small proteins that play a role in the migration of leukocytes), transforming growth factors (TGFα/β stimulate the growth of a variety of cells), and tumor necrosis factors (proteins released by immune cells and regulate other cells of the immune system to trigger an inflammatory response).

The Janus kinase (JAK) family consists of four members: JAK1, JAK2, JAK3, and TYK2 (Tyrosine Kinase 2) [6]. These nonreceptor protein-tyrosine kinases consist of seven distinct JAK homology (JH1-JH7) domains. The JAK family enzymes possess an inactive pseudokinase domain (JH2) amino-terminal to an active carboxyterminal protein kinase domain (JH1). The pseudokinase domain negatively regulates the activity of the functional JH1 protein kinase domain. Janus is a two-faced Roman God (looking forwards and backwards) whose name was ascribed to this family because of the existence of two protein kinase domains within a single polypeptide chain. JAK was whimsically conceived as Just Another Kinase [7,8]. JAK1, JAK2, and TYK2 are ubiquitously expressed in nearly all types of cells (excluding mature erythrocytes) whereas JAK3 activity is confined to myeloid, lymphoid, and hematopoietic cells [9]. Mature blood cells have a limited life span and are continuously renewed by an intricate multistep process. The Janus kinases play an essential role in hematopoiesis; accordingly, Janus kinase dysregulation can result in a wide variety of hematological illnesses. These protein kinases also regulate many other activities including metabolism, post-natal growth, and satiety (leptin signaling).

Manning et al. identified 478 classical and 40 non-classical or atypical human protein kinase family genes (total 518) that correspond to about 2.5% of the human protein-encoding genome [10]. Based upon the nature of the substrate hydroxyl group, these catalysts are classified as protein-tyrosine kinases (90 members), tyrosine-kinase like enzymes (43), and protein-serine/threonine kinases (385). A small group of enzymes including MEK1 and MEK2, which catalyze the phosphorylation of both tyrosine and then threonine residues of the activation segment of their target proteins, are classified as dual specificity kinases. Of the 90 protein-tyrosine kinases, 58 are transmembrane receptors and 32 are intracellular nonreceptor proteins, including the four members of the Janus kinase family. This enzyme family catalyzes the following reaction:

$$MgATP^{1-} + \text{protein-tyrosine–O:H} \rightarrow \text{protein-tyrosine–O:}PO_3^{2-} + MgADP + H^+$$

Note that the phosphoryl group ($PO_3^{2-}$) and not the phosphate group ($OPO_3^{2-}$)is transferred from ATP to a tyrosine residue within the protein substrate. Divalent cations such as $Mg^{2+}$ or $Mn^{2+}$ are required for the activity of almost all protein kinases; however, $Mg^{2+}$ is the physiological ion owing to its significantly greater intracellular content.

The JAK-STAT (signal transducer and activator of transcription) pathway conveys extracellular signals from a variety of cytokines to the nucleus and is responsible for the expression of hundreds of protein-encoding genes [11]. Each Janus kinase binds to the intracellular juxtamembrane region of their cognate cytokine receptor. The genes specifying selected high-affinity cytokine receptors are listed in Table 2. The cytokine receptors – which lack catalytic activity – consist of an extracellular domain, a transmembrane segment, and an intracellular domain that interacts with their corresponding Janus kinases.

Many steps are required for the conversion of an extracellular signal into a transcriptional response. First, ligand binding produces conformational changes in the cytokine receptors that lead to JAK activation as a result of the phosphorylation of two tyrosine residues within the activation segment of the JH1 domains as catalyzed by a partner Janus kinase JH1 enzyme. Such intermolecular reactions represent phosphorylation in *trans*. Following activation, the JH1 protein kinase domains catalyze the phosphorylation of cytokine receptor tyrosine residues that attract the SH2 domain of their corresponding STATs. The activated JH1 domain then mediates the phosphorylation of the STAT molecules themselves. The phosphorylated STATS then form homo- or heterodimers that are translocated into the nucleus where they mediate the transcription of target genes (Fig. 1). On the other hand, STATs may preexist as dimers and phosphorylation may produce a conformational

**Table 2**
Cytokine stimulation of selected JAK-STAT signaling pathways involving TYK2 and JAK family kinases[a].

| Cytokine | *Genes* of human receptor subunits | Downstream JAK/TYK2 dimers | Downstream STATs | Selected functions |
|---|---|---|---|---|
| IL-11 | *IL6ST* and *IL11RA* | JAK1 +TYK2, JAK2 +TYK2 | STAT3 | Stimulates the proliferation of hematopoietic stem cells and megakaryocyte progenitor cells and induces megakaryocyte maturation resulting in increased platelet production. |
| IL-12 is made of IL-12A and IL-12B | *IL12R1* and *IL12R2* | JAK1 +TYK2, JAK2 +TYK2 | STAT4 | IL12A heterodimerizes with IL12B to form the IL-12 cytokine or with EBI3/IL27B to form the IL-35 cytokine. Induces production of IFN-γ. |
| IL-23 is made from IL-12B & IL-23A | *IL12RB1* and *IL23R* | JAK1 +TYK2, JAK2 +TYK2 | STAT4 | Promotes production of pro-inflammatory cytokines and induces autoimmune inflammation. Pro-inflammatory T helper type 17 cell (Th17 cell) maintenance and expansion. |
| IL-13 | *IL4R* and *IL13RA1* | JAK1 +TYK2 | STAT6 | Stimulates B-cell proliferation and activation of eosinophils, basophils, and mast cells. Synergizes with IL-2 in regulating IFN-γ synthesis. |
| INFα/β | *INFAR1* and *INFAR2* | JAK1 +TYK2 | STAT1/2 | TYK2 binds to IFNAR1 and JAK1 binds to IFNAR2. STAT signaling results in transcriptional activation or repression of interferon-regulated genes that encode the activation of innate immune responses that promote not only cytokine production but also natural killer cell functions and antigen presentation (the interferon response). |

[a] Ref. [6] and https://www.uniprot.org/.

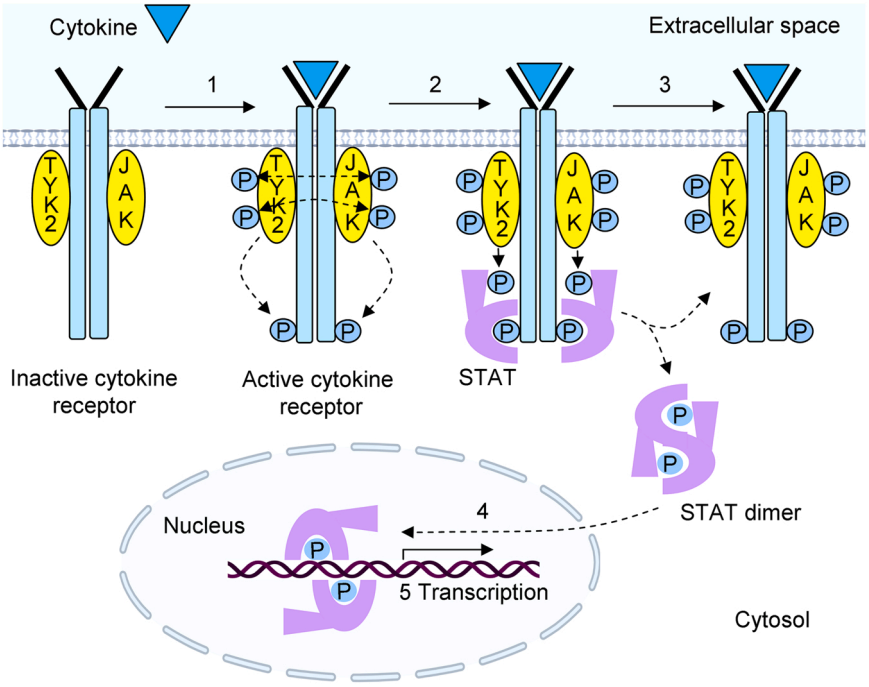


**Fig. 1.** Cytokine signaling by the JAK-STAT pathway. The JAK enzymes work in pairs and TYK2 pairs with JAK1 or JAK2, but not JAK3 or TYK2.

change that results in activation [12]. Janus kinase activation by EGFR and PDGF is downstream from the activated receptor and may involve other protein kinases such as Src [11,13].

Humans have seven STAT genes that are named *STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B,* and *STAT6*. Each STAT protein contains six domains (from the N- to C-terminus) including an N-terminal domain, a coiled-coil segment, a DNA-binding domain, a linker segment, an SH2 domain, and a transcriptional activation domain (TAD), which were first described for STAT1 (Fig. 2A) [14]. The transcriptional activation domain contains a tyrosine residue (Y) that is a target for an upstream Janus kinase. After their phosphorylation, STATs form homo- or heterodimers that result from the binding of a phosphotyrosine (pY) to their partner's SH2 domain. The dimer is translocated into the nucleus where it binds to DNA target sequences alone or in combination with other transcription factors that regulate DNA transcription (Fig. 1).

The STATs have specific roles in signaling. For example, STAT1 and STAT2 participate in interferon signaling [15–17] and STAT3 participates in the signaling pathways initiated by IL-11. Furthermore, STAT4 participates in the signaling by IL-12 and IL-23 and STAT6 participates in IL-13 signaling. The material given in Table 2 is a general overview and the precise pathway from the stimulatory ligand and receptor to a specific Janus kinase and its specific STAT depends upon the physiologic context and cellular milieu. See Refs. [1,6,17] for more comprehensive discussions of cytokine and JAK-STAT signaling pathways involving JAK1/2/3 and TYK2.

The catalytic activity of all protein kinases is exactingly regulated because of their general importance in numerous signaling pathways [18]. Multiple phosphoprotein phosphatases including SHP1/2 (Sh2-containing phosphatase), PTP1B (protein tyrosine phosphatase), and TCPTP (T-cell protein tyrosine phosphatase) catalyze the dephosphorylation and inactivation of Janus kinases [12]. CD45 is a receptor phosphoprotein phosphatase that catalyzes the hydrolytic

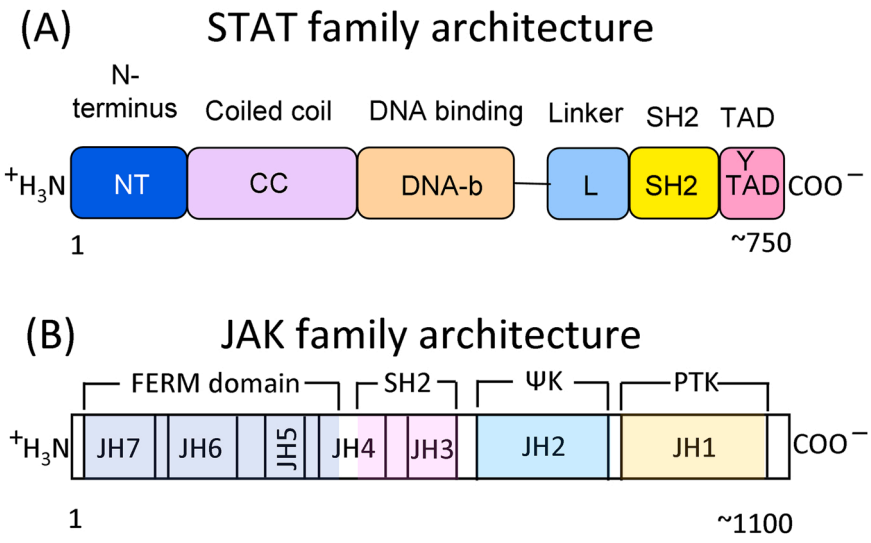


**Fig. 2.** Overview of (A) STAT and (B) JAK family architecture.

dephosphorylation of the activation segment phosphotyrosines of all four Janus kinase members [19]. The SOCS (suppressor of cytokine signaling) family of eight proteins also negatively regulates cytokine and Janus kinase [11,12]. The SOCS proteins are ubiquitin ligases that catalyze the proteasomal degradation of Janus kinase-associated cytokine receptors. Moreover, SOCS1 and SOCS3 bind to the protein-substrate binding grove within the kinase activation segment and sterically block the catalytic activity of JAK1/2 and TYK2, but not JAK3.

Psoriasis is associated with increased activity of TNF-α, IL-17A, IL-17F, IL-22, and IL-23 [20]. One of the inflammatory pathways in psoriasis is driven by type I interferons (IFN-α/β), which are produced by dendritic cells during disease development [21]. These interferons are upstream of JAK1, TYK2, and STATS1/2/4 (Table 2). Type I interferons activate dendritic cells to produce TNF and IL-23, which drives plaque formation in psoriasis. IL-23 is upstream of JAK2, TYK2, and STAT3/4. Subsequently, TNF/IL-23/IL-17 promote later plaque development. There are several IL-17 ligands (A–F), but their association with the JAK-STAT pathway is currently unknown. The association of several other cytokines with the JAK and TYK2 pathways are provided in Table 2, but their role in the pathogenesis of psoriasis – if any – is unknown.

## 3. Janus kinase (JAK) biochemistry

### 3.1. Janus kinase family architecture

The Janus kinases are large intracellular enzymes containing about 1100 amino acid residues. They contain seven JAK homology modules named JH7-JH1 as we go from the N-terminus to the C-terminus. These proteins are divided into four functional domains [22]. The N-terminal JH7-JH6 module corresponds to a FERM domain (where F is for 4.1 protein, E refers to ezrin, R is for radixin and M refers to moesin); FERM is a protein module consisting of ≈ 350 amino acids and it directs proteins to the plasma membrane. The JH5-JH3 module is an atypical SH2 domain in that it fails to bind to protein-tyrosine phosphates (it interacts with negatively charged glutamate residues). The JH5-JH3 domain is followed by a JH2 pseudokinase and the C-terminal JH1 segment is the catalytically active protein-tyrosine kinase domain (Fig. 2B).

TYK2 JH1 and JH2 domains contain a small amino-terminal lobe and large carboxyterminal lobe that contains several conserved α-helices and β-strands as first described by Knighton et al. for PKA in 1991 [23,24]. The amino-terminal lobe consists of a twisted five-stranded antiparallel β-sheet (β1–β5) [15] and a regulatory αC-helix (Fig. 3A and D). The N-lobe of the TYK2 JH1 domain contains a conserved glycine-rich

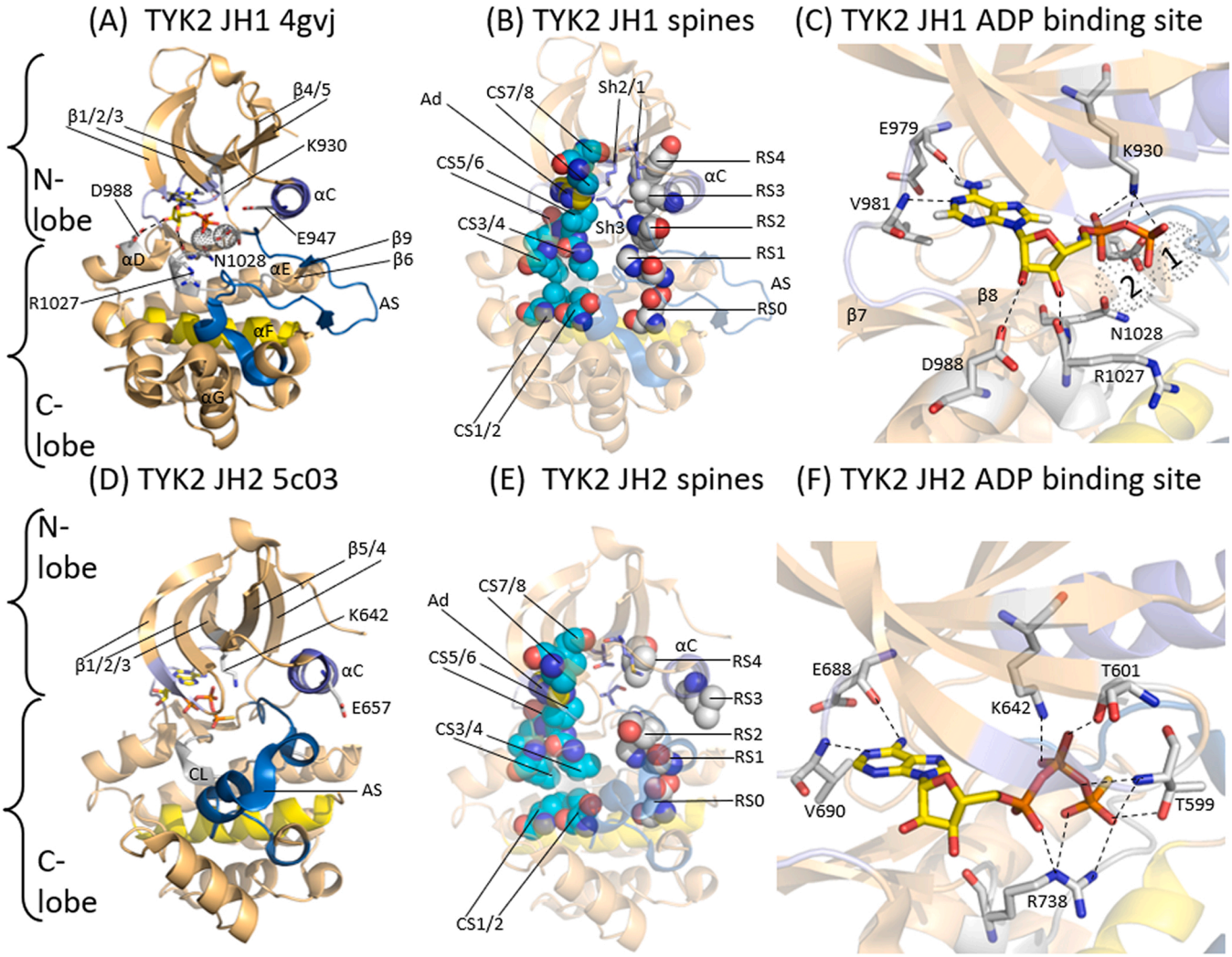


**Fig. 3.** (A) Overview of the active TYK2 JH1 domain. (B) Spine and shell residues of active TYK2 JH1. (C) Interaction of ADP with the active TYK2 JH1 domain. (D) Overview of the inactive TYK2 JH2 domain. (E) Spine and shell residues of inactive TYK2 JH2. (F) Interaction of ADP with the inactive TYK2 JH2 domain. 1 and 2 refer to different magnesium ions.
Figs. 3, 4, and 6 were prepared with the PyMOL Molecular Graphics System Version 1.5.0.4 Schrödinger, LLC.

(GEGHFG) ATP-phosphate-binding loop (GRL – glycine-rich loop – sometimes called the P-loop for ATP-phosphate), which links the β1- and β2-strands. The signature sequence of the glycine-rich loop is GxGxΦG, where x refers to any amino acid and Φ is a hydrophobic residue, usually phenylalanine or tyrosine. The β1- and β2-strands are found above the adenine base of ATP/ADP. The β3-strand normally contains an Ala-Xxx-Lys (AxK) sequence, the lysine of which (TYK2 K930) links the α- and β-phosphates of ATP/ADP to the αC-helix (Fig. 3C). A conserved glutamate residue occurs near the center of the αC-helix (TYK2 E947) in protein kinases. A salt-bridge between the β3-lysine and the αC-glutamate is required for the formation of an active state, which corresponds to an "$\alpha C_{in}$" conformation (Fig. 3A). In contrast, the corresponding K642 and E657 of the TYK2 JH2 pseudokinase domain fail to make contact, and this corresponds to an "$\alpha C_{out}$" conformation (Fig. 3D). Furthermore, the glycine-rich loop in the TYK2 JH2 domain consists of a shortened GQG sequence between the β1- and β2-strands. The $\alpha C_{in}$ structure is necessary, but not sufficient, for full protein kinase activity.

The large lobe of the TYK2 JH1 protein kinase domains is mostly α-helical with six conserved segments (αD-αI), which occur in all functional protein kinases [25]. The C-lobe also contains four short conserved β-strands (β6-β9). The β-strand amino acids are found between the αE- and αF-helices and include residues proximal to the catalytic loop (β6), after the catalytic loop and below the adenine ring of ATP (β7 and β8), and within the activation segment (β9). The activation segment of the TYK2 JH1 active state forms an open structure that facilitates protein substrate binding (Fig. 3A). In contrast, the activation segment of the dormant TYK2 JH2 domain forms a closed structure that sterically blocks protein binding (Fig. 3D).

Hanks and Hunter identified 12 subdomains (I–VIa, VIb–XI) with signature sequences that make up the catalytic and regulatory residues of protein kinases [26]. The following four amino acids, which form a K/E/D/D core, represent the functional properties of the TYK2 JH1 protein-tyrosine kinase domain. An invariant β3-strand lysine (K930, the K of K/E/D/D) forms a salt bridge with the αC-glutamate (E947 the E of K/E/D/D) and also the α- and β-phosphate groups of ATP/ADP. The catalytic loop surrounding the actual site of phosphoryl transfer consists of HRDLAARN in JAK1, JAK3 and TYK2; the sequence HRDLATRN occurs in JAK2. The TYK2 JH2 pseudokinase domain contains HGNVCGRN. The catalytic aspartate in TYK2 (D1023), which is the first D of K/E/D/D, serves as a base that abstracts a proton from the tyrosyl –OH moiety. The corresponding residue in the pseudokinase domain of Janus kinases is an asparagine, which cannot abstract a proton from the phenolic hydroxyl, thereby contributing to the catalytic dormancy of the pseudokinase domain. The AAR sequence in the JAK/TYK2 catalytic loop is a receptor protein-tyrosine kinase signature and RAA is a nonreceptor protein kinase signature; the occurrence of AAR in the JAK nonreceptor protein kinases is thus anomalous.

The second aspartate of the K/E/D/D signature of TYK2 (D1041) is the first residue of the activation segment. The activation segment of nearly all protein kinases including the Janus kinase family begins with DFG (Asp-Phe-Gly) and ends with APE (Ala-Pro-Glu) or a related triad signature such as PPE. DFG-D1041 binds $Mg^{2+}$(1), which in turn interacts with the α- and β-phosphates of ATP/ADP. The HRDxxxxN (N1028) binds $Mg^{2+}$(2), which in turn interacts with the α- and γ-phosphates of ATP (Fig. 3C). In the active conformation, the DFG-D is directed inward toward the active site (DFG-$D_{in}$) where it can bind $Mg^{2+}$(1). In contrast, when the DFG-D is pointed outward, the resulting DFG-$D_{out}$ structure is catalytically impaired owing to the blockade of ATP and protein substrate binding. The 6-amino portion of ATP generally forms a hydrogen bond with the C═O backbone residue of the first hinge residue (E979 of the TYK2 JH1 domain) that connects the N- and C-lobes of the protein kinase domain and the *N*1 nitrogen of the adenine moiety hydrogen bonds with the N–H group of the third hinge residue (V981) (Fig. 3C). Most steady-state ATP competitive small molecule Janus kinase inhibitors also hydrogen bond with backbone residues of the connecting hinge. The end of the activation segment of the carboxyterminal lobe generally binds and positions the protein substrates.

The activation segment is an important mediator of protein-substrate binding and catalysis [27]. This segment in Janus kinases contains two phosphorylatable tyrosines [28]. The proximal activation segment is located sterically near the N-terminus of the αC-helix and the HRD signature of the catalytic loop. These components interact hydrophobically. As for most protein kinases [18], phosphorylation of residues within the activation segment shifts the equilibrium from an inactive JAK JH1 domain to an active one [29]. Several important human and mouse Janus kinase residues are listed in Table 3.

### 3.2. Hydrophobic spines in active JH1 and inactive JH2 domains

Kornev et al. examined the structures of active and inactive conformations of 23 protein kinases and they discovered the role of several critical residues by a local spatial pattern alignment algorithm [30,31]. Their study led to a group of four hydrophobic residues that form a regulatory or R-spine and eight hydrophobic residues that form a catalytic or C-spine. The adenine moiety of ADP/ATP is one component of the catalytic spine. Each spine consists of amino acids that are found in both the N- and C-lobes. The regulatory spine contains a residue from the activation segment and αC-helix, both of which are important in determining active and dormant states. The catalytic spine positions ATP and the regulatory spine positions the protein substrate to facilitate catalysis. The structure of the spines differs between the active JH1 and dormant JH2 domains. Moreover, the correct alignment of both the regulatory and catalytic spines is necessary for the assembly of an active protein kinase JH1 domain.

The protein kinase regulatory spine signature consists of the histidine of the catalytic loop HRD, the phenylalanine of the activation segment DFG, the amino acid four residues carboxyterminal to the conserved αC-glutamate of the αC-helix, and a residue at the N-terminus of the β4-strand. The backbone of the catalytic loop HRD-His is linked to the very hydrophobic αF-helix by a hydrogen bond to a conserved aspartate side chain. Moving from the αF-helix aspartate to the top residue of the spine within the β4-strand, Meharena et al. named the regulatory spine residues RS0, RS1, RS2, RS3, and RS4 [27]. The R-spine of the functional JH1 TYK2 kinase domain is nearly linear while that of the dormant TYK2 pseudokinase domain is broken with RS3 displaced (Figs. 3B and 3E).

The protein kinase catalytic spine is made up of residues from both the small and large lobes; the catalytic spine is completed by the adenine moiety of ATP (Figs. 3B and 3E) [27,31]. The two N-lobe residues of protein kinase domains that make hydrophobic contact with the adenine base of ATP include a valine at the beginning of the β2-strand (CS7) and an alanine from the conserved AxK of the β3-strand (CS8). Additionally, a hydrophobic residue from the β7-strand (CS6) interacts with the adenine (Ad) moiety. This CS6 residue is adjacent to CS5 and above CS4; CS5 interacts with the CS3 residue near the beginning of the αD-helix. Lastly, CS3 and CS4 interact hydrophobically with CS1 and CS2 of the αF-helix to fabricate a completed C-spine [32]. Of significance, the internal αF-helix anchors both the R- and C-spines. Moreover, each spine positions the protein kinase catalytic residues in their functional and active state. When comparing the locations of the spinal residues, the greatest difference in the structures of the TYK2 JH1 and JH2 domains involves RS3 (Fig. 3B and 3E).

Using site-directed mutagenesis, Meharena et al. identified three residues in murine PKA that stabilize the regulatory spine – which they labeled Sh1, Sh2, and Sh3 – where Sh refers to shell [27]. Sh2 is the gatekeeper residue which describes the role that this residue plays in restricting access to the back pocket. The back pocket is also called the back cleft or hydrophobic pocket II (HPII). The residues that form the spines were identified by their locations in active and inactive enzymes based upon their X-ray crystallographic three-dimensional structures [30,31]. This contrasts with the classification of the HRD, DFG, or APE amino acid signatures based upon their primary structures [26]. A

**Table 3**
Important residues in selected human and mouse JAK family members.

| | JAK1 | JAK2 | JAK3 | TYK2 | mJAK1 | Inferred function | Hanks no. |
|---|---|---|---|---|---|---|---|
| FERM domain | 34–420 | 37–380 | 24–356 | 26–431 | 34–420 | Interacts with receptor | – |
| SH2-like | 439–544 | 410–482 | 375–475 | 450–529 | 439–542 | Binds glutamate | – |
| ΨK | 583–855 | 545–809 | 521–781 | 589–875 | 582–854 | Regulation | – |
| PTK | 875–1153 | 849–1124 | 822–1111 | 897–1176 | 874–1152 | Catalysis | – |
| *N-Lobe* | | | | | | | |
| Glycine-rich loop: GxGxΦG | $^{882}$GEGHFG$^{887}$ | $^{856}$GKGNFG$^{861}$ | $^{829}$GKGNFG$^{834}$ | $^{904}$GEGHFG$^{909}$ | $^{881}$GEGHFG$^{886}$ | Anchors ATP β-phosphate | I |
| β3-K (K of K/E/D/D) | K908 | K882 | K855 | K930 | K907 | Forms ion pair with ATP α- and β-phosphates | II |
| αC-E (E of K/E/D/D) | E925 | E898 | E871 | E947 | E924 | Forms ion pair with β3-K | III |
| Hinge residues | $^{957}$EFLPSG$^{962}$ | $^{930}$EYLPYG$^{935}$ | $^{903}$EYLPSG$^{908}$ | $^{979}$EYVPLG$^{984}$ | $^{956}$EFLPSG$^{961}$ | Connects N- and C-lobes | V |
| *C-Lobe* | | | | | | | |
| Catalytic loop HR*D* (first *D* of K/E/*D*/D) | D1003 | D976 | D949 | D1023 | D1002 | Catalytic base (abstracts proton) | VIb |
| Catalytic loop Asn (N) | N1008 | N981 | N954 | N1028 | N1007 | Chelates $Mg^{2+}$(2) | VIb |
| Activation segment | 1021–1051 | 994–1024 | 967–997 | 1041–1071 | 1020–1050 | Positions protein substrate | VII-VIII |
| AS *D*FG (second *D* of K/E/D/*D*) | D1021 | D994 | D967 | D1041 | D1020 | Chelates $Mg^{2+}$(1) | VII |
| AS phosphorylation site | Y1034/Y1035 | Y1007/Y1008 | Y980/Y981 | Y1054/Y1055 | Y1033/Y1034 | Stabilizes the AS after phosphorylation | VIII |
| APE, end of AS | 1049–1051 | 1022–1024 | 995–997 | 1069–1071 | 1048–1050 | Interacts with the αHI loop and stabilizes the AS | VIII |
| JH7 | 44–117 | 51–124 | 37–110 | 40–113 | 44–117 | FERM domain | – |
| JH6 | 147–180 | 142–170 | 126–154 | 141–169 | 147–180 | FERM domain | – |
| JH5 | 309–324 | 282–299 | 265–282 | 310–327 | 309–324 | FERM domain | – |
| JH4 | 378–452 | 329–411 | 304–387 | 389–462 | 378–452 | FERM + SH2 like | – |
| JH3 | 500–550 | 455–506 | 432–482 | 508–558 | 500–550 | SH2 like | – |
| JH2 | 583–855 | 545–809 | 521–781 | 589–875 | 582–854 | Pseudokinase | – |
| JH1 | 875–1153 | 849–1124 | 822–1111 | 897–1176 | 874–1152 | Protein-tyrosine kinase | – |
| UniProt KB ID | P23458 | O60674 | P52333 | P29597 | P23458 | | |

[a] AS, activation segment;
Φ, hydrophobic residue.

summary of the spine and shell residues of the JAK family JH1 and JH2 domains is given in Table 4. Therapeutic small molecule protein kinase blockers usually interact with residues that make up the C-spine and shell residues and sometimes those of the R-spine [33].

The protein kinase spine and shell residues perform an important role in determining the structure and activity of these enzymes; one cannot overemphasize their importance in supporting the activity of this enzyme superfamily as well as their participation in their interactions with almost all small molecule protein kinase antagonists. For an examination of the properties of the spine and shell residues and their interactions with low molecular weight inhibitors of important members of the protein kinase superfamily, see the following articles: Refs. [34–36] for the ALK pleotrophin and midkine receptor protein-tyrosine kinase, Refs. [37–40] for the EGFR family of protein-tyrosine kinases, Ref. [41] for the PDGFRα/β protein-tyrosine kinases, Ref. [42] for the fibroblast growth factor receptor family of protein-tyrosine kinases, Ref. [43] for the Kit stem cell receptor protein-tyrosine kinase, Ref. [44] for the RET glial-cell derived receptor protein-tyrosine kinase, Ref. [45] for the VEGFR1/2/3 protein-tyrosine kinases, Ref. [46] for the ROS1 orphan receptor protein-tyrosine kinase, Ref. [47] for the Flt3 receptor protein-tyrosine kinase, Refs. [33,48] for the BCR-Abl nonreceptor protein tyrosine kinases, Refs. [15,16] for the Janus nonreceptor protein-tyrosine kinase, Ref. [49] for the Bruton nonreceptor protein-tyrosine kinase, Refs. [50,51] for the Src nonreceptor

**Table 4**
Spine and shell residues of selected murine (m) and human Janus kinase and pseudokinase (Ψ) domains.

| | Symbol | KLIFS No. | mJAK1 Ψ | mJAK1 | JAK1 Ψ | JAK1 | JAK2 Ψ | JAK2 | JAK3 Ψ | JAK3 | TYK2 Ψ | TYK2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Regulatory spine* | | | | | | | | | | | | |
| β4-strand (N-lobe) | RS4 | 38 | L652 | Y939 | L653 | Y940 | N612 | Y913 | L587 | Y886 | V673 | Y962 |
| C-helix (N-lobe) | RS3 | 28 | M640 | L928 | M641 | L929 | M600 | L902 | L575 | L875 | L661 | L951 |
| Activation loop DFG-F (C-lobe) | RS2 | 82 | P739 | F1021 | P740 | F1022 | P700 | F995 | P672 | F968 | P760 | F1042 |
| Catalytic loop HRD-H (C-lobe) | RS1 | 68 | H711 | H1000 | H712 | H1001 | H671 | H974 | H645 | H947 | H732 | H1021 |
| F-helix (C-lobe) | RS0 | None | D774 | D1062 | D775 | D1063 | D735 | D1036 | D707 | D1009 | D796 | D1083 |
| *R-shell* | | | | | | | | | | | | |
| Two residues upstream from the gatekeeper | Sh3 | 43 | M664 | L953 | M665 | L954 | L624 | L927 | M598 | L900 | M685 | L976 |
| Gatekeeper, end of β5-strand | Sh2 | 45 | E666 | M955 | E667 | M956 | Q626 | M929 | Q600 | M902 | T687 | M978 |
| αC-β4 loop | Sh1 | 36 | V650 | V937 | V651 | V938 | V610 | V911 | V585 | V884 | A671 | I960 |
| *Catalytic spine* | | | | | | | | | | | | |
| β3-AxK-A (N-lobe) | CS8 | 15 | I619 | A905 | I620 | A906 | L579 | A880 | L554 | A853 | V640 | A928 |
| β2-strand (N-lobe) | CS7 | 11 | I596 | V888 | I597 | V889 | I559 | V863 | I535 | V836 | V603 | V911 |
| β7-strand (C-lobe) | CS6 | 77 | L720 | L1009 | L721 | L1010 | L680 | L983 | L654 | L956 | L741 | L1030 |
| β7-strand (C-lobe) | CS5 | 78 | L721 | V1010 | L722 | V1011 | L681 | V984 | L655 | V057 | L742 | L1031 |
| β7-strand (C-lobe) | CS4 | 76 | L719 | V1008 | L720 | V1009 | I679 | I982 | V653 | I955 | I740 | V1029 |
| D-helix (C-lobe) | CS3 | 53 | L674 | L963 | L675 | L964 | L634 | L937 | I608 | L910 | L695 | L986 |
| F-helix (C-lobe) | CS2 | None | T781 | T1069 | I782 | T1070 | T742 | V1043 | T714 | S1012 | T803 | T1090 |
| F-helix (C-lobe) | CS1 | None | I785 | L1073 | I786 | L1074 | I746 | L1047 | V718 | V1016 | I807 | L1094 |

[a] From Refs. [27,30,31], https://klifs.net/, and https://www.uniprot.org/uniprotkb/.

protein-tyrosine kinase, Refs. [52,53] for the MEK1/2 dual specificity protein kinases, Refs. [54,55] for the cyclin-dependent protein-serine/threonine kinase family, Refs. [56,57] for the ERK1/2 protein-serine/threonine kinases, Refs. [58,59] for the RAF protein-serine/threonine kinases, and Ref. [60] for PI 3-kinase, a member of the atypical protein kinase group.

### 3.3. The role of the cytokine receptors in JAK family regulation

Owing to the importance of protein kinase activities in the regulation of a myriad of cellular processes, it is necessary that they are stringently regulated [18]. Moreover, dysregulation of protein kinases leads to many pathological conditions. In contradistinction to metabolic enzymes such as hexokinase, which mediate the phosphorylation of thousands of molecules per minute, protein kinases catalyze the phosphorylation of limited amounts of protein substrates. For example, when TYK2 is activated by a cytokine receptor, the main product is phospho-TYK2 that results from the trans-phosphorylation of TYK2 by JAK1 or JAK2 [15,16]. TYK2 and JAK1/2 also mediate the phosphorylation of the activating cytokine receptors and STATS. The concentrations of TYK2 and JAK1/2 and their substrates are nearly equivalent and the high turnover of substrates is unnecessary. The mechanisms for the interconversion of an inactive and active protein kinase vary with each kinase and are generally quite intricate as observed for the Janus kinases.

Glassman et al. hypothesize that inactive JAK protein kinases occur in an autoinhibited monomeric form in which the FERM-SH2 region occludes the active site of the protein-tyrosine kinase JH1 domain [61]. This closed state disallows TYK2 and JAK1/2 dimerization because of a steric clash between the pseudokinase domain and the FERM domain of a potential dimer partner. The closed state "breaths," however, to form transient open states. Activation by cytokine-mediated receptor dimerization results in the formation of a JAK1-TYK2 or JAK2-TYK2 dimer and shifts the equilibrium from the autoinhibited state to an open state that promotes maximum activity of the JH1 kinase domain while concomitantly promoting the proximity of partner protein-tyrosine kinases to facilitate transphosphorylation.

Using cryo-electron microscopy, Glassman et al. determined the structure of the complete active dimer of (murine) JAK1 [61]. They used a human interferon lambda receptor (IFNλR) dimer linked to a GCN4 leucine zipper to generate an intracellular receptor dimer. This receptor construct bound to JAK1 dimers and was even used in dimer purification. The authors provided the reasoning for using this particular mouse JAK1 construct, which has better expression and stability properties when compared with other JAK family members. Note, however, that JAK1 forms physiological heterodimers with other members of the JAK family owing to receptor-receptor interactions and not homodimers as used here. Going from the amino-terminus to the carboxyterminus of JAK1 are the FERM, SH2, the JH2 pseudokinase domain, and finally the JH1 protein-tyrosine kinase domain (Fig. 4).

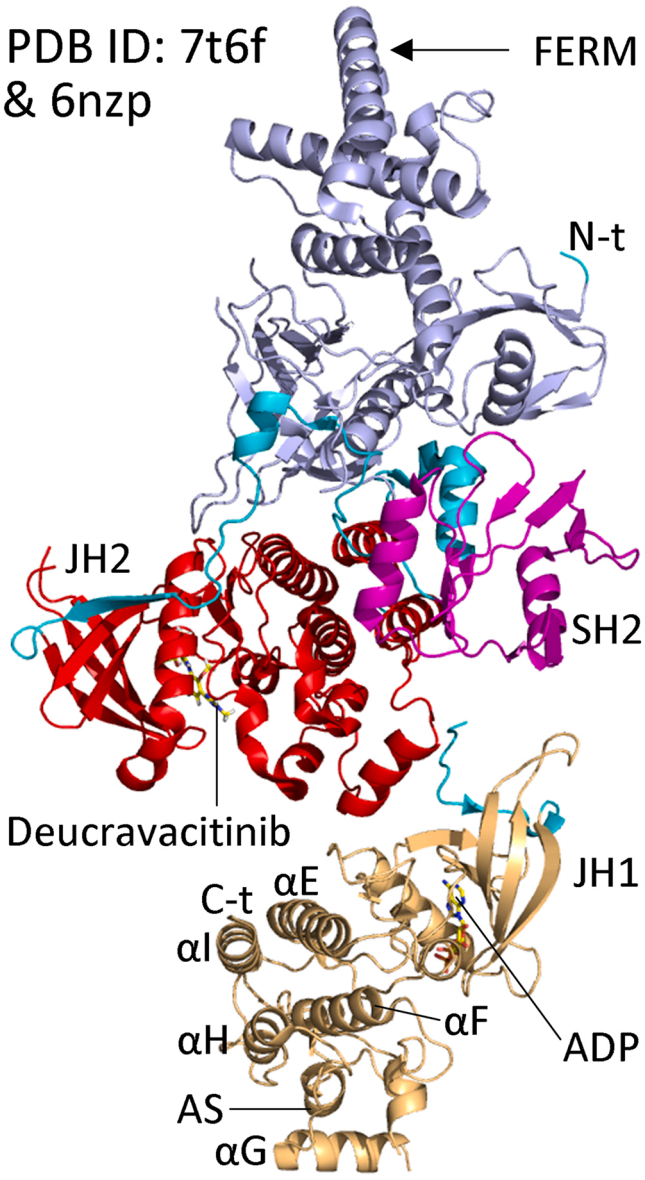


**Fig. 4.** Overview of mouse JAK1 with the FERM, SH2-like, JH2, and JH1 domains. AS, activation segment; C-t, carboxyterminus; N-t, amino-terminus. The distance from deucravacitinib to the ADP site in the operational protein kinase site is about 46 Å.
The figure was made by the superposition of drug-human JH2 TYK2 (PDB ID: 6nzp) with mouse JAK1 holoenzyme (7t6f).

## 4. Protein kinase-inhibitor classification

Based upon the work of other investigators [62–65], we divided the small molecule protein kinase inhibitors into seven major groups including both reversible (Groups I, I½, II, III, IV, and V) and irreversible inhibitors (VI) (Table 5). Type I and I½ inhibitors bind to kinases with the DFG-$D_{in}$ conformation and the type II inhibitors bind to kinases with the DFG-$D_{out}$ conformation. We further divided the type I½ and type II inhibitors into A and B subtypes [32]. Subtype A drugs extend past the gatekeeper residue into the back cleft while subtype B drugs do not enter the back cleft. Based upon preliminary data, the potential significance of this difference is that subtype A inhibitors bind to their enzyme target with longer residence times than subtype B inhibitors [32].

Most protein kinase antagonists bind to the ATP-binding site. Allosteric antagonists do not bind to the ATP site and are non-competitive steady-state inhibitors with respect to ATP. These inhibitors fall into two classes. Those that bind near the ATP binding site are type III allosteric inhibitors and those that bind far from the site are type IV allosteric inhibitors. Binimetinib, cobimetinib, selumetinib and upadacitinib are type III allosteric inhibitors that target MEK1/2, dual specificity kinases [66]. Everolimus, sirolimus, and temsirolimus are three macrolides classified as type IV allosteric inhibitors. These drugs bind to FKBP12 and the drug-protein complex inhibits the catalytic activity of mTOR (mammalian target of rapamycin); as such, these three drugs are indirect blockers of their target protein kinase and are classified as type IV inhibitors [48]. In contrast, asciminib is a type IV allosteric inhibitor that binds directly to BCR-Abl, its drug target. The drug binds to an allosteric site that is far from the ATP-binding site (28 Å) and is one of the best examples of an FDA-approved type IV antagonist. Asciminib is a STAMP (specifically targeting the Abl myristoyl pocket) inhibitor that is FDA-approved for the third line treatment of Philadelphia

**Table 5**
Classification of small molecule protein kinase inhibitors[a].

| Inhibitor type | Properties |
|---|---|
| I | Binds in and around the ATP-binding pocket of an active enzyme |
| I½ A/B | Binds in and around the ATP-binding pocket of an inactive DFG-$D_{in}$ enzyme |
| I½ A | Extends into the back cleft |
| I½ B | Does not extend into the back cleft |
| II A/B | Bind in and around the ATP-binding site of an inactive DFG-$D_{out}$ enzyme |
| II A | Extends into the back cleft |
| II B | Does not extend into the back cleft |
| III | Allosteric inhibitor bound next to the ATP-binding site |
| IV | Allosteric inhibitor bound away from the ATP-binding site |
| V | Bivalent inhibitor spanning two kinase domain regions |
| VI | Covalent inhibitor |

[a] Adapted from Ref. [32].

chromosome-positive CML and the first line treatment of Philadelphia chromosome-positive CML with the *T315I* mutation [48]. Deucravacitinb is another example of a type IV allosteric inhibitor; it targets the JH2 domain of TYK2 and is FDA-approved for the treatment of psoriasis.

Modi and Dunbrack performed a comprehensive analysis of the interactions of drugs and ligands with active and inactive conformations of protein kinases based upon the structure of the activation segment, which begins with the DFG signature [67]. These investigators observed a cluster of protein kinase conformations based upon the disposition of the phenylalanine side chain (DFG-$D_{in}$, DFG-$D_{out}$, and DFG-$D_{inter}$ or intermediate) and the backbone dihedral angles of xDF, where x is the residue proximal to DFG. They detected eight different conformations and classified them based on the Ramachandran regions (A, alpha; B, beta; L, left) of the xDF motif and the χ1 phenylalanine rotamer (minus, plus, trans). Their clustering protocol divided the DFG-$D_{in}$ conformation into six groups or clusters including BLAminus, which contains active structures, and two common inactive forms – BLBplus and ABAminus. $DFG_{out}$ structures are primarily in the BBAminus cluster. The inactive enzymes have features that preclude their binding to $Mg^{2+}$, ATP, and/or their protein substrates. These authors created a useful and noncommercial web site (http://dunbrack3.fccc.edu/kincore/) that enables one to determine whether a protein kinase conformation corresponds to an active (DFG-$D_{in}$, BLAminus) or an inactive enzyme (otherwise). We used this web site to determine whether the structure of the various kinases of the drug-enzyme complexes that we are considering are functional (DFG-$D_{in}$, BLAminus) or inactive.

Carles et al. created a comprehensive listing of therapeutic protein kinase inhibitors that are in clinical trials or have been approved by various international agencies [68]. They produced a searchable and noncommercial web site that is regularly updated that provides the inhibitor structures, trade name, therapeutic indications, physical properties, year of first approval (if applicable), their protein kinase targets, and their protein kinase family (http://www.icoa.fr/pkidb/). The Blue Ridge Institute for Medical Research maintains a web site that lists the FDA-approved protein kinase inhibitors and provides their (i) chemical structures, (ii) number of hydrogen bond donors/acceptors, (iii) calculated log of the distribution coefficient, (iv) number of rings and rotatable bonds, (v) year of initial approval, (vi) protein kinase targets, (vii) clinical indications, and (viii) links to their FDA labels. This web site, which is updated following the approval of a new drug, is found at www.brimr.org/PKI/PKIs.htm.

## 5. Mechanism of deucravacitinb inhibition of TYK2

Deucravacitinib is an *N*-trideuteromethyl-pyridazine derivative (Fig. 5C) that targets TYK2 and is FDA-approved for the treatment of psoriasis. Wrobleski et al. determined the X-ray structure of this drug bound to the JH2 domain of TYK2 and they demonstrated that an amino N–H forms a hydrogen bond with the side chain carbonyl moiety of E688, the first hinge residue (Fig. 6) [69]. Furthermore, the pyridazine nitrogen atoms hydrogen bond with the N–H backbone of V690 and an N–H from the drug hydrogen bonds with the same the third hinge residue. The β3-strand K642 hydrogen bonds with two of the drug-carbonyl groups. The 2-triazole nitrogen interacts with the side chain of R738. The drug makes hydrophobic contact with the first two shell residues (Sh1/2) and three catalytic spine residues (CS6/7/8) and KLIFS residue 3. The KLIFS-3 residue occurs immediately before the G-rich loop. The drug also interacts hydrophobically with $^{596}$GQG$^{598}$ of the glycine-rich loop, the β3-strand K642, Y689 of the hinge, $^{691}$EHGP$^{694}$ of the linker, R738 and N739 of the catalytic loop, L741 of the β7-strand, and S758 (the x of xDFG). The drug occupies the front pocket of the pseudokinase JH2 domain and it is about 46 Å from the ATP-binding site of the functional JH1 TYK2 domain; it is therefore classified as a type IV inhibitor [32]. The presence of the drug on the ATP-binding site of TYK2 JH2 stabilizes the inhibitory action of the JH2 domain over the functional JH1 domain. See Refs. [70–72] for information on clinical trials that led to the approval of deucravacitinib in 2022.

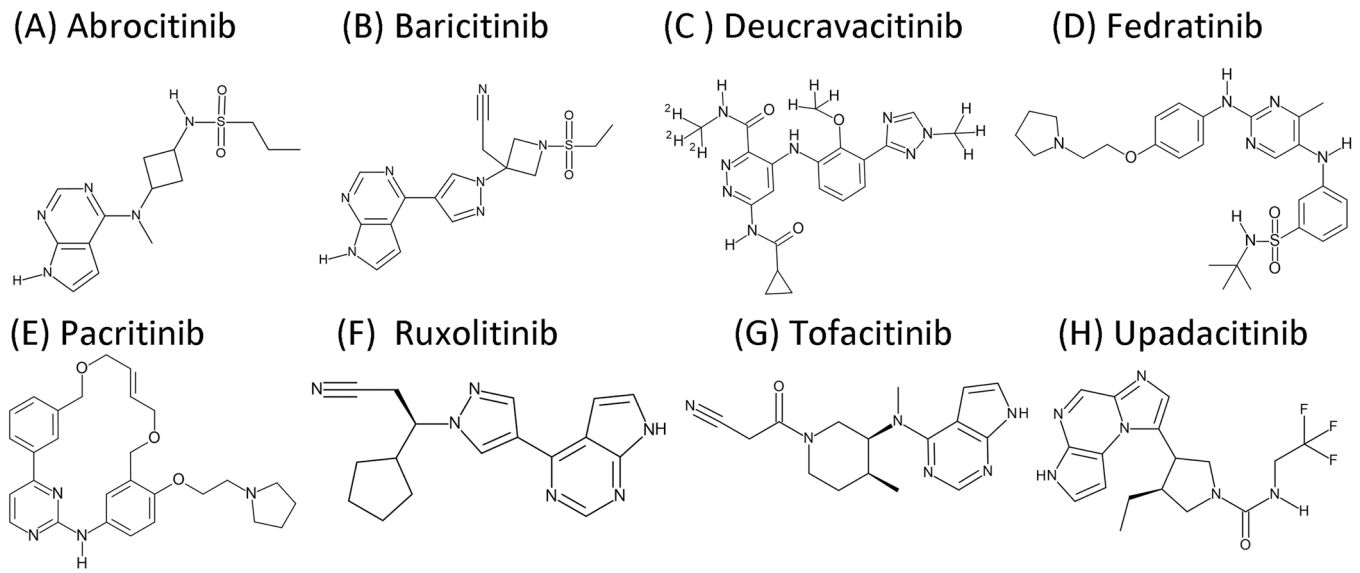


**Fig. 5.** Structure of selected JAK family inhibitors.

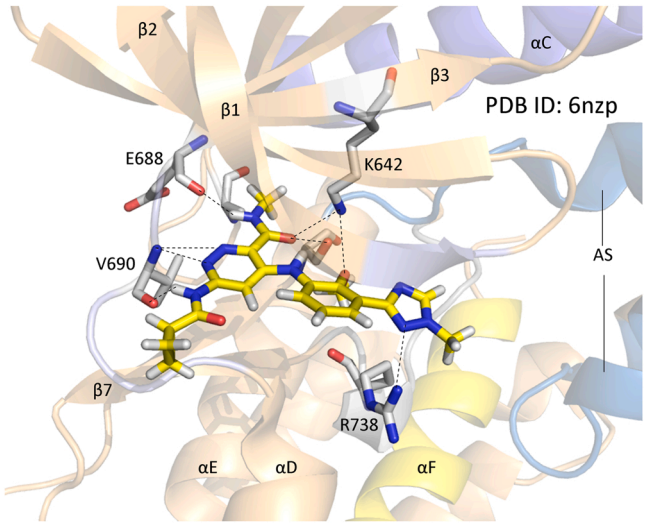


**Fig. 6.** Polar interactions of deucravacitinib with the inactive TYK2 JH2 domain.

## 6. An overview of the JAK family inhibitors

The eight JAK-family inhibitors that are FDA-approved for human use are depicted in Fig. 5 and are listed in Table 6 along with their therapeutic indications and targets. These drugs are used for the treatment of both neoplastic and inflammatory diseases [15,16]. Deucravacitinib is the only JAK family kinase inhibitor prescribed for the treatment of plaque psoriasis. Upadacitinib and abrocitinib inhibit JAK1. In contrast, pacritinib blocks the activity of JAK2 and fedratinib inhibits JAK2 and JAK3. With low nanomolar effectiveness, ruxolitinib and tofacitinib are pan-JAK family inhibitors while baricitinib blocks the activity of JAK1, JAK2, and TYK2 (Table 7). These seven drugs target the ATP-binding site of the JH1 domain of their target enzymes while deucravacitinib binds to the JH2 domain of its target (TYK2); it also binds weakly to the JH2 domain of JAK1 [74]. In cell-based assays, deucravacitinib showed 100- to 200-fold greater selective inhibition for TYK2 than JAK1/3 and was 3000-fold more selective for TYK2 than JAK2 [75,76].

Ruxolitinib, tofacitinib, and upadacitinb are three JAK inhibitors whose FDA label contains black box warnings against serious infections, myocardial infarction, stroke, lymphoma and other malignancies, deep vein thrombosis, pulmonary embolism, and arterial thrombosis. The "black box" appears in the FDA label in bold type expressing these warnings. The deucravacitinib label lacks black box warnings and adverse events associated with deucravacitinib are generally less severe than those of the other JAK inhibitors [70,76]. Complications associated with deucravacitinib include nasopharyngitis, upper respiratory infections, headache, and elevated creatine phosphokinase activity. Anemia is one of the adverse events produced by JAK inhibitors. The bone marrow responds to erythropoietin leading to the production of red blood cells; the erythropoietin receptor uses the JAK2/JAK2 pair for signaling [76] and JAK JH1 inhibitors generally produce anemia (regardless of their selectivity or proposed mechanism of action). However, anemia does not occur in people receiving deucravacitinib. Lymphocyte homeostasis is regulated in part by IL-7 and IL-15. Their signaling involves the JAK1-JAK3 pair. Lymphocyte, natural killer cell, B-cell, and neutrophil counts, which are markers of JAK1, JAK2, or JAK3 inhibition, remained stable in patients with psoriasis undergoing treatment with deucravacitinib [76]. Inhibition of IL-6 signaling results in increased circulating lipids and decreased C-reactive protein. Mean total cholesterol, triglycerides, and C-reactive protein levels were unchanged in patients undergoing deucravacitinib treatment. These studies indicate that deucravacitinib therapy produces fewer side effects than the traditional JAK JH1 antagonists.

**Table 6**
FDA-approved JAK family inhibitors and their indications[a].

| Drugs | Targets | Diseases | Approval date | Clinical trials |
|---|---|---|---|---|
| Abrocitinib | JAK1, JAK2 | Atopic dermatitis | 2022 | NCT03627767<br>NCT03720470 |
| Baricitinib | JAK1, JAK2, TYK2 | Rheumatoid arthritis | 2018 | NCT02265705<br>NCT01710358 |
| | | Covid 19 | 2022 | NCT04421027<br>NCT04401579 |
| Deucravacitinib | TYK2 | Psoriasis | 2022 | NCT04036435<br>NCT04772079 |
| Fedratinib | JAK2, JAK2 V617F, JAK3 | Myelofibrosis | 2019 | NCT00724334<br>NCT00631462<br>NCT01437787 |
| Pacritinib | JAK2, JAK2 V617F | Myelofibrosis | 2022 | NCT04884191 |
| Ruxolitinib | JAK1, JAK2, JAK2 V617F, JAK3, TYK2 | Myelofibrosis | 2011 | NCT00952289 |
| | | Polycythemia vera | 2014 | NCT02038036 |
| | | Acute and chronic graft vs. host disease | 2019, 2021 | NCT03112603<br>NCT03147742 |
| Tofacitinib | JAK1, JAK2, JAK3, TYK2 | Rheumatoid arthritis | 2012 | NCT02187055 |
| | | Psoriatic arthritis | 2017 | NCT01877668 |
| | | Ulcerative colitis | 2018 | NCT03281304 |
| | | Juvenile idiopathic arthritis | 2020 | NCT02592434 |
| | | Ankylosing spondylitis | 2021 | NCT03502616 |
| Upadacitinib | JAK1 | Rheumatoid arthritis | 2019 | NCT02706847 |
| | | Psoriatic arthritis | 2021 | NCT03104400 |
| | | Atopic dermatitis | 2022 | NCT03738397 |
| | | Ulcerative colitis | 2022 | NCT02819635 |

[a] Data from Refs. [72,73].

## 7. Epilogue

Medicinal chemists and pharmacologists have studied the physicochemical properties of drugs that are orally bioavailable such as deucravacitinib. Lipinski's rule of five (Ro5) is a computational and experimental procedure that is used to characterize membrane permeability, solubility, and effectiveness during drug discovery and development [77]. It is a rule of thumb that determines whether an agent with specific pharmacological actions has properties suggesting that it would be orally effective. The Lipinski benchmarks were based on findings indicating that most orally effective medicinals are relatively small and

**Table 7**
Janus kinase JH1 and JH2 inhibitor $EC_{50}$ values (nM)[a, b, c].

| Drug | JAK1-JH1 | JAK2-JH1 | JAK3_JH1 | TYK2-JH1 |
|---|---|---|---|---|
| Abrocitinib | 5.01 | 39.8 | 501 | 1260 |
| Baricitinib | 1.00 | 0.79 | 25.1 | 7.94 |
| Fedratinib | 100 | 3.16 | 1.00 | 20.0 |
| Pacritinib | 1280 | 23 | 520 | 50 |
| Ruxolitinib | 0.079 | 0.039 | 2.51 | 0.40 |
| Tofacitinib | 0.50 | 0.50 | 0.16 | 3.98 |
| Upadacitinib | 43 | 120 | 2300 | 4700 |
| Deucravacitinib | > 10,000<br>JAK1-JH2 | > 10,000<br>JAK2-JH2 | > 10,000<br>JAK3-JH2 | > 10,000<br>TYK2-JH2 |
| Deucravacitinib[c] | 0.2 | 15 | ? | 0.0038 |

[a] $EC_{50}$ (effective concentration) refers to $IC_{50}$, $K_d$, or $K_i$.
[b] Ref. [73] for JH1 values.
[c] Ref. [74] for JH2 values.

moderately lipophilic substances. The Ro5 criteria indicates that less than ideal oral bioavailability is more likely to occur when (i) the atom-based calculated Log P (ALogP) is greater than 5 (it is 1.7 for deucravacitinib), when (ii) there are more than 5 hydrogen-bond donors (3 for deucravacitinib), when (iii) there are more than 5 × 2 or 10 hydrogen-bond acceptors (8 for deucravacitinib), and when (iv) the molecular weight is more than 5 × 100 or 500 (425 for deucravacitinib) [77]. This drug thus fulfills each Ro5 criterion.

Although medicinal chemists have produced drugs containing deuterium since the 1970 s, deucravacitinib is the first that has been initially designed with the heavy isotope in place during the drug development process [78]. The use of deuterium in other cases has been used in modifying an already approved medicine. The rationale for using deuterium in the trimethylamide was to decrease the rate of demethylation and to slow the production of a metabolite that is active against a variety of targets in addition to TYK2. The second unique aspect in the development of deucravacitinib is the targeting of a pseudokinase domain. Over 50 human protein kinases contain pseudokinase domains and these proteins have been implicated in various diseases and thus represent new drug targets. This is challenging owing to the need to laboriously determine the biology of each pseudokinase domain and the impact of any of its ligands. The successful development of deucravacitinib may stimulate the development of additional pseudokinase ligands for the JAK family and for other kinase families as well.

## Conflict of interest

The author is unaware of any affiliations, memberships, or financial holdings that might be perceived as affecting the objectivity of this review.

## Data Availability

No data was used for the research described in the article.

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