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JAK2 mutants (e.g., JAK2V617F) and their importance as drug targets in myeloproliferative neoplasms

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Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; Bad, Bcl2-associated agonist of cell death; Bax, Bcl2-associated X protein; Bcl-2, B-cell CLL/Lymphoma 2; Bcl-xL, B-cell lymphoma-extra large; Bcr, breakpoint cluster region protein; Bcr-Abl, Bcr/Abl fusion protein; Bim, Bcl-2 interacting mediator of cell death; c-Abl, Abelson tyrosine protein kinase 1; cA/cD, cyclin A/cyclin D; CBL, Casitas B-cell lymphoma; CD, cluster of differentiation; Cdc25A, cell division cycle 25 homolog A; Cdk, cyclin-dependent kinase; CML, chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; EGF, epidermal growth factor; eIF2α, eukaryotic translation initiation factor 2-alpha; Epo, erythropoietin; ET, essential thrombocythemia; FDA, US Food and Drug Administration; FERM, four point one protein, ezrin, radixin, moesin; FOXO, forkhead box protein O; GCN2, general control non-derepressible 2 = eIF2α kinase 4; G-CSF, granulocyte colony stimulating factor; GM-CSF, granulocyte macrophage colony stimulating factor; Grb2, growth factor receptor-bound protein 2; Gsk3, glycogen synthase kinase 3; HES, hypereosinophilic syndrome; HSP90, heat shock protein 90; IL, interleukin; IFN, interferon; KAK, Janus kinase; JMML, juvenile myelomonocytic leukemia; KD, kinase domain; LNK, lymphocyte linker protein; MCL1, myeloid cell leukemia sequence 1; MDS, myelodysplastic syndrome; mdm2, mouse double minute 2 homolog; MEK1, MAP kinase/Erk kinase 1; MPN, myeloproliferative neoplasm; mTOR, mammalian target of rapamycin; mTORC, mammalian target of rapamycin complex; PDGF, platelet-derived growth factor; PI3K, phosphoinositide 3 kinase; Pim, proviral integration site for Moloney murine leukemia virus; PTEN, phosphatase and tensin homolog; PV, polycythemia vera; PDK1, 3-phosphoinositide-dependant protein kinase-1; PCM1, pericentriolar material 1; PIAS, protein inhibitor of activated STAT; PKD, pseudokinase domain; PMF, primary myelofibrosis; PPT, protein phosphatase; RARS (-T), refractory anemia with ringed sideroblasts (-with thrombocytosis); SCID, severe combined immunodeficiency; SH2, scr homology 2; SNP, single nucleotide polymorphism; SOCS, suppressor of cytokine signaling; Sos, son of sevenless; STAT, signal transducers and activators of transcription; Tpo, thrombopoietin; TSC, tuberous sclerosis; TSLP, thymic stromal lymphopoietin; Tyk2, tyrosine kinase 2; VEGF, vascular endothelial growth factor; XIAP, X-linked inhibitor of apoptosis

The Janus kinase 2 (JAK2) mutant V617F and other JAK mutants are found in patients with myeloproliferative neoplasms and leukemias. Due to their involvement in neoplasia and inflammatory disorders, Janus kinases are promising targets for kinase inhibitor therapy. Several small-molecule compounds are evaluated in clinical trials for myelofibrosis, and ruxolitinib (INCB018424, Jakafi®) was the first Janus kinase inhibitor to receive clinical approval. In this review we provide an overview of JAK2V617F signaling and its inhibition by small-molecule kinase inhibitors. In addition, myeloproliferative neoplasms are discussed regarding the role of JAK2V617F and other mutant proteins of possible relevance. We further give an overview about treatment options with special emphasis on possible combination therapies.

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Introduction

Soon after their discovery[1] the Janus kinases were found to be involved in cytokine signaling.[2] The phenotypic analysis of knock-out mice for all four JAKs revealed that the lack of each JAK protein is linked to deficiencies in the signaling of specific cytokines using these JAKs in their receptor complexes[3-8] (reviewed in refs. 9 and 10). Janus kinase 2 is essential in the signaling of cytokines using homodimeric receptors (Epo, Tpo, prolactin, leptin, and growth hormone). It has been shown that JAK2 plays a crucial role in hematopoiesis as JAK2 knockout mice die at day 13 of gestation due to failure of the development of definite hematopoiesis.[4,5] JAK2 also plays a central role in the signaling of cytokines employing the common β chain receptor (IL3, IL5, and GM-CSF), of certain members of the IL10 type cytokine family (IFNγ, IL19, IL20, and IL24), of the IL12 type family members (IL12 and IL23) and in TSLP signaling.[11]

Many detailed studies have shown how the four members of the Janus kinase family mediate cytokine-induced signal transduction through cytokine receptors and regulate proliferation, differentiation, survival, and cell migration and thereby play a

major role in hematopoiesis and the immune system. Due to this immunomodulatory role it is evident that Janus kinases are major regulators of inflammatory disorders (e.g., rheumatoid arthritis and psoriasis[12]) and cytokine-dependent cancers (e.g., multiple myeloma[13]) and, thus, have long been identified as druggable targets. Mutations in JAKs have first been described for JAK3 and have been found to elicit severe combined immunodeficiency (SCID).[14] Fusion of JAK2 with certain proteins (e.g., Tel, Bcr, or PCM1) resulting in constitutively active signaling molecules has been described in a variety of hematopoietic malignancies as CML, AML, or ALL.[15-18]

Additionally, a point mutation in JAK2—JAK2V617F—was discovered in the majority of Philadelphia chromosome-negative myeloproliferative neoplasm (MPN) patients in 2005.[19-23] JAK2V617F is found with high incidence in patients with polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In different murine models, it has been shown that the expression of JAK2V617F is sufficient to induce a MPN-like phenotype.[24-29] JAK2V617F is also, albeit rarely, found in other hematologic malignancies like the hypereosinophilic syndrome (HES), chronic or juvenile myelomonocytic leukemia (CMML or JMML), acute myeloid leukemia (AML), and refractory anemia with ringed sideroblasts (with thrombocytosis) (RARS or RARS-T) (reviewed in ref. 11). The JAK2V617F mutation is an acquired somatic event of the hematopoietic compartment, where it has been identified in hematopoietic stem cells ($CD34^+CD38^-CD90^+lin^-$) and multi-potent progenitor cells[22,30] as well as in differentiated cells like granulocytes.[20] It was also found in cells from the lymphoid lineage (e.g., natural killer cells) in a considerable amount of MPN patients[31,32] suggesting that JAK2V617F occurs in multi-potent hematopoietic progenitor cells, although the phenotype of MPN is related to a selective proliferative advantage of the myeloid lineages. In the last years, many more genetic alterations affecting all members of the Janus kinase family have been discovered in leukemias and other hematopoietic neoplasia.[11]

JAK-STAT Signaling and the JAK2V617F Mutant

Structural organization of JAKs. The size of Janus kinases ranges from 120 to 140 kDa. All JAK family members share a similar sequence consisting of seven JAK homology (JH) domains,[33] which only partially match the JAK domain structure. The JH1 and JH2 domains represent the adjacent kinase and pseudokinase domain, a feature only found in five kinases (in the four JAKs and in GCN2). The domains JH3 to JH7 correspond to the SH2 and FERM domains[33,34] and are involved in cytokine receptor binding. Structural aspects of receptor binding have been reviewed recently[11,35,36] and will not be covered here. Since the discovery of JAK2V617F, a great number of mutations (~70) have been described throughout all the structural domains of the JAKs and many (~30) have been biochemically validated to lead to constitutively active proteins.[37] Mutations in the kinase domain can have direct consequences on kinase domain conformation and activation, but the molecular consequences of mutations in other domains of the JAKs are not as easily understood. The pseudokinase domain mutations (e.g., V617F) are thought to relieve the negative regulatory interaction between the pseudokinase domain and the kinase domain[36,38] and result in constitutive activation of the kinase. Recently, the pseudokinase domain has been described to have residual kinase activity and to phosphorylate inhibitory amino acid residues within JAK2 (serine 523 and tyrosine 570).[39] This might imply that mutations in the pseudokinase domain could alternatively represent loss-of-function mutations regarding the pseudokinase domain's remaining kinase activity. Still, the pseudokinase domain mutations are not fully understood, while the consequences of the mutations within the FERM and SH2 domains are not understood at all. This is due to the lack of detailed structural information concerning the full-length JAK proteins. Structural models of JAK2[40,41] have been used to explain the molecular details of processes involved in JAK2V617F activation.[42-44] However, 3D reconstructions of isolated JAK1 from an electron microscopy imaging approach[45] have shown that the pseudokinase and kinase domain form a closely associated cluster, the conformation of which does not correspond to the molecular model described above. The isolated JAK1 showed great flexibility and could adopt different conformations from an "open" conformation (relatively linear with contacts between the adjacent domains in the polypeptide chain) to a "closed" conformation (in addition to contacts between adjacent domains, the FERM, SH2 domains are in contact with the kinase and pseudokinase domains). Although mutational studies have already suggested these contacts between the FERM and kinase domains,[46-48] there is no certainty that the conformation of the JAKs bound to a cytokine receptor is entirely comparable to these conformational states. Unfortunately, the conformation of JAK1 bound to gp130 could not be resolved in this study. This might show that even when bound to a cytokine receptor the JAKs have great conformational flexibility.

JAK activation at the receptor. Janus kinases are tightly associated to the intracellular parts of cytokine receptors mediated by their FERM and SH2 domains and are maintained in an inactive state, when no cytokine is bound to the receptor.[35] Binding of a cytokine to a cytokine receptor leads to conformational changes in the receptor which are transmitted to the cytoplasmically associated JAKs, leading to their activation and phosphorylation (reviewed in refs. 11 and 35). Recently, a study using kinase-inactive and constitutively active mutants of JAK1 and JAK3 in the context of IL-2 receptor signaling suggested that the conformational and phosphorylation events of JAK activation are independent of one another (**Fig. 1**), and that both events are necessary to induce full activation of the JAKs.[37] However, the exact molecular details of JAK activation upon binding of a cytokine to the receptor remains elusive, because of lacking structural information of the full-length protein bound to a receptor. The transformation potential of JAK2V617F is also dependent on binding to a cytokine receptor (EpoR, thrombopoietin receptor [TpoR], or G-CSF receptor)[49] and it has been demonstrated that a functional FERM domain as well as an intact SH2 domain are required for the JAK2V617F-mediated transformation.[50,51]

JAK2V617F-mediated activation of diverse signaling pathways. The activated JAKs phosphorylate tyrosine residues in

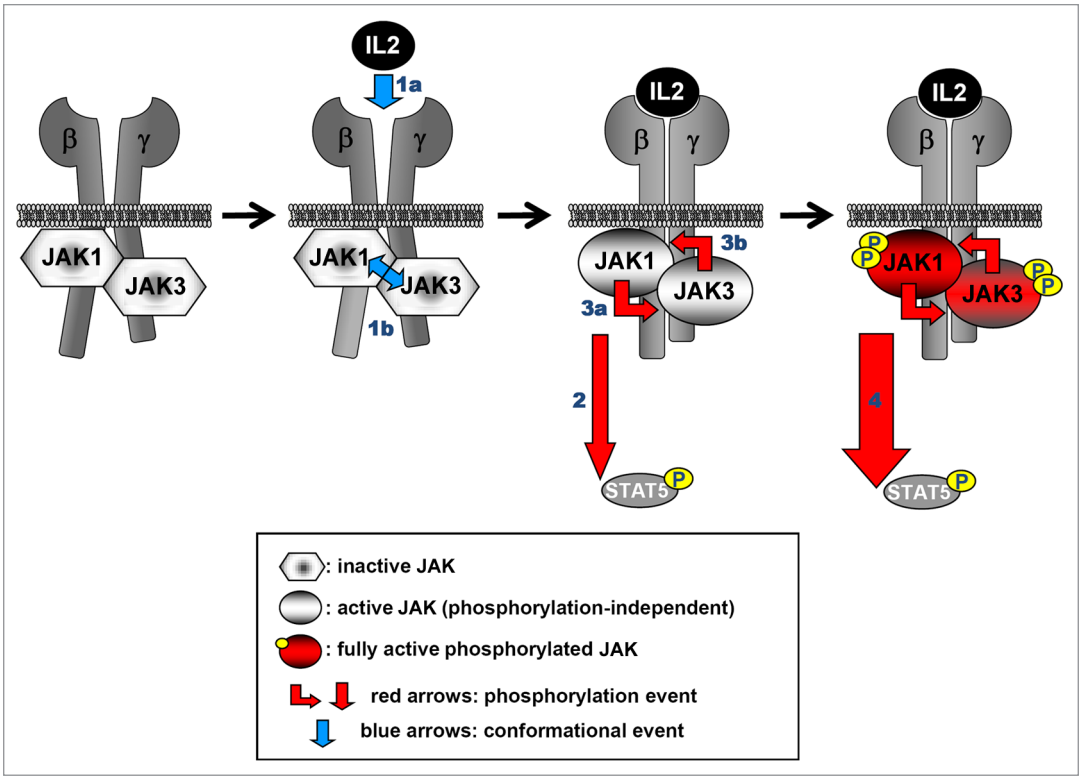

Figure 1. Conformational and phosphorylation events leading to JAK activation. IL2 signaling is used as an example. For clarity only the signal transducing receptor chains (IL2Rβ [β] and IL2Rγ [γ]) are shown. The scheme on the left shows the inactive state of a receptor/JAK complex. The binding of the cytokine (1a) impinges conformational changes in the receptor complex. JAKs are sensitive to these changes since they bind to the membrane-proximal region of cytokine receptors. This results in a conformational, phosphorylation-independent activation of the two JAKs (1b). Activation of downstream signaling is already promoted at this stage (2), although at a non-maximal level. The now activated JAKs phosphorylate each other "in *trans*" (3a and 3b). This leads to a full-fledged activation of the JAKs and maximal downstream signaling (4) (here: STAT5 phosphorylation). The different steps of the activation process are derived from a study using kinase-inactive, constitutively active and analog-sensitive mutants of JAK1 and JAK3 in the context of IL2 signaling.[37]

the cytoplasmic part of the receptor, thereby providing docking sites for SH2 domain-containing signaling molecules (**Fig. 2**). JAK2V617F leads to constitutive activation of downstream signaling through the JAK-STAT (STAT5 and STAT3), the MAPK, and the PI3K/Akt pathways,[23,49,52,53] which lead to the expression of the mitotic serine/threonine-protein kinases Pim, anti-apoptotic genes (BclxL and Bcl2), and cell cycle regulatory proteins (cyclin D1 and Cdc25A).[54-58] This results in a proliferative advantage of the affected cells.[23] It has recently been shown that STAT5 is absolutely essential for the cellular transformation mediated by JAK2V617F,[59-61] whereas activation of Akt might also play a role in the process of transformation.[62] JAK2V617F has been implicated in promoting transition from G1 to S phase of the cell cycle which could be reverted by the inhibition of JAK2V617F with a small molecule JAK inhibitor.[63] The inhibition of JAK2V617F correlated with a decreased expression of cyclin D2 and an increased expression of the cyclin-dependent kinase (Cdk) inhibitor 1B ($p27^{Kip1}$) (**Fig. 2**). p27 expression could also be blocked by Akt- or Erk1/2-mediated phosphorylation and subsequent degradation of FOXO transcription factors.[64,65] JAK2 has also been reported to phosphorylate $p27^{Kip1}$, thereby impairing its function and stability, which then leads to partial activation of Cdk and cell cycle progression.[66] Pim kinases, which are upregulated by JAK2V617F-mediated signaling,[50,57] have been described to inactivate Bad by phosphorylation, thereby activating the anti-apoptotic BclxL.[57] Akt can also display its anti-apoptotic role via phosphorylation of BH3-only proteins resulting in a recruitment of Bcl2 and BclxL to the mitochondrial membrane.[64] Furthermore Akt can inactivate Gsk3 by phosphorylation, thus impairing normal downstream Gsk3 functions such as inhibition of the cell cycle (e.g., by phosphorylation of cyclin/cyclin-dependent kinase complexes or by inhibition of mitotic transcription factors) or promotion of apoptosis (e.g., by increasing BH3-only protein [Bax/Bak]-mediated apoptosis or by inhibition of Bcl2 family member expression).[64,67,68] Inhibition of FOXO by Akt is also known to lead to a downregulation of pro-apoptotic BH3-only proteins. Interestingly, the activation of Gsk3 by DNA damage stress was shown to synergize with JAK inhibitors in inducing apoptosis in cells expressing JAK2V617F.[69]

Additionally, it has also been described that JAK2V617F phosphorylates a histone arginine methyltransferase (PRMT5) and thus inhibits its activity resulting in altered chromatin modifications and gene expression.[70] This contributes then to myeloproliferation and erythroid differentiation in JAK2V617F-positive cells. JAK2 has been described to phosphorylate histone H3 at tyrosine 41 resulting in the displacement of heterochromatin

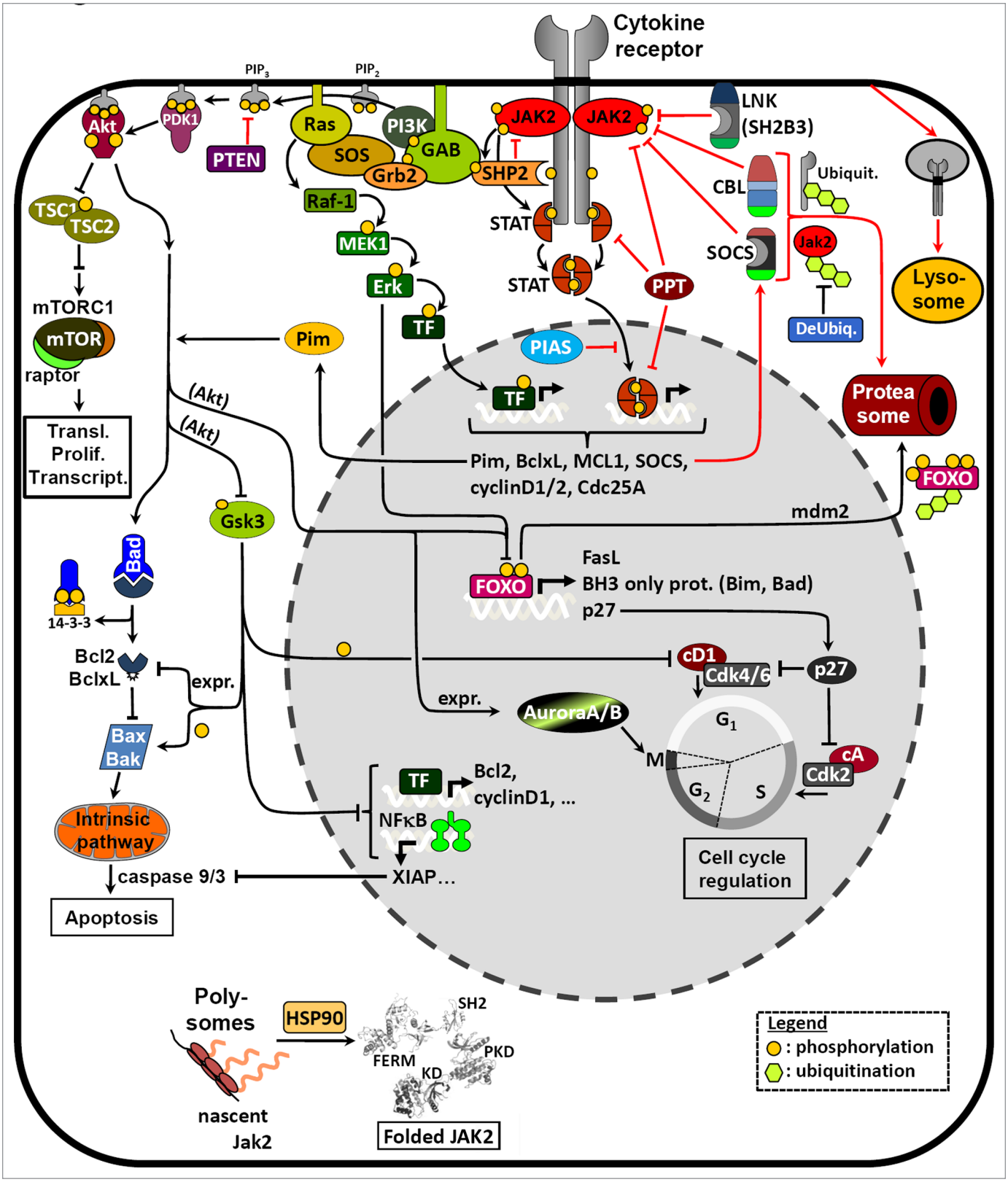

Figure 2. For figure legend, see page 5.

protein (HP) 1α[71] leading to expression of leukemogenic oncogenes like LMO2. However, the direct implication of JAK2V617F in this process remains controversial,[72] and it cannot be excluded that a kinase downstream of JAK2V617F may be involved in

Figure 2 (See previous page). Schematic representation of pathways related to signaling of JAK2V617F and regulation of its expression levels. A number of possible pharmacological approaches have been described that target proteins in the scheme (e.g., mTOR, MEK, HSP90, Aurora A/B, …). Other kinases involved in these pathways (e.g., PI3K, Akt, Pim, Erk1/2, …) might also be promising targets for combination treatments. In addition to Aurora kinases, further kinases influencing cell cycle progression also represent interesting targets (cyclin-dependent kinases [Cdk] and polo-like kinases [Plk]). Inhibition of the Bcl-2 family members might counteract anti-apoptosis. Interference with JAK expression levels has been shown to suppress JAK-STAT signaling either by inhibiting chaperone functions (HSP90) or by using deubiquitinase inhibitors.[216] Future approaches could also involve the targeting of adaptor proteins such as GAB1/2 which orchestrate the activation of the different signaling pathways in the signalosome at the receptor.

promoting this nuclear function. An active JAK homolog, HOP, in *Drosophila* has also been implicated in changes of chromatin condensation and STAT-independent gene transcription.[73]

Negative Regulatory Mechanisms of JAK Activity

To prevent a permanent and/or excessive activation of JAK-STAT signaling a number of negative regulatory mechanisms that modulate the pathway at different levels have been reported.

Phosphatases and PIAS proteins. Negative regulatory mechanisms include the dephosphorylation of cytokine receptors, JAKs or STATs by protein tyrosine phosphatases (PTP)[74] or the prevention of STAT factors to bind DNA by protein inhibitors of activated STAT (PIAS).[75] No specific regulations of JAK-STAT phosphatases or PIAS family members have been reported for JAK2V617F to our knowledge.

SH2B protein family members. LNK (SH2B3), an adaptor protein comprising a dimerization domain, proline-rich regions, a PH domain, and an SH2 domain, negatively regulates activated JAK2 by directly binding to the phosphorylated tyrosine residue 813 via its SH2 domain.[76,77] LNK has been reported to negatively regulate TpoR and EpoR signaling.[78,79] LNK mutations have been detected in JAK2V617F-positive and -negative myeloproliferative neoplasms[80-83] and LNK mRNA in MPN patients was reported to positively correlate with JAK2V617F allele burden.[84] Interestingly, other family members, SH2B1 (SH2Bβ) and SH2B2 (APS), have been described to associate with Janus kinases and to positively[85-87] or negatively[88-90] regulate their kinase activity. Concerning EpoR signaling, however, all three family members have been reported to act as negative regulators (SH2B1[90]). Moreover, SH2B2 was reported to cooperate with CBL (see below) in doing so.[88]

Regulation of JAK and receptor protein expression (internalization, SOCS, and CBL). On the cellular[52] and the organism level as well as in patients (see sections below) it is well established that the levels of mutant JAK2V617F protein influence the signaling intensity and its pathological consequences. This underscores the importance of understanding the regulation of the cytokine receptor/JAK complexes at the protein level.

Cytokine signaling can be regulated on the level of plasma membrane localization of receptor/JAK complexes. Cytokine receptor/complexes can be internalized and processed either for recycling back to the plasma membrane or be targeted for degradation of their components via the lysosome or proteasome[91-93] (reviewed in ref. 94). JAK2V617F has been described to lead to the internalization, ubiquitination, and degradation of TpoR.[95]

Downregulation by ubiquitination in the JAK-STAT pathway has been described to be mediated by two families of proteins, SOCS proteins and CBL proteins. Both types of proteins possess E3 ubiquitin ligase activity. Among the two types of ubiquitin ligases, SOCS and CBL proteins are both part of the RING finger E3 family, but they belong to different subgroups. While CBL proteins are single subunit E3s (having the RING finger and the substrate recruiting subunit on the same polypeptide chain), the SOCS proteins are part of the multi-subunit E3s (including a small RING finger protein, a member of the Cullin family, and multiple other subunits among which there is the substrate recruiting domain).[96]

The suppressor of cytokine signaling (SOCS) protein[97] family (all having a central SH2 domain and a C-terminal SOCS box) comprises eight family members (SOCS1–7 and CIS) that can suppress JAK-STAT signaling by inhibiting JAK kinase activity, by competing with STAT factors for docking sites on the cytokine receptor and/or by facilitating the proteasomal degradation of signaling proteins. Constitutively active JAK2 mutants are susceptible to negative regulation by SOCS proteins, show decreased stability, increased ubiquitination, and are degraded via the proteasome.[52] Thus, mechanisms interfering with this negative regulation could considerably contribute to the development and progression of MPNs by increasing the levels of constitutively active JAK2 mutants, although this is still under debate.[98] Mechanisms that were reported to interfere with SOCS function are methylation,[99-101] mutations,[102] and deletions[103] of SOCS genes. Importantly, epigenetic silencing of SOCS3 and SOCS1 was recently reported in about 40% of patients with Philadelphia chromosome-negative chronic myeloid disorders.[104,105] The Casitas B-cell lymphoma (CBL) family consists of 3 mammalian members, CBL, CBL-b, and CBL-c. All CBL proteins have a conserved N-terminal tyrosine kinase binding domain (TKB) (itself comprised of a 4-helix bundle [4H], an EF-hand [EF] and an atypical SH2 domain) connected by an α-helical linker to a RING finger (RF) domain. C-terminally to the RF, CBL proteins contain proline-rich sequences, tyrosine residues and an ubiquitin-associated domain (UBA). CBL proteins can function as ubiquitin ligases but are also adaptor proteins which can mediate signal transduction events by offering binding sites for SH3 and SH2 domain-containing proteins.[106] CBL proteins are known to mediate ubiquitination and degradation of kinases and were described to interact with many receptor tyrosine kinases, cytokine receptors, and cytoplasmic kinases (including the JAKs) and oncogenic mutants of CBL have been reported to uncouple kinases from degradation.[107-109] CBL mutations are also found in myeloproliferative neoplasms[110-113] and have been associated with a poor prognosis.

Myeloproliferative Neoplasms and JAK2 Mutations

Myeloproliferative neoplasms. Myeloproliferative neoplasms are characterized by a dysregulated enhanced proliferation of one or

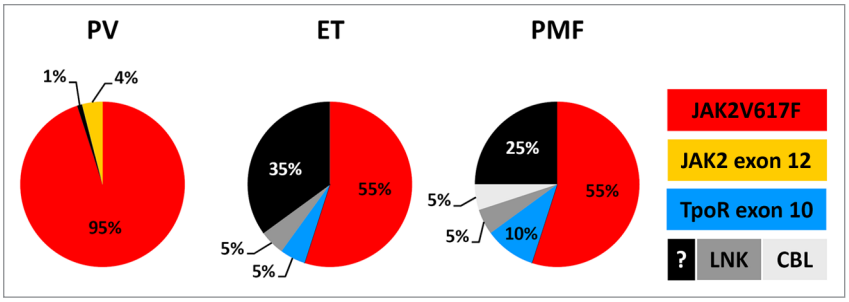

Figure 3. Proportion of patients with PV, ET, or PMF carrying different genetic abnormalities related to JAK-STAT signaling.

more of the myeloid lineages (i.e., the erythroid, granulocytic, megakaryocytic, and monocytic lineages), which is considered to result from genetic abnormalities at the level of hematopoietic stem/progenitor cells. Myeloproliferative neoplasms comprise chronic myeloid leukemia (Bcr-Abl-positive) (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL-NOS), mast cell disease, and unclassified myeloproliferative neoplasms (MPN-U). CML, PV, ET, and PMF were known since long to be clonal stem cell disorders.[114-117] Patients suffering from MPN usually show an increased amount of functional and terminally differentiated myeloid cells (i.e., erythrocytes, granulocytes, monocytes, and/or platelets) in their peripheral blood. However, the diseases can progress to ineffective hematopoiesis and failure of the bone marrow due to myelofibrosis and/or transformation to acute leukemia.

In addition to CML (for which the fusion protein kinase Bcr-Abl was already identified as the disease-causing mutation[118]), three other MPNs (PV, ET, and PMF) were shown to harbor a mutated kinase—JAK2V617F,[19,20,22,23,119] which can result from a heterozygous or homozygous mutation. Cells homozygous for JAK2V617F can be found in most of the PV patients but only rarely in ET patients.[120] The homozygous mutation was demonstrated to result from a duplication of the mutant allele by mitotic recombination.[20-23]

Polycythemia vera. Polycythemia vera (PV) is the only acquired primary polycythemia. It has an incidence of 1–3 per 100 000 people per year and is most frequently diagnosed in people aged between 60 and 70 y. The vast majority of PV patients is positive for the JAK2V617F mutation and most of them bear cells which are homozygous for the mutation.[120] PV patients, who do not carry the JAK2V617F mutant, mostly display other activating mutations in exon 12 of JAK2 (see **Fig. 3**).[121]

Polycythemia vera is characterized by the dysregulated proliferation of the erythroid, granulocytic, and/or megakaryocytic lineages. This leads to the hypercellularity of the bone marrow (i.e., panmyelosis) and an increase of the red cell mass in the peripheral blood as well as leukocytosis and thrombocytosis. However, patients with mutations in JAK2 exon 12 mainly demonstrate an isolated erythrocytosis without associated increase of platelet number or white blood count.[122,123] In contrast to PMF and ET, the megakaryocytes in PV show mainly a normal phenotype and size.

The course of PV can be divided into three phases:[124] (1) the pre-polycythemic phase characterized by a borderline or mild erythrocytosis often in combination with significant thrombocytosis (sometimes associated with thrombotic events), (2) the apparent polycythemic phase, and (3) the post-polycythemic phase defined by cytopenia (including anemia), bone marrow fibrosis, and extramedullary hematopoiesis (post-polycythemia myelofibrosis). Almost all patients are diagnosed when they are in the polycythemic phase and the first symptoms appear. These include e.g., headache, dizziness, paresthesia, aquagenic pruritus, and erythromelalgia mainly due to thrombotic events in the microvasculature. However, a thrombosis of major blood vessels (e.g., splanchnic vein thrombosis) can occur as well. Additionally, many patients suffer from splenomegaly and/or hepatomegaly. Upon appropriate treatment the survival time of PV is very much prolonged, but life expectancy of PV patients is nevertheless reduced when compared with that of the general population.[125]

The probability of PV patients to develop a post-polycythemic myelofibrosis is ~15% at 10 y and ~35% at 15 y after the initial diagnosis.[126] A major risk factor to progress to myelofibrosis seems to be the JAK2V617F allele load since the incidence is much higher in patients with a high JAK2V617F allele burden compared with those with a low allele load.[126,127] On the other hand, the incidence of progression to myelodysplastic syndromes (MDS) or acute myeloid leukemia (AML) is very low, but is increased with higher age at diagnosis or due to treatment with certain cytotoxic agents (e.g., busulfan or pipobroman[128]).

Essential thrombocythemia. ET has an annual incidence of 0.5–2.5 per 100 000 people.[129] It can occur at any age (including children), but the disease is mostly diagnosed in patients who are in their sixties or around 30 y old.[130] Approximately half of the ET patients carry the JAK2V617F mutation; these patients mainly bear cells that are heterozygous for the mutation.[120] About 5% of the ET patients are positive for a mutation in exon 10 of the Tpo receptor and additional 5% bear a mutation in the adaptor protein LNK. The remaining ET patients (~1/3) do not display any known mutation affecting the JAK-STAT signaling pathway (see **Fig. 3**).

Essential thrombocythemia is mainly characterized by an enhanced proliferation of the megakaryocytic lineage leading to sustained thrombocytosis (with a platelet count ranging from more than 450 to more than 2000 × 10^9/l). The platelets are not equal in size ranging from small to giant and display abnormal functions (e.g., spontaneous aggregation and activation) resulting in an increased risk of thrombosis and/or bleeding.[131] The bone marrow of ET patients is typically normal or slightly hypercellular apart from the megakaryocytic lineage. The number of megakaryocytes is elevated and megakaryocytes in ET patients have extremely lobulated nuclei and their size is increased varying from large to giant.

In general, ET is a rather indolent disorder with long symptom-free periods and only occasional events of thrombosis or

bleeding. Up to 50% of the patients are asymptomatic at diagnosis; the disease is then mostly detected by a routine examination. The other patients demonstrate symptoms related to thrombotic events in the microvasculature. However, the thrombosis of major blood vessels (leading to, e.g., seizures, stroke, myocardial infarct, and deep-vein thrombosis) can occur as well. The life expectancy of the majority of ET patients is near normal[132] and only a minority of patients either progress to post-ET myelofibrosis or to AML.[133]

Primary myelofibrosis. Myelofibrosis is defined as an increase in quantity and density of extracellular matrix proteins, which normally provide a scaffold for the hematopoietic (stem and progenitor) cells in the bone marrow. Myelofibrosis can occur secondary to, e.g., infections and inflammatory or neoplastic disorders.

Primary myelofibrosis (PMF) occurs with an incidence of 0.5–1.5 per 100 000 people per year. The median age at diagnosis is usually > 70 y.[134] Importantly, the clinical characteristics of post-polycythemic or post-ET myelofibrosis are the same as for PMF in the fibrotic phase and can only be distinguished when the initial disease was well diagnosed. Approximately half of the patients with PMF carry the JAK2V617F mutant, whereas approximately 10% are positive for a mutation in exon 10 of the Tpo receptor. Additionally, mutations in the adaptor proteins LNK or CBL can be found in PMF patients as well (each ~5%). The remaining PMF patients (~25%) do not display any known mutation affecting the JAK-STAT signaling pathway (see **Fig. 3**).

Primary myelofibrosis is characterized by enhanced proliferation mainly of the megakaryocytic lineage and the alteration of the bone marrow structure including progressive myelofibrosis and hyperactive angiogenesis, which is often accompanied by extramedullary hematopoiesis. The disease course can be divided in two phases:[124] The prefibrotic or early phase is characterized by a hypercellular bone marrow (due to an increase of the megakaryocytic and the granulocytic lineages; erythropoiesis is often decreased) with no or slight reticulin fibrosis and an increased platelet count in the peripheral blood. The fibrotic phase displays a hypocellular bone marrow with marked reticulin and/or collagen fibrosis and also osteosclerosis. Megakaryocytes and platelets for instance produce PDGF, TGFβ, or OSM,[135,136] which stimulate fibroblast proliferation and activity. The peripheral blood of PMF patients in the fibrotic phase demonstrates decreased erythrocyte levels up to anemia, low levels of large abnormal platelets, and also leukopenia. Moreover, the plasma levels of inflammatory cytokines (e.g., IL1β, IL6, IL8, IFNγ, and TNFα) are highly increased.[137,138] In the advanced stages, bone marrow failure results in relocation of the hematopoiesis to other organs. Most common sites of extramedullary hematopoiesis are the spleen and the liver, but any other organ (e.g., kidney, lung, or the gastrointestinal tract) can be affected. Bone marrow failure also leads to high levels of $CD34^+$ cells in the peripheral blood, which normally reside in the bone marrow.

The median overall survival of PMF patients who have been diagnosed in the fibrotic phase is approximately five years. However, the survival times can be much longer if the disease has been diagnosed in the prefibrotic stage.[132,139] The main causes of death for PMF patients include the progression to acute leukemia (observed in 20% of the patients at 10 y of diagnosis), infection, and bleeding secondary to bone marrow failure, and portal hypertension or hepatic failure caused by hepatic vein thrombosis or extramedullary hematopoiesis.[125]

JAK2 mutations and other mechanisms contributing to PV, ET, and PMF. The discovery of an activating mutation downstream of cytokine receptors playing an essential role in myeloid hematopoiesis was a major breakthrough in understanding the development of the Philadelphia chromosome-negative MPNs. However, this raised the question of how a single mutation can lead to the development of three distinct diseases (PV, ET, and PMF). Subsequently, it was demonstrated in a murine bone marrow transplantation model introducing JAK2V617F-positive cells that the MPN phenotype was influenced by the genetic background of the respective mouse strain.[140] Furthermore, the amount of JAK2V617F seems to play a role in the pathogenesis as well, given that cells that are homozygous for JAK2V617F are more often found in PV patients than in ET patients.[120] This could be recapitulated in a JAK2V617F transgenic mouse model, which allowed the expression of varying ratios of JAK2 wild-type to JAK2V617F.[25] Low expression of JAK2V617F resulted in a MPN phenotype resembling human ET, while higher expression of JAK2V617F led to a PV-like phenotype.

Since 2005, many more mutations affecting proteins important in JAK-STAT signaling have been identified in JAK2V617F-negative MPN patients (for a review see ref. 11). Scott and colleagues discovered several additional mutations in exon 12 of JAK2 including K539L by sequencing JAK2 in JAK2V617F-negative PV patients. Several more point mutations, deletions, and insertions affecting JAK2 exon 12 have been identified in PV patients since then.[11,122] Somatic gain of function mutations often affecting the amino acid residues W515 and S505 have been found in the Tpo receptor gene (MPL) of patients with ET and PMF.[141-145] Both JAK2 exon 12 (K539L) and Tpo receptor (W515K/L) mutants have been shown to lead to the transformation of BA/F3 cells and induce a MPN-like phenotype in murine bone marrow transplantation models.[122,142] Furthermore, mutations in adaptor proteins involved in the negative regulation of cytokine signaling, i.e., LNK and CBL, have been described in ET and PMF patients.[80,112]

A great variety of new data could contribute to understand the development of the three diseases with differing phenotypes. The high proportion of patients with ET and PMF not displaying any known mutation affecting JAK-STAT signaling shows that for these two diseases at least other players can be sufficient to induce the disease state. Indeed, further recurrent somatic mutants of different proteins (e.g., ASXL1, EZH2, IKZF1, IDH1/2, RUNX1, and TET2) have been found with variable frequency in PV, ET, and PMF.[111,146] Some of the affected proteins are implicated in the epigenetic regulation (e.g., ASXL1 and TET2), whereas IKZF1 and RUNX1 are transcription factors. The different mutations are not specific for any of the MPN subtypes and can occur concomitantly with JAK2V617F or the other

mutations. However, these mutations and/or their accumulation might partially explain the clinical differences among PV, ET, and PMF.[147] Furthermore, some of the mutations are associated to disease progression and are more frequently found in post-MPN acute leukemia (e.g., mutants of IDH1/2, IKFZ1, and also LNK[146]).

Additional mechanisms like epigenetic silencing, post-transcriptional regulation or post-translational modifications could account for the development of different phenotypes. For instance, it has been reported that the SOCS1, SOCS2, and SOCS3 genes are hypermethylated in MPN.[104,105,148-150] Furthermore, the comparison of microRNA expression in MPN patients and healthy controls identified among others miRNA-150 to be differentially expressed.[151-153] Interestingly, miRNA-150 has been reported to regulate the lineage fate in megakaryocyte-erythrocyte progenitor cells.[154] Furthermore, Pardanani and colleagues found several germline single nucleotide polymorphisms (SNPs) in the region of the JAK2 gene that are different in PV and ET patients and could contribute to the differences in MPN phenotype.[155] Subsequently, several groups reported that a common haplotype (referred to as "46/1") in the JAK2 locus is associated with the acquisition of JAK2V617F as well as the development of MPN.[156-158] They demonstrated that patients who were heterozygous for this haplotype were significantly more likely to acquire JAK2V617F. The same haplotype also predisposes to mutations in JAK2 exon 12 as well as in the Tpo receptor.[159,160] However, the mechanism by which a germline SNP in the 46/1 haplotype increases the risk to develop MPN or acquire JAK2V617F or other mutations is not known. In general, the 46/1 haplotype seems to be a major germline factor involved in MPN development and to date no other common SNP associated with MPN has been reported.[147] The newly discovered genetic abnormalities also played a central role in the revision of the WHO classification for MPN in 2008[161] as they can be used as diagnostic parameters. The new classification includes CML, the "classic" Philadelphia chromosome-negative MPN (PV, ET, and PMF) and several other rare diseases that demonstrate many features of MPN.

Inflammation and an aberrant activation of the JAK-STAT signaling pathway are also hallmarks of MPN[162-165] irrespective of mutations influencing the JAK-STAT pathway. The JAK-STAT pathway not only drives myeloproliferation but also mediates the activity of inflammatory cytokines, whose levels are commonly increased in myelofibrosis patients.[137,138] Since an initiating event in MPN is not known, inflammation has also been discussed to be an incipient event. It has been reviewed recently[166] that inflammation can induce epigenetic changes and genomic mutations. High levels of inflammatory cytokines and chemokines are found in the plasma of MPN patients and in supernatants of cells expressing JAK2V617F[136-138,167-170] and a number of cytokines, e.g., IL6, IL11, TNFα, and HGF have been reported to promote survival of cells carrying JAK2V617F.[171-173] Cytokines are involved in the development of fibrosis, e.g., megakaryocytes and platelets produce PDGF, TGFβ, or OSM,[135,136] which stimulate fibroblast proliferation and activity. On the other hand, the stroma also secretes cytokines, which regulate the behavior of JAK2V617F mutated cells.[171-173]

Janus Kinase Inhibitors in the Treatment of MPN

Classic treatment of MPNs. For PV and ET the treatment rationale is mainly the prevention of thrombotic complications which is the major reason for morbidity and mortality in these patients.[174] Low-risk patients with PV are normally treated with phlebotomy and low-dose aspirin. High-risk PV patients additionally receive hydroxyurea or pegylated IFN-α as first-line treatment. ET patients at low thrombotic risk are either monitored without therapeutic intervention or they receive low-dose aspirin as well. High-risk patients with ET are usually treated with hydroxyurea, pegylated IFN-α, or anagrelide.

There are several treatment approaches for patients with myelofibrosis that are primarily aimed at relieving the diverse disease symptoms and improve the patient's quality of life. The only curative treatment of myelofibrosis is allogeneic hematopoietic stem cell transplantation (HSCT). However, the mortality and morbidity of this procedure is still very high and it is questionable if it leads to substantial increase in overall survival for eligible patients.[174] The main issues that are targeted by conventional treatment strategies are anemia and splenomegaly/extramedullary hematopoiesis. Blood transfusion or treatment with corticosteroids, androgens or erythropoiesis-stimulating agents is used to treat the anemia. Anemia as well as splenomegaly can be treated with immunomodulatory agents like thalidomide or lenalidomide. Furthermore, cytoreductive drugs as hydroxyurea or pegylated IFNα or chemotherapeutic agents (e.g., cladribine or decitabine) are used to reduce the spleen size. Alternative treatment options for splenomegaly/extramedullary hematopoiesis are radiation therapy or splenectomy, either of which is rare and only performed if no other treatment option is feasible. However, there is no evidence that any conventional treatment approach improves the constitutional symptoms.[175] In addition, none of the conventional treatment strategies except allogeneic stem cell transplantation shows durable effects/benefits and they also demonstrate significant toxicities.[176-179]

Treatment of MPN with JAK inhibitors. The discovery of the JAK2V617F mutant defined JAK2 as "druggable" target for Philadelphia chromosome-negative MPNs. Although JAK2V617F is not found in all patients with ET and PMF, an aberrant activation of the JAK-STAT signaling pathway plays a central role in the pathogenesis of most PV, ET, and PMF patients.[162] The JAK-STAT pathway not only drives myeloproliferation but also mediates the activity of inflammatory cytokines, whose levels are commonly increased in myelofibrosis patients.[137,138] Since 2005, many inhibitors of JAK(2) have been developed; several of those are currently evaluated in clinical trials (see **Table 1**).

INCB018424 (ruxolitinib, Jakafi®). To date, only ruxolitinib (INCB018424) received approval by the FDA (in November 2011) and the European Commission (in August 2012) for the treatment of intermediate- and high-risk myelofibrosis (primary and post-PV/ET). Ruxolitinib is a JAK1 and JAK2 inhibitor.[180] The basis of its approval were two phase III clinical studies for myelofibrosis (COMFORT I and II) which provided evidence that application of ruxolitinib leads to the reduction of spleen size

Table 1. JAK inhibitors in clinical trials for myelofibrosis

Compound	Targets	Clinical trial	Responses observed so far
AZD1418	JAK1/2[217]	I/II	No information available
BMS911543	JAK2[218]	I/II	No information available
CEP701	JAK2/3[219]	II	Splenomegaly ↓[220]
CYT387	JAK1/2, Tyk2[168]	II	Splenomegaly ↓ Improvement of constitutional symptoms Improvement of anemia[221]
LY2784544	JAK2[222]	II	Splenomegaly ↓ Improvement of constitutional symptoms (improvement of bone marrow fibrosis)[223]
NS-018	JAK2[224]	I/II	No information available
SB1518 (Pacritinib)	JAK2, Tyk2[225]	I/II	Splenomegaly ↓ Improvement of constitutional symptoms[226]
TG101348 (SAR302503)	JAK2[227]	III	Splenomegaly ↓ Improvement of constitutional symptoms Normalization of leukocytosis and thrombocytosis (JAK2V617f allele burden ↓)[189]
XL019	JAK2[228]	I/terminated	Neuronal toxicity[229]

Status: October 2012, adapted from references 177, 190, 191, and 193.

and an improvement of symptoms.[181,182] In addition, ruxolitinib decreases leukocytosis and thrombocytosis as well as inflammatory cytokine levels and thereby enhances the patients' quality of life. Recently, long-term results from the before mentioned studies have shown that ruxolitinib-treated patients have a survival advantage over the control groups (placebo in COMFORT I, best available therapy [BAT] in COMFORT II) and that the JAK2V617F allele burden was reduced (> 20% in 13% of the patients).[181,183-186] Interestingly, also the requirement of blood transfusions (due to the side effects of anemia and thrombocytopenia) observed in the early phases for patients receiving ruxolitinib decreased to rates similar to the control groups.

It will be interesting to determine to what extent the relief of symptoms in myelofibrosis patients by ruxolitinib is in fact due to the inhibition of inflammatory cytokine action (via its role as JAK1 and JAK2 inhibitor). This will probably only be recognized when data from studies with more JAK2-specific inhibitors advance to the same stage in clinical studies. As mentioned before, inflammatory cytokines are a hallmark of myelofibrosis (even in those cases without apparent mutations affecting the JAK-STAT pathway).

Also for the treatment of PV it will be interesting to follow the performance of specific JAK2 vs. multi-JAK inhibitors since PV patients do not generally demonstrate elevated serum levels of inflammatory cytokines. In fact, the phenotype of PV is mainly characterized by myeloproliferation resulting in the increase of red blood cell count often accompanied by leukocytosis and/or thrombocytosis. However some studies have shown that inflammatory cytokines are also detectable in PV and contribute to the growth of clonal erythroblast independently of JAK2V617F.[169,173] Additionally, the underlying mechanism of PV is more closely connected to hyperactivated JAK2, since almost all PV patients either bear the JAK2V617F mutant or a mutation in exon 12 of JAK2. Thus, one might speculate that in the treatment of PV a JAK2-specific inhibitor (e.g., TG101348) might be more efficient; however, this remains to be shown. Ruxolitinib has been assessed in a phase II clinical trial in PV and ET patients intolerant or resistant to treatment with hydroxyurea.[187] Application of ruxolitinib led to a decrease of hematocrit levels, platelet count, and JAK2V617F allele burden.[188] The most common side effect was anemia for both patient cohorts, which was clinically well manageable. Two clinical studies on PV patients (http://www.clinicaltrials.gov/, NCT01243944 [RESPONSE] and NCT01632904 [RELIEF]) are currently being conducted.

TG101348 (SAR302503). TG101348, an inhibitor described to be specific for JAK2, is also evaluated in a phase II clinical trial in patients with PV and ET (http://www.clinicaltrials.gov/, NCT01420783). When tested in a phase I/II clinical trial in myelofibrosis patients, it led to the normalization of leukocytosis and thrombocytosis, while a decrease in inflammatory cytokine levels could not be observed for this compound.[189] This suggests that TG101348 acts rather anti-proliferative than anti-inflammatory. So it will be very interesting, how this inhibitor with a stronger preference for JAK2 in in vitro kinase assays will perform in myelofibrosis, PV, and ET patients in comparison to ruxolitinib.

Other JAK inhibitors. Many potent JAK inhibitors (showing nanomolar activities in intact cell assays) have been developed in the last years and several are evaluated in clinical trials.[177,190-193] **Table 1** shows promising JAK(2) inhibitors in clinical trials for MPN. More comparative studies of these inhibitors are needed to show possible differences of potency and to uncover potential additional activities of these compounds (see ref. 194). For instance CEP701, a JAK2 inhibitor, was recently shown to also

Table 2. A selection of potent commercially available JAK inhibitors

Compound	JAK1	JAK2	JAK3	Tyk2	References
AZ960	-	3	9	-	57
BMS911543	356	1	73	66	218
CEP33779	~72	1.8	85	~1440	230
JAK inhibitor 1 (JI1, pyridone 6, CMP6)	15	1	5	1	231
NVP-BSK805	~32	0.5	~19	~11	232
TG101209	-	6	169	-	233

IC_{50} values in nM obtained in in vitro kinase assays.

target Aurora kinases in the sub-micromolar concentration range in intact cells.[194]

However, most of the JAK inhibitors demonstrate inhibitory activity toward more than one JAK family member (or other kinases), which, on the other hand, might be beneficial in the setting of inflammatory disorders. In line with this, tofacitinib (CP-690550, a pan-JAK inhibitor) has been successfully applied in patients with rheumatoid arthritis[195] and has recently been approved by the FDA (Xeljanz®) for the treatment of patients with moderately to severely active rheumatoid arthritis.

The majority of ATP-competitive kinase inhibitors bind the kinase domain of their respective targets in the active state (also known as type I inhibitors); the clinically approved drugs gefitinib, erlotinib, and sunitinib are prominent examples of this inhibitor class.[196] Most inhibitors developed against Janus kinases are type I inhibitors.[197] Since kinase domains in their active conformation are highly similar to each other it is especially difficult to accomplish high selectivity by using type I inhibitors. A strategy to gain selectivity would be the targeting of the inactive conformation of a kinase domain. This class of compounds (type II inhibitors) also acts ATP-competitively but targets an extended ATP-binding site by spreading into the hydrophobic deep pocket which is only accessible in the inactive conformation of the kinase.[196] Recently, NVP-BBT594 (originally designed to target Bcr-Abl) was described as first compound to bind JAK2 in its inactive conformation.[197]

Some of the JAK targeting compounds (including some that are not tested in clinical trials) are also very valuable tools for research: some by their pan-JAK activity and some by their specificity for individual JAKs. **Table 2** shows some of these potent inhibitors of Janus kinases that are commercially available.

Combination treatment with JAK2 inhibitors. Combinations of different kinase inhibitors have been shown to have beneficial effects on growth inhibition of JAK2V617F-expressing cells. The combination of an Aurora kinase inhibitor with a JAK2 inhibitor has recently been shown to synergistically reduce the proliferation of JAK2V617F-positive cells.[194] Also the use of a JAK2 inhibitor in combination with the suppression of the PI3K/Akt/mTOR pathway synergistically reduces the proliferation of JAK2V617F-positive cells.[198,199] Moreover, a combined application of an inhibitor of the dual specificity mitogen-activated protein kinase kinase (MEK)—selumetinib (AZD6244)—and a JAK2 inhibitor has been demonstrated to act synergistically on the proliferation of JAK2V617F-positive cells.[200]

Additionally, compounds modifying the epigenome have been tested for their potential therapeutic activity in MPN. However, it is not clear if there is a therapeutic indication for DNA demethylation in MPN since the reports on alterations in DNA methylation patterns are controversial. Demethylating agents as azacitidine and decitabine are tested as single drug or in combination with JAK2 inhibitors in MPN patients.[177] Barrio and colleagues found a homogeneous and very similar methylation pattern in MPN compared with healthy control populations.[201] On the other hand, it was described that PV and ET are characterized by an aberrant hypermethylation while PMF is characterized by both aberrant hyper and hypomethylation.[202] Histone deacetylases (HDACs) are also known to epigenetically regulate gene expression by removing acetyl groups from lysine residues on histone proteins and also non-histone proteins like transcription factors.[203,204] It has been shown that both the level and activity of HDACs are elevated in primary myelofibrosis patients.[205] Therefore the potent pan-HDAC inhibitor panobinostat (LBH589) has been evaluated in vitro in JAK2V617F-positive cells.[206] The treatment with panobinostat decreased JAK2V617F expression levels and its downstream signaling probably by mediating hyperacetylation of heat shock protein (HSP) 90 and thereby disrupting the association between JAK2 and the chaperone, leading to its proteasomal degradation. Myelofibrosis patients treated with panobinostat as a single agent experienced an improvement of constitutional symptoms and a reduction of spleen size.[205,207] Moreover, when applying a JAK2 inhibitor and panobinostat in combination, the proliferation of JAK2V617F-positive cells was synergistically suppressed[206] and demonstrated enhanced efficacy in comparison to each single agent in murine MPN models.[208] Based on these findings a phase I clinical trial was initiated to test the combination of ruxolitinib and panobinostat in myelofibrosis patients (http://www.clinicaltrials.gov/, NCT01433445). As mentioned, the disturbance of the association between JAK2V617F and its chaperone HSP90 can lead to lower JAK2V617F expression levels. This can also be achieved by inhibiting HSP90. It has been shown that the inhibition of HSP90 chaperone function by e.g., PU-H71 or AUY922 leads to the loss of binding to JAK2 resulting in attenuated expression of JAK2 (V617F) and inhibition of JAK-STAT signaling. The combination of a JAK2 inhibitor and a HSP90 inhibitor showed enhanced efficacy in the proliferation of JAK2V617F-positive cells in comparison to each single compound.[209,210] Furthermore, AUY922 was demonstrated to overcome resistance to JAK2 inhibitor treatment in cells expressing JAK2V617F.[209,211] Taken together, inhibition of HSP90 and/or the combination with JAK2 inhibitors might be a valuable treatment approach to test in MPN patients, especially in those who do not respond to JAK2 inhibitory treatment. However, it has to be considered that HSP90 has many other client proteins besides JAK2 that are prone to degradation upon inhibition of HSP90 as well. This might lead to additional side effects compared with a more specific treatment.

In conclusion, a combination of JAK2 inhibitors with other agents that have demonstrated a clinical benefit in MPN patients might help to further improve the treatment outcome

in comparison to JAK2 inhibitors as single drug. Thereby, the efficacy of the treatment can be enhanced while possibly decreasing the drug dosage resulting in reduced toxicity. In addition, combining two compounds with different mechanisms of action would decrease the probability of developing resistance to either of the drug.

Perspectives

The clinical development of ruxolitinib and other JAK inhibitors appears to be a breakthrough in the treatment of myelofibrosis patients. These drugs significantly improve the patients' quality of life, which is remarkable progress over conventional treatment strategies. In addition to the reduction of symptoms, the recent data indicate that ruxolitinib treatment leads to a reduction of the JAK2V617F allele load and presents a survival advantage. It will be interesting to follow up to what extent the ruxolitinib-induced relief of symptoms and decrease of JAK2V617F allele load in myelofibrosis and PV is due to the inhibition of inflammatory cytokine action (since ruxolitinib targets both JAK1 and JAK2). This will probably only be recognized when data from studies with more JAK2-specific inhibitors (e.g., TG101348 [SAR302503] or BMS911543) will have reached comparable stages in clinical studies. It is conceivable that a JAK2-specific inhibitor might actually perform less well in comparison to ruxolitinib, due to a lack of activity against JAK1. It could also be possible, that a specific JAK2 inhibitor might be more adequate for the treatment of PV, as almost all PV patients carry a mutant of JAK2 (V617F or exon 12 mutations) and the inflammatory cytokine levels are much lower in PV patients than in myelofibrosis patients. For PV and JAK2V617F-positive ET patients a JAK1-targeting inhibitor might also have more undesired side effects.

No JAK2-specific compound has yet been approved for clinical application and the development of specific JAK inhibitors also for other indications besides MPN is still required. Additionally, the generation of a JAK2-specific inhibitor targeting the inactive state of the kinase (type II inhibitor)[197] is especially interesting. If type II inhibitors are more efficient in inhibiting JAK2 (V617F) activity and reducing the JAK2V617F allele burden compared with a type I compound remains to be elucidated. The occurrence of JAK2 mutations in MPN patients conferring resistance to JAK2 inhibition has not been described so far. However, the acquisition of secondary mutations to evade therapeutic targeting is a common mechanism in cancer.[212] Nevertheless, several mutations in the JAK2 kinase domain that evade JAK2 inhibition have been identified in in vitro studies.[211,213-215] These mutations may also emerge in patients under prolonged JAK2 inhibitory treatment. More specific JAK inhibitors are necessary to investigate the above mentioned issues and will provide more insight in understanding the perspective of JAK inhibitors in the treatment of MPN.

Furthermore, the disease-driving mechanisms in the three MPNs with high JAK2V617F levels have not been fully elucidated. It is not well understood how the various genetic aberrations interact and contribute to the pathogenesis of MPN. Thus, the elucidation of underlying molecular mechanisms including the interplay between the JAK-STAT signaling pathway (with JAK2 as central node), other signaling pathways and epigenetic abnormalities remains a major subject of research in the field of MPN. Better therapies for MPN patients are sought, which provide better treatment of symptoms, can efficiently change the course of these disorders and increase the patients' survival time. The development of combination treatment approaches affecting key cellular regulators (including JAK2 inhibitors as well as other drugs) might contribute to reach this goal.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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