

Molecular Pathways: Molecular Basis for Sensitivity and Resistance to JAK Kinase Inhibitors

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Abstract

Janus-activated kinases (JAK) are the mediators of a variety of cytokine signals via their cognate receptors that result in activation of intracellular signaling pathways. Alterations in JAK1, JAK2, JAK3, and TYK2 signaling contribute to different disease states, and dysregulated JAK–STAT signaling is associated with hematologic malignancies, autoimmune disorders, and immune-deficient conditions. Genetic alterations of JAK2 occur in the majority of patients with myeloproliferative neoplasms and occur in a subset of patients with acute leukemias. JAK-mediated signaling critically relies on STAT transcription factors, and on activation of the MAPK and PI3K/Akt signaling axes. Hyperactive JAK at the apex of these potent oncogenic signaling pathways therefore represents an important target for small-molecule kinase inhibitors in different disease states. The JAK1/2 inhibitor ruxolitinib and the JAK3 inhibitor tofacitinib were recently approved for the treatment of myelofibrosis and rheumatoid arthritis, respectively, and additional ATP-competitive JAK inhibitors are in clinical development. Although these agents show clinical activity, the ability of these JAK inhibitors to induce clinical/molecular remissions in hematologic malignancies seems limited and resistance upon chronic drug exposure is seen. Alternative modes of targeting JAK2 such as allosteric kinase inhibition or HSP90 inhibition are under evaluation, as is the use of histone deacetylase inhibitors. Combination therapy approaches integrating inhibition of STAT, PI3K/Akt, and MAPK pathways with JAK kinase inhibitors might be critical to overcome malignancies characterized by dysregulated JAK signaling. *Clin Cancer Res*; 20(8); 2051–9. ©2014 AACR.

Background

A modular receptor tyrosine kinase

Janus-activated kinases (JAK) are cytoplasmic tyrosine kinases that associate with transmembrane class I/II cytokine receptors. The JAK–cytokine receptor complex equals a functional receptor tyrosine kinase and propagates extracellular cytokine signals across the cell membrane to activate intracellular messenger pathways. JAK kinases mediate a variety of cytokine signals affecting cellular growth, differentiation, and survival predominantly in hematopoiesis and immune response (1). Dysregulated JAK activity is involved in hematologic malignancies, autoimmune disorders, and immunodeficient conditions and has been implicated in the pathogenesis of a subset of solid tumors. Most prominent is the role of activated JAK2 signaling due to the V617F mutation observed in the majority of patients with myeloproliferative neoplasms (MPN; refs. 2–5).

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The JAK family

Numerous cytokines signal through the four JAK family members. JAK1, JAK2, JAK3, and TYK2 range from 120 to 140 kDa in size and share seven JAK homology domains (JH1–7), which include the C-terminal kinase domain, an adjacent pseudokinase domain and the N-terminal Src homology 2 (SH2) and FERM (Band-4.1, ezrin, radixin, and moesin)-like domain mediating the association with the cytokine receptor. The kinase domain contains an N- and C-lobe surrounding the ATP-binding site and an activation loop with tandem tyrosine residues Y1007/Y1008 that regulate kinase activity through autophosphorylation (6). The pseudokinase domain, which classically has been thought to be deficient of catalytic activity, negatively regulates the kinase domain by phosphorylation of S523 and Y570 (7). However, recent studies have suggested that the pseudokinase domain might indeed have catalytic activity, which is required for autoinhibition of the JAK kinase domain (7, 8). The crystal structures of complete JAK molecules will be critical to clarify the JAK structure–function relationship in more detail and to reveal specific structural differences between the JAK family members.

JAK1, JAK2, JAK3, and TYK2 associate with different cytokine receptors and activate specific members of the STAT family as downstream effectors and are thus critically involved in different aspects of hematopoiesis and immune response. JAK2 is the most extensively investigated of the JAK family of kinases due to its pathogenic role in MPNs and

other malignancies. JAK2 is essential for signaling through hematopoietic cytokine receptors, including type I homodimeric erythropoietin (EpoR) and thrombopoietin receptors (TPOR or MPL) and the heterodimeric granulocyte macrophage colony-stimulating factor (GM-CSFR), interleukin (IL)-3 and IL-5 receptors. JAK2 also mediates signaling from the prolactin, growth hormone, and leptin receptors and is involved in signaling through IFN- γ and members of the IL-10 and IL-12-type cytokine receptor family. The critical relation of JAK2 and hematopoietic cytokine signaling is exemplified by its interaction with the EpoR. In the absence of JAK2 expression, EpoR signaling is abolished and the germline JAK2 knockout mouse is embryonically lethal at day 12.5 of embryogenesis due to loss of definitive erythropoiesis (9). Germline-activating mutations in JAK2 lead to inherited polycythemia, whereas acquired JAK2 mutations are critical in the pathogenesis of MPN and are also seen in acute leukemia. The transforming capacity of JAK2 in hematopoietic cells is restricted to its EpoR- or MPL-bound form, highlighting the functional interdependence of JAK2 and hematopoietic cytokine receptors (10).

JAK1 is critical for IFN- γ and IFN- α/β signaling, mostly as part of a heterodimer with JAK2 or TYK2, and is involved in IL-2 receptor signaling as a heterodimer with JAK3. Somatic gain-of-function mutations in JAK1 have been identified in acute leukemia (11), whereas JAK1 deficiency is perinatally lethal due to impaired lymphopoiesis and central nervous system (CNS) development (12).

JAK3 mutations were the first human germline JAK mutations reported and give rise to severe combined immunodeficiency (SCID) with absent T and natural killer (NK) cells (13). JAK3 associates with cytokine receptors containing the IL-2 common γ chain in hematopoietic cells, which includes the IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 receptors.

TYK2 binds a variety of receptors including the IFN, IL-10, and IL-6 family and the IL-12/23/27 group. Germline *Tyk2* deficiency in murine models results in defective viral, bacterial, and fungal immune defense (14), whereas inherited TYK2 mutations have been described in autosomal recessive hyperimmunoglobulin E syndrome in humans. The current understanding of the different JAK family members implicates JAK1, JAK3, and TYK2 mainly in lymphopoiesis and immune response, whereas JAK2 is most critically involved in the development, production, and function of the myeloid lineages.

The JAK signaling cascade

The JAK kinases, in association with dimeric cytokine receptors, are activated upon ligand binding, leading to JAK autophosphorylation predominantly at the tandem tyrosines in the activation loop. JAKs then phosphorylate intracellular receptor tyrosines, creating binding sites for SH2 domain-containing proteins such as the STATs. In case of JAK2, STAT3 and STAT5 are recruited and directly phosphorylated, while TYK2 mostly acts through STAT4 and STAT1 phosphorylation. STATs then dimerize and translocate to the nucleus to initiate transcription of effector genes involved in cell-cycle regulation, apoptosis, and proteaso-

mal degradation (Fig. 1). JAK2 also activates the PI3K/Akt and the mitogen-activated protein kinase (MAPK) signaling pathways, which further promote proliferation and survival (refs. 5, 10, 15; Fig. 1). Subsequent expression of the serine/threonine kinase Pim favors cell survival by phosphorylation of Bad and increased activity of antiapoptotic Bcl2 and BclxL, whereas increased expression of cyclin D1/2 and Cdc25A promotes cell-cycle progression from G₁ to S phase (16).

In contrast, expression of the suppressor of cytokine signaling proteins (SOCS) forms a negative feedback loop. SOCS 1 and 3 compete with STATs for docking at the cytokine receptor, promote proteasomal degradation of JAK2 by ubiquitination, and interfere with its catalytic function via their kinase inhibitory region (17). JAK2 is also negatively regulated by the Casitas B-cell lymphoma (CBL) proteins, which act as ubiquitin ligases for numerous tyrosine kinases, and by the adaptor protein LNK (also called SH2B3), which sequesters JAK2 (18). Further regulatory control of JAK signaling comes from protein tyrosine phosphatases (PTP) that dephosphorylate receptors, JAKs and STATs, and from protein inhibitors of activated STAT (PIAS), which prevent STATs from binding to target DNA.

Beyond activating cytoplasmic signaling cascades, JAK2 has recently been shown to translocate to the nucleus and to have a direct impact on chromatin state. Nuclear JAK2 phosphorylates histone H3 at Y41, leading to the displacement of heterochromatin protein 1 (19, 20), and PRMT5, a histone arginine methyltransferase (21). JAK2 also phosphorylates p27Kip1, a cyclin-dependent kinase inhibitor involved in cell-cycle regulation. These nuclear functions of JAK2 warrant further investigation and may represent a novel therapeutic opportunity.

Oncogenic JAK2 signaling in MPNs

Dysregulated JAK2 signaling is a hallmark of MPNs, clonal stem cell disorders characterized by excessive proliferation of differentiated hematopoietic cells derived from one or more myeloid lineages. Dameshek in 1951 recognized polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis as interrelated diseases due to a characteristic "pancytosis" with proliferation of the erythroid, megakaryocyte, and granulocyte lineages (20). Patients with polycythemia vera present with erythrocytosis, patients with essential thrombocythemia with thrombocytosis, and patients with primary myelofibrosis with splenomegaly, a leukoerythroblastic blood smear, and bone marrow fibrosis. These MPNs share cardinal clinical features, including an increased risk of thrombosis and bleeding, development of bone marrow fibrosis, splenomegaly, and a risk of transformation to acute leukemia. While patients with polycythemia vera or essential thrombocythemia may transform to a post-PV/ET myelofibrotic phase similar to primary myelofibrosis, a subset of patients with essential thrombocythemia subsequently develop erythrocytosis and are diagnosed with polycythemia vera. These clinical observations highlight the biologic overlap between the different forms of MPNs.

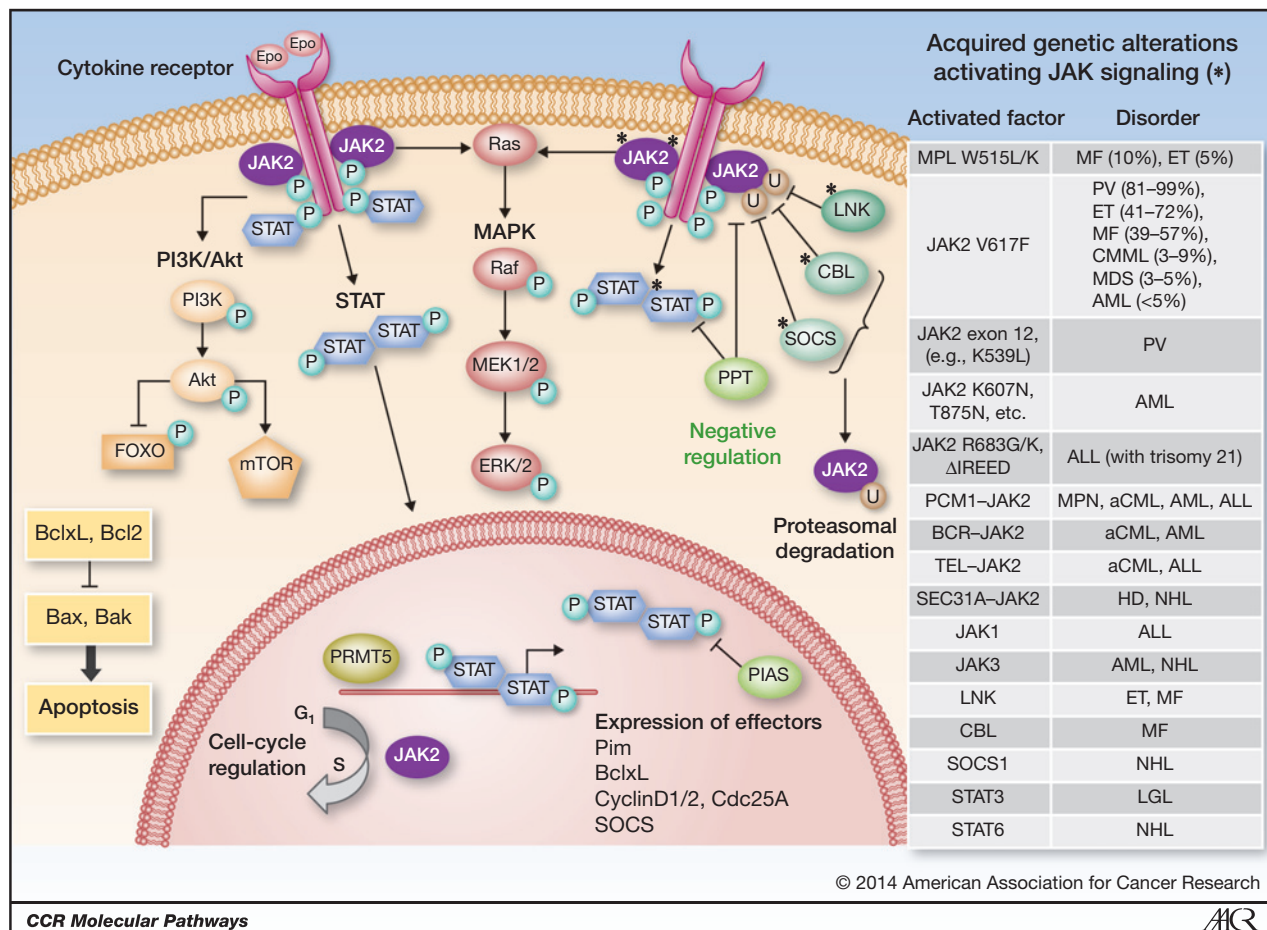


Figure 1. Overview of molecular JAK signaling. Ligand binding to cell surface cytokine receptors initiates autophosphorylation of JAK2 and phosphorylation of cytoplasmic receptor tyrosines. STATs bind receptor phosphotyrosines via SH2 domains and translocate to the nucleus to induce expression of effector genes such as antiapoptotic Pim kinase and BclxL, cyclins promoting cell-cycle progression, and SOCS forming a negative feedback loop. JAK2 also activates the PI3K/Akt and the MAPK pathways promoting proliferation and survival. Nuclear JAK2 is involved in epigenetic modifications. The negative regulators SOCS and CBL mark JAK2 for proteasomal degradation, LNK sequesters JAK2 by direct binding, and PTPs dephosphorylate cytokine receptors, JAK, and STATs. The protein inhibitors of STATs (PIAS) prevent STATs from binding target DNA. Stars indicate signaling components with reported genetic alterations. Transcription factors are shown in blue, negative regulators in green. CMML, chronic myelomonocytic leukemia; HD, Hodgkin disease; LGL, large granular lymphocyte leukemia; MF, myelofibrosis; NHL, non-Hodgkin lymphoma.

On a molecular level, the first insight into the molecular cause of these diseases came in 2005 with the discovery of the somatic V617F mutation in exon 14 of *JAK2* in more than half of all patients with MPN (2–5). Crystal structures of the wild-type and mutant pseudokinase domain have revealed that the valine-to-phenylalanine substitution rigidifies JH2 and relieves negative feedback regulation of the tyrosine kinase (8). In addition, *JAK2* V617F escapes negative feedback by SOCS3 as, in contrast with the wild-type kinase, phosphorylation of *JAK2* V617F is paradoxically increased upon overexpression of SOCS3 *in vitro*. *JAK2* V617F constitutively activates JAK–STAT signaling and, in combination with expression of a hematopoietic cytokine receptor, leads to transformation of hematopoietic stem cells (10).

The *JAK2* V617F mutation is seen in 81% to 99% of patients with polycythemia vera, 41% to 72% of patients with essential thrombocythemia, and 39% to 57% of

patients with primary myelofibrosis and has greatly facilitated the diagnostics of MPNs. However, it is incompletely understood how one specific mutation contributes to the pathogenesis of three phenotypically distinct disease entities. *JAK2* V617F gene dosage may influence the phenotype of MPNs, as hematopoietic cells are homozygous for V617F in most patients with polycythemia vera, but rarely in patients with essential thrombocythemia (22). High V617F allele burden is associated with an increased risk for thrombotic complications and progression to myelofibrosis, while the impact of allele burden on clinical outcome remains controversial (23). Homozygosity for V617F does not arise from sequential mutation events, but rather as a result of mitotic recombination and resultant uniparental disomy of the 9p24 locus (24). Recent studies have identified a specific "46/1" haplotype at the *JAK2* locus prone to acquire *JAK2* V617F (25). Analysis of familial MPNs suggests that there are additional, unknown inherited alleles

that predispose to familial MPN. Genetic events preceding *JAK2* V617F are also suggested by cases of secondary acute myeloid leukemia (AML) that are *JAK2* V617F-negative, in contrast with the preceding myeloproliferative neoplasm. In such cases, the presence of shared additional cytogenetic and molecular alterations demonstrates that the myeloproliferative neoplasm and subsequent AML arise from the same clone (26).

In *JAK2* V617F-negative polycythemia vera, missense mutations in *JAK2* exon 12 in the pseudokinase-SH2 linker region are identified in nearly all cases (27). They analogously lead to constitutive kinase activation and dysregulated JAK signaling, but result in isolated erythrocytosis, whereas V617F-positive polycythemia vera mostly shows concomitant thrombo- and leukocytosis. *JAK2* exon 12 mutations are not found in essential thrombocythemia and primary myelofibrosis.

Essential thrombocythemia is characterized by thrombocytosis due to increased proliferation of the megakaryocytic lineage and by an increased risk for thrombotic and bleeding complications. In V617F-negative essential thrombocythemia, 5% of patients carry acquired mutations in exon 10 of the thrombopoietin receptor *MPL*, most commonly *MPL* W515L or W515K (28). They result in constitutive receptor activation and downstream JAK2 signaling, including the STAT-, PI3K/Akt-, and MAPK-axis, and transform hematopoietic cells analogously to *JAK2* mutations. In contrast with murine models of *JAK2* V617F, which largely result in a polycythemia vera-like phenotype, expression of *MPL* W515L *in vivo* causes pronounced leukocytosis and thrombocytosis. Another 5% of V617F-negative essential thrombocythemia harbors mutations in *LNK*, affecting the negative regulation of JAK2 activity.

Myelofibrosis may develop as a primary manifestation of MPNs or develop from preceding polycythemia vera or essential thrombocytosis. Beyond leukocytosis and thrombocytosis, splenomegaly and progressive bone marrow fibrosis leading to extramedullar hematopoiesis and ultimately cytopenias are characteristic of primary myelofibrosis. In addition, the levels of circulating inflammatory cytokines such as INF- γ , TNF- α , IL-1, IL-6, and IL-8 are elevated (29). The *MPL* W515L mutation is identified in 10% of *JAK2* V617F-negative myelofibrosis, while mutations in the negative regulators *LNK* and *CBL* are found in approximately 5% of patients with myelofibrosis. Very recently, two groups independently identified somatic mutations in exon 9 of *CALR* encoding the endoplasmic reticulum (ER) chaperone calreticulin, in 70% to 84% of *JAK2*-unmutated myelofibrosis and essential thrombocythemia (30, 31). Several variants due to indels with subsequent frameshift all displayed an altered C-terminus deficient of an ER retention signal. The discovery of calreticulin mutations in *JAK*-unmutated MPNs for the first time implicates an ER chaperone in leukemogenesis and will further improve the diagnostics of MPNs. Although the functional effects of *CALR* mutations require further investigation, first *in vitro* studies suggest a critical involvement of STAT5 activation.

The STAT-, PI3K/Akt-, and MAPK pathways represent major routes of oncogenic signaling in many tyrosine kinase-driven systems, including JAK kinase signaling. The essential requirement of the STAT axis downstream of activated JAK2 is highlighted by the critical role of STAT5 for the transforming activity of *JAK2* V617F and of *TEL-JAK2* in murine bone marrow transplantation models (32, 33). The PI3K/Akt pathway is involved in malignant transformation by *JAK2* V617F (34), while the relative significance of the MAPK pathway requires more detailed evaluation.

Beyond MPNs: oncogenic JAK signaling in hematologic malignancies

Hyperactive JAK signaling is not exclusive to MPNs. *JAK2* V617F is found in other myeloid malignancies, including 7.8% to 13% of chronic myelomonocytic leukemia and 1% to 4.2% of myelodysplastic syndrome (35). *JAK2* V617F-positive AML is mostly observed in patients with a previous myeloproliferative neoplasm, but cases of V617F in *de novo* AML have been described. Alternative *JAK2* mutations cluster in the pseudokinase domain, including the K607N mutation observed in AML (36) and L611S allele observed in acute lymphatic leukemia (ALL; ref. 37). A gain-of-function mutation in the kinase domain, T875N, was reported in acute megakaryoblastic leukemia (38). *JAK2* mutations are relatively rare in lymphoid malignancies except for ALL, most commonly in Down syndrome-associated ALL, which has a high frequency of mutations at R682 in the pseudokinase domain. *JAK1*, *JAK2*, and *JAK3* mutations and translocations are observed in high-risk BCR-ABL B-cell ALL, and it is important to identify patients with mutations in JAK kinases as these alleles have prognostic and therapeutic relevance (39, 40). *JAK2* may alternatively acquire constitutive activation as a fusion partner in oncogenic translocations described in both myeloid and lymphoid neoplasms. A *TEL-JAK2* fusion combining the catalytic domain of *JAK2* with the oligomerization domain of an *ETS* family transcription factor was described in childhood T-cell ALL (41) and atypical chronic myeloid leukemia (aCML), while the *PCM1-JAK2* fusion between *JAK2* and the centrosomal factor *PCM1* has been identified in a variety of hematologic malignancies (42). *JAK2* has also been partnered by *BCR* in aCML and AML and by *SEC31A* in Hodgkin lymphoma. Amplification of the *JAK2* locus has been detected in Hodgkin lymphoma and mediastinal B-cell lymphoma (43). The diversity of JAK2 dysregulation highlights its significance as an oncogenic driver and as a prominent therapeutic target in hematologic malignancies.

Beyond *JAK2*, oncogenic mutations occur in other *JAK* family members and their regulators. Gain-of-function mutations in *JAK3* occur in megakaryoblastic leukemia (44) and NK-T-cell lymphoma (45) and cutaneous T-cell lymphoma. *JAK1* mutations are seen in ALL (11), and biallelic inactivating mutations of *SOC31* in mediastinal B-cell lymphoma (46). Several missense mutations in *STAT3* and *STAT5b* have been identified in large granulocytic leukemia and (47) recurrent mutations of the *STAT6* DNA-binding domain in mediastinal B-cell lymphoma.

JAK2 mutations and translocations are very rare in solid cancers. However, activated JAK signaling in certain solid tumors in the absence of JAK2 mutations fosters interest into JAK2 inhibition as a therapeutic strategy. Ectopic expression of EpoR was shown to desensitize HER2-positive breast cancer to the HER2 inhibitor trastuzumab (48), and is also seen in head and neck squamous cell carcinoma in which it correlates with tumor aggressiveness. Reduced levels of the microRNA miR-375, which negatively regulates JAK2 expression, represent another mechanism of JAK2 activation seen in gastric cancer (49). In non-small cell lung cancer, IL-6/JAK2/STAT3 signaling was shown to promote dedifferentiation and to correlate with microvessel density. JAK signaling is increasingly implicated in the pathogenesis of several solid tumors, but its role is incompletely understood and requires detailed investigation to validate JAK2 as a therapeutic target in these malignancies.

Clinical-Translational Advances

Therapeutic JAK inhibition

The identification of the JAK2 V617F mutation in patients with MPNs fueled the development of small-molecule JAK inhibitors to specifically target hyperactive JAK signaling. Several compounds differing in structure and JAK selectivity profile are at different stages of clinical development (Table 1) after the previous standard of care in MPNs had been restricted to palliation of symptoms and to prevention and treatment of thrombohemorrhagic events by low-dose aspi-

rin, phlebotomy, and hydroxyurea, while allogeneic stem cell transplantation represented the only curative approach in these patients. Beyond MPNs, there is an increasing interest in JAK inhibitors for targeted therapy of other hematologic malignancies, certain solid cancers and, given the role of JAK-mediated cytokine signaling in immune response, in autoimmune disorders. The compounds currently in clinical testing target both wild-type and mutated JAKs in an ATP-competitive manner by occupying the ATP-binding pocket. This type I mode of inhibition stabilizes the kinase in its active conformation and results in paradoxically increased phosphorylation of the JAK2 activation loop, a phenomenon whose relevance is incompletely understood (50).

Ruxolitinib is the first JAK inhibitor approved by the U.S. Food and Drug Administration (FDA) in 2011 and by the European Medicines Agency in 2012 for treatment of intermediate and high-risk myelofibrosis. Two phase III studies demonstrated superiority of ruxolitinib over placebo (COMFORT I, ref. 51) and best available therapy (COMFORT II, ref. 52) in treatment of myelofibrosis. A >35% reduction of spleen size was achieved in 28% to 41% of patients at 24 weeks of therapy and substantial improvement of constitutional symptoms was observed in 46% of patients treated with ruxolitinib. Leukocytosis and thrombocytosis were decreased and JAK inhibitor therapy resulted in a marked reduction in inflammatory cytokine levels. Ruxolitinib is well tolerated with grade 3 and 4 adverse events due to myelosuppression in <10% of patients.

Table 1. JAK inhibitors in clinical development

Compound	Previous name	Targets	Disorder	Stage	Trade name (manufacturer)
Ruxolitinib	INCB18424	JAK1, JAK2	Myelofibrosis	FDA approved	Jakafi (Incyte)
			PV	Phase III	
			ET	Phase II	
			MDS	Phase II	
			AML	Phase II	
SAR302503	TG101348	JAK2, FLT3	Lymphoma	Phase II	
			Myelofibrosis	Phase III terminated ^a	
Momelotinib	CYT387	JAK1, JAK2	PV and ET	Phase II terminated ^a	
			Myelofibrosis	Phase II	
Pacritinib	SB1518	JAK2, FLT3	Myelofibrosis	Phase III	
			MDS	Phase I/II	
			AML	Phase I/II	
			Lymphoma	Phase I/II	
AZD1480	—	JAK1, JAK2, FLT3, aurora	Myelofibrosis	Phase I/II	
BMS911543	—	JAK2	Myelofibrosis	Phase I/II	
Lestaurtinib	CEP701	JAK2, FLT3	Myelofibrosis, PV, and ET	Phase I/II	
Baricitinib	—	JAK1, JAK2	Rheumatoid arthritis, psoriasis	Phase II	
Tofacitinib	—	JAK1, JAK3	Rheumatoid arthritis	FDA approved	Xeljanz (Pfizer)
			Psoriasis, IBD	Phase II	

NOTE: Small-molecule JAK inhibitors and their stage of clinical development are indicated.

Abbreviations: IBD, inflammatory bowel disease; MDS, myelodysplastic syndrome; MF, myelofibrosis.

^aThe phase II and III studies of SAR302503 in PV, ET, and MF were terminated in November 2013 due to suspected CNS toxicity.

Anemia and thrombocytopenia are most probably due to the essential role of JAK2 in hematopoiesis. Consequently, platelet counts <200 g/L require treatment at a reduced dose, whereas platelets <100 g/L preclude the use of ruxolitinib therapy outside of a clinical trial. Ruxolitinib is currently under evaluation in a phase III trial in polycythemia vera and a phase II study in essential thrombocythemia. As the significance of activated JAK signaling is increasingly recognized across hematologic and solid cancers as well as autoimmune disorders, phase II studies of ruxolitinib in AML, myelodysplastic syndrome, lymphoma, multiple myeloma, androgen-independent prostate cancer, rheumatoid arthritis, and psoriasis are under way.

SAR302503 (formerly TG101348) selectively inhibits JAK2 at an IC_{50} value of 3 nmol/L as compared with 105 nmol/L for JAK1 and also shows Flt3 inhibitory activity. A phase I trial on 59 patients with myelofibrosis showed prompt response of splenomegaly, leukocytosis, thrombocytosis, and constitutional symptoms as well as significant reduction of V617F allele burden (53). Inflammatory cytokine levels were not affected which might relate to the more JAK2-specific action. Besides cytopenias, gastrointestinal side effects were most prominent. A phase III study comparing SAR302503 with placebo in myelofibrosis was completed (JAKARTA, NCT01437787), whereas a phase II study in polycythemia vera and essential thrombocythemia was previously initiated (NCT01420783). However, recent reports have suggested that this agent is associated with an increased risk of Wernicke encephalopathy, and clinical development is currently on hold pending further evaluation of these CNS toxicities.

Momelotinib (CYT387) is a JAK1/2 inhibitor that reduced splenomegaly, constitutional symptoms, and inflammatory cytokines in a single-center phase I/II study in myelofibrosis (54). Of note, 70% of patients with transfusion-dependent anemia at initiation of the study became transfusion-independent for a minimum of 3 months. Results of multicenter follow-up phase II/III studies are awaited. Confirmation of a beneficial anemia response would render momelotinib a favorable treatment in transfusion-dependent patients with myelofibrosis.

Pacritinib (SB1518) is a pyrimidine-based inhibitor of JAK2 and FLT3 with efficacy in a phase I/II study of myelofibrosis (55). Its safety profile is favorable, particularly regarding hematologic toxicity. While type I JAK2 inhibitors in current clinical evaluation typically cause anemia and thrombocytopenia, pacritinib shows less myelosuppression with sustained platelet counts. Adverse events are mainly gastrointestinal, but low grade. A phase III trial in myelofibrosis has been initiated (NCT01773187). The compound is also being evaluated in phase I/II studies for myelodysplastic syndrome, AML, and lymphoma.

Additional JAK1 and JAK2 inhibitors in clinical development. AZD1480 is a JAK1/JAK2 inhibitor with activity also toward FLT3 and aurora kinases. It has shown inhibition of the TEL-JAK2 fusion in AML and a phase I/II study in myelofibrosis is ongoing. AZD1480 is being

evaluated as a targeted therapy in Hodgkin lymphoma, multiple myeloma, and certain solid tumors. BMS911543 is a JAK2 selective inhibitor, which is currently evaluated in myelofibrosis. Baricitinib is a JAK1/JAK2 inhibitor with favorable effects in murine models of arthritis (56), which has entered clinical trials for rheumatoid arthritis and psoriasis.

Tofacitinib is a JAK1/JAK3 inhibitor recently approved for therapy of rheumatoid arthritis (57). JAK3 represents a favorable target for novel immunosuppressive agents due to its expression restricted to the hematopoietic system. Tofacitinib interferes with stimulation of T lymphoblasts via IL-2/STAT5 and also with expression of inflammatory cytokines via IL-6/STAT3 and STAT1. Clinical phase II and III studies in rheumatoid arthritis, renal transplant rejection, psoriasis, and inflammatory bowel disease were favorable and additional trials are under way. Adverse events include increased incidence of viral infections and decreased hemoglobin most probably due to an inhibitory effect on JAK2 at clinically active doses.

Limitations of type I JAK inhibition in MPNs

The translation of type I JAK inhibitors to clinical application in myelofibrosis represents a significant advance for the treatment of patients with MPNs who benefit from improved quality of life. Ruxolitinib's success has led to the rapid development of additional JAK inhibitors that will extend the armamentarium for targeted therapy of MPN and other malignancies driven by JAK signaling. However, the curative potential of type I JAK inhibitors in MPNs seems limited, as reductions of JAK2 V617F allele burden are modest and effects on disease pathology and survival remain controversial with limited follow-up. Hematologic toxicities due to the essential role of JAK2 in hematopoiesis may contribute by limiting dose escalation and limiting the extent of target inhibition.

Furthermore, chronic exposure to JAK inhibitors leads to a loss of response *in vitro*, in animal models as well as in patients with myelofibrosis. Acquisition of secondary kinase mutations during tyrosine kinase inhibition is a well-known phenomenon, as illustrated by BCR-ABL tyrosine kinase inhibitor resistance mutations in CML. Saturation mutagenesis screens in JAK2 V617F- or R683G-mutated and TEL-JAK2--translocated cell lines identified a small set of secondary mutations in the JAK2 kinase domain, which inferred resistance to ruxolitinib and caused cross-resistance to other type I JAK2 inhibitors (58). So far, none of these mutations has been identified in JAK inhibitor-resistant patients, suggesting that mutation-independent mechanisms likely mediate the survival of MPN cells in the setting of chronic JAK kinase inhibition. We recently described functional adaptation and reactivation of JAK-STAT signaling as an escape mechanism from chronic type I JAK inhibition. This resulted from heterodimeric activation of JAK2 by other JAK family members such as JAK1 and TYK2 that reactivate downstream signaling in the absence of secondary mutations. The phenomenon is seen in cell lines, murine models, and in patient samples and is reversible,

as after drug withdrawal, sensitivity to type I JAK inhibitors is restored (59). A recent study suggested intrinsic resistance to JAK inhibitors in myelofibrosis as an additional mechanism. Primary cells from patients with myelofibrosis showed more modest responses, as assessed by the degree of inhibition of STAT phosphorylation to ruxolitinib *ex vivo*, compared with polycythemia vera or essential thrombocytopenia patient samples. (60).

Novel concepts to target JAK signaling

Although type I JAK inhibition has led therapy of MPNs to a new stage of molecularly targeted cancer treatment, its potential is limited due to insufficient efficacy. Several alternative options to target myeloproliferative neoplasms are being explored.

Type II JAK inhibition. Novel JAK inhibitors such as BBT594 bind JAK2 in its inactive state at a hydrophobic pocket adjacent to the ATP-binding site that is uncovered by a conformational change of the activation loop with dislocation of a DFG motif. In contrast with type I JAK inhibition, inactive JAK2 is stabilized and activation loop phosphorylation is decreased (50). BBT594 was initially designed as an inhibitor of T315I mutated bcr-abl, similar to nilotinib, which also acts via a type II mode of kinase inhibition. BBT594 can overcome persistence to ruxolitinib in JAK2 V617F- and MPL W515L-positive cells and represents an interesting candidate chemical scaffold for further evaluation (59).

HSP90 inhibitors such as PU-H71 and AUY922 attenuate JAK2 expression by interference with the chaperone function of HSP90 and subsequent degradation of JAK2. They efficiently impair JAK-mediated signaling (61) and overcome resistance due to secondary mutations (58) and through JAK heterodimer formation *in vitro* (59). A phase II study of AUY922 in myelofibrosis has recently been initiated (NCT01668173). HSP90 inhibition as a monotherapy or in combination with a JAK inhibitor will provide a favorable option for treatment of MPNs if not compromised by side effects due to inhibition of other HSP90 client proteins.

Histone deacetylase inhibition. Histone deacetylase (HDAC) inhibitors, including panobinostat, reduce JAK2 expression most probably due to effects on JAK2 mRNA expression, and through increased proteasomal JAK2 deg-

radation. Phase II studies of givinostat and panobinostat in myelofibrosis showed improvement of splenomegaly and constitutional symptoms (62) and are now followed by a phase I study of panobinostat combined with ruxolitinib (NCT01433445).

Combined pathway inhibition. Dysregulated JAK2 is at the apex of three classic oncogenic signaling cascades including STAT, PI3K/Akt/mTOR, and MAPK pathways. Their interrelationship and dependencies downstream of JAK2 in MPN cells is incompletely understood. *In vitro* studies suggest synergistic effects of PI3K/mTOR inhibitors such as BEZ235 (63) or of the MEK1/2 inhibitor selumetinib with JAK2 inhibitors (64). Synergism of JAK and PI3K inhibition was also seen in a murine model of myelofibrosis. A phase I/II trial of the mTOR inhibitor everolimus showed reduction of splenomegaly and constitutional symptoms, but was not as efficient as with JAK inhibition (65). Combination therapy might prove beneficial due to a synergistic impact on functionally redundant oncogenic axes, might allow for lower doses of the different agents with better tolerability, and might avoid or delay the development of resistance.

Perspectives

The ongoing molecular dissection of JAK signaling has enabled the development of specifically targeted therapies for patients with MPNs. The interest in applications of clinical JAK inhibition in other hematologic malignancies and solid tumors is increasing. The next challenges will be to define the signaling dynamics of JAK-driven malignancies, to test novel JAK inhibitor scaffolds, and to test targeted combination therapies to improve outcomes for patients with JAK-dependent malignancies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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