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# JAK, an Oncokinase in Hematological Cancer

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## Abstract

Janus kinases (JAKs) play an essential role in the regulation of cytokine signaling. They control cell survival, proliferation, differentiation, immune response, and hematopoiesis. Deregulation of JAK signaling has been associated to the pathogenesis of numerous immune-inflammatory diseases, hematological malignancies, and solid tumors. Thus, JAK proteins have emerged as attractive therapeutic targets in the last decade. The discovery of the gain-of-function JAK2 mutation (JAK2 V617F) as the main cause of polycythemia vera—a chronic myeloproliferative syndrome—led to the development of the JAK inhibitor ruxolitinib. This key finding opened the door to the search for new therapeutic agents able to suppress the constitutive activation of JAK signaling in hematological cancers and other tumors. However, given the conserved nature of the kinase domain among JAK family members, and the interrelated roles of JAK kinases in many physiological processes, including hematopoiesis and immunity, the broad usage of JAK inhibitors in hematology is challenged by their narrow therapeutic window. Novel therapies are, therefore, needed. This chapter focuses on the understanding of the complex signaling of JAK proteins in cancerous cells, the various JAK aberrations implicated in myeloproliferative neoplasms, leukemia, and lymphoma, and the clinically available JAK inhibitors in cancer therapy.

**Keywords:** blood cancer, hematological tumor, JAK, STAT, mutation, JAK2 V617F

## 1. Introduction

The Janus kinase (JAK) signal transducer and activators of transcription (STAT) intracellular pathway connects the signaling from extracellular cytokines, hormones, and growth factors, with the nuclear transcriptional machinery [1]. It is expressed in animals from flies to humans, being highly evolutionarily conserved [2]. The cascade consists of the tyrosine kinase JAK, the transcription factor STAT, and different regulatory proteins. In mammals, four JAKs and seven STATs have been identified [3]. JAK/STAT signaling controls numerous essential cellular responses, including cell proliferation, differentiation, migration, immune response, apoptosis, and cell survival, according to the signal, cell context, and tissue [4, 5]. These cellular events are crucial to a wide range of biological functions

like hematopoiesis, immune development, inflammatory response, adipogenesis, and angiogenesis, among others [6]. Under normal physiological conditions, JAK/STAT pathway signaling is strictly regulated. However, in different pathological conditions such as cancer, atherosclerosis, rheumatoid arthritis, or diabetes, an “aberrant” regulation of JAK/STAT signaling has been described [6]. Mutations on JAK proteins have been reported in certain cancers, highlighting hematological cancers (HCs). Generally, these are JAK gain-of-function mutations that promote constitutive STAT activation, which triggers tumorigenesis, high-grade inflammation, or hypergrowing, among other pathological consequences [7]. As consequence, JAK inhibitors are gaining prominence in clinical use, mainly in the treatment of HCs driven by JAK mutations, or in those tumors in which JAK/STAT pathway is determinant for the pathogenesis [8, 9]. Interestingly, not only in HCs therapy, but also in the treatment of advanced solid tumors such as pancreatic cancer and triple-negative breast cancer, and certain autoimmune and inflammatory diseases such as rheumatoid arthritis, JAK inhibitors are under clinical trial [10, 11].

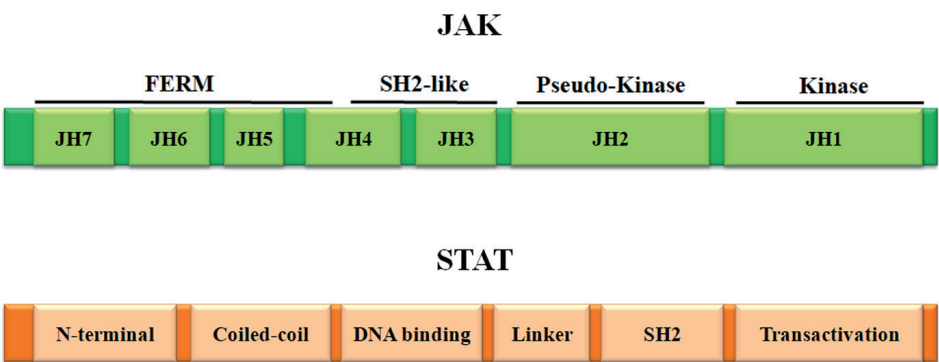
## 2. The JAK/STAT pathway

### 2.1 JAKs

JAK proteins are nonreceptor tyrosine kinases that are essential for the activation of signaling mediated by receptors for cytokines, hormones, or several growth factors. The family includes four 120–130 kDa proteins, named JAK1, JAK2, JAK3, and TYK2, with seven defined regions of homology, called JAK homology (JH) domains (JH1–JH7) [5] (**Figure 1**). The C-terminal region includes the kinase (JH1) and the pseudokinase (JH2) domains. JH1 domain contains tyrosine residues in the activation loop, essential for JAK activation. The pseudokinase domain JH2 is structurally analogous to JH1 and participates on its activity regulation but lacks characteristic residues of tyrosine kinases, which makes it catalytically inactive [12]. Next, the SH2-related domain is constituted by JH3 and part of JH4; this region mediates JAK docking to phosphorylated tyrosine residues [13]. The other half of JH4 to JH7 domains compose the N-terminal region, called FERM (four-point-one, ezrin, radixin, and moesin), which are involved in the association between JAK and cytokine receptors [12].

### 2.2 STATs

The STAT family consists of seven members, named STAT1 to STAT4, STAT5A, STAT5B, and STAT6, of 80–100 kDa, which share highly conserved homology



**Figure 1.**  
*JAKs and STATs structural domains.*

regions. These include (a) an N-terminal domain, (b) a spiral domain, (c) a DNA-binding domain, (d) a SH2 domain, and (e) a transactivation domain at the C-terminal end [7] (**Figure 1**). The N-terminal region is the less conserved one among the STATs, and it is implicated in some STAT dimer-dimer and other protein interactions. The spiral coiled-coil domain is responsible for many other protein-protein interactions [6]. The STAT binding to DNA is mediated by the DNA-binding domain, which defines that STAT dimers recognize an 8- to 10-base pair inverted repeat DNA element with a consensus sequence of 5'-TTCN<sub>2</sub>-4GAA-3'. Differential binding affinity of an activated STAT dimer for a single target DNA sequence is determined by the number of nucleotides between TTC and GAA [14]. The SH2 domain is responsible to target STATs to specific tyrosine-phosphorylated peptide sequences within their binding molecules, thus controlling a broad range of intracellular signaling functions [7]. The transactivation domain holds two aminoacidic residues (tyrosine and serine) essential for STAT activity; so that JAK-promoted tyrosine phosphorylation leads to STAT dimerization, whereas STAT serine phosphorylation mediated by mitogen-activated protein kinases (MAPKs) enhances its transcriptional activity [7, 15]. All these domains are essential for STAT biological functions in response to extracellular stimuli such as cytokines or growth factors.

### 2.3 Pathway signaling

External stimuli (i.e., cytokines, growth factors) bind their receptors in the cellular membrane activating receptor-associated JAK autophosphorylation and subsequent activation. This event triggers a conformational change in JAK structure, which gets it ready for binding substrate and exerting its kinase activity. JAK binding sites are then exposed to the cytoplasm, where STAT monomers are found themselves in latency. STATs are recruited to the recognition areas at JAK-binding sites being phosphorylated by JAKs, which triggers their dimerization in homodimers (STAT1, STAT3, STAT4, STAT5A, and STAT5B) or heterodimers (STAT1-STAT2 and STAT1-STAT3). Consequently, active STAT dimers translocate into the nucleus where they bind to DNA, activating or repressing the transcription of their target genes [3, 6] (**Figure 2**). According to the cellular context, the external stimuli implicated, and the receptors engaged, different JAKs and STATs can be activated [16, 17] (**Table 1**).

Interestingly, through a noncanonical signaling, other tyrosine kinases different from JAKs can activate STAT factors, including membrane-bound growth factor receptor tyrosine kinases (e.g., epidermal growth factor receptor—EGFR, platelet-derived growth factor receptor—PDGFR) and nonreceptor tyrosine kinases (e.g., the proto-oncogene tyrosine kinases Src and Bcr-Abl) [2, 18]. Furthermore, STAT has been shown to be able to form dimers and exert biological activity in absence of canonical JAK tyrosine phosphorylation [19]. In fact, activated JAK2 has been reported that it can enter the nucleus where it mediates epigenetic modifications of histones [20]. Furthermore, a fraction of inactive STAT5 has been found to be localized in the nucleus (instead of in the cytoplasm as the canonical signaling describes), where it is not susceptible of being phosphorylated by tyrosine kinases, mediating chromatin stabilization [21, 22].

### 2.4 Regulation of JAK/STAT pathway

Owing to the implication of JAK/STAT pathway in many relevant biological processes, its endogenous regulation is tight and precise. Besides, since deregulated JAKs and STATs have been associated with several pathological disorders, most of JAK/STAT modulators have been largely assessed as interesting therapeutic approaches. One of the conventional JAK/STAT modulators is protein tyrosine



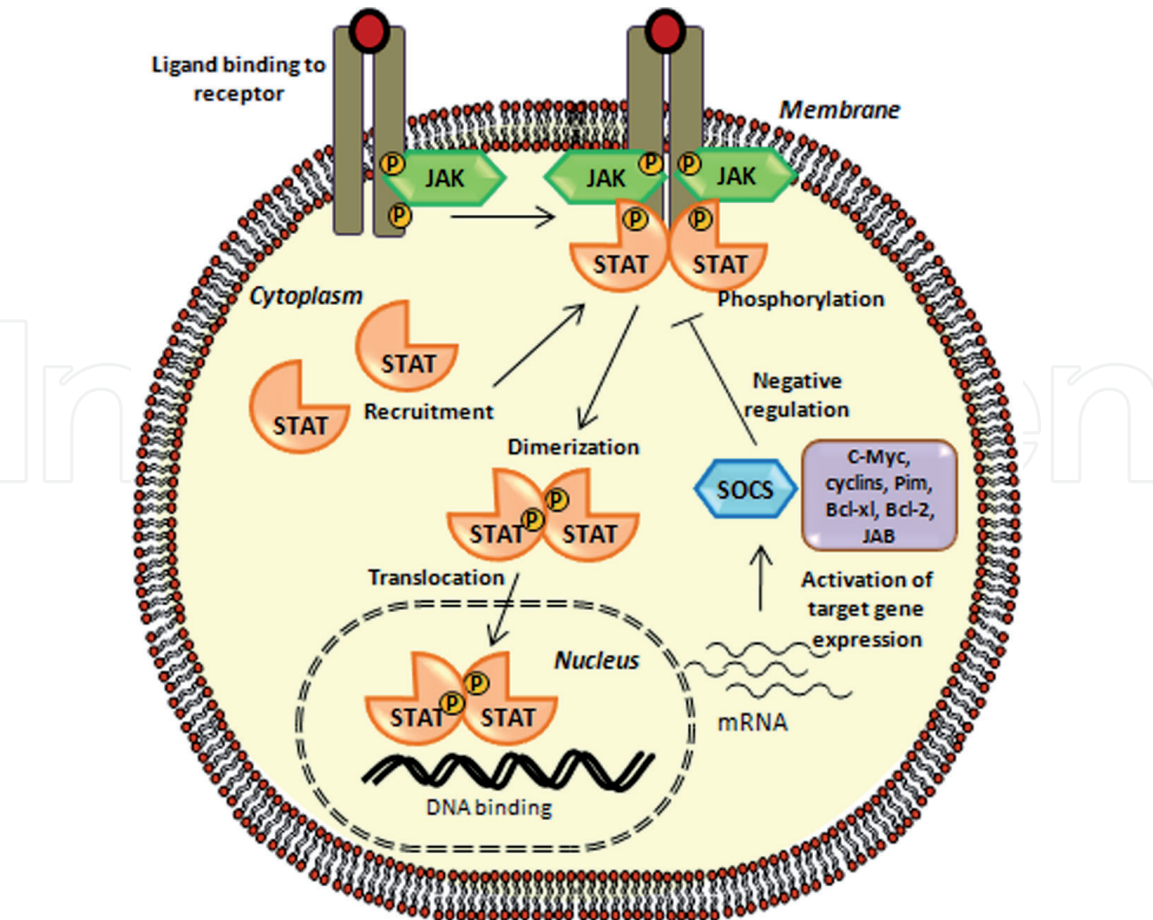


Figure 2.  
JAK/STAT signaling pathway.

Cytokine/growth factor	Activated JAK	Activated STAT	Therapeutic area
IFN ( $\alpha,\beta,\gamma$ )	JAK1, JAK2, TYK2	STAT1, STAT2	Immune regulation, cancer
IL-10	JAK1, TYK2	STAT1, STAT3	Immune regulation
IL-2, IL-7, IL-9, IL-15	JAK1, JAK3	STAT5, STAT3	Immune regulation, asthma
IL-4, IL-13	JAK1, JAK2, JAK3	STAT6	Allergy, asthma
IL-3, IL-5, GM-CSF	JAK2	STAT5	Myeloid reconstitution, asthma
IL-12, G-CSF, EPO, TPO, PRL, GH	JAK2, JAK1, TYK2	STAT5, STAT3, STAT4, STAT1	Immune regulation, myeloid reconstitution, anemia, platelet production, growth, aging
CSF-1/M-CSF, EGF, PDGF, insulin	JAK1	STAT1, STAT3, STAT5	Cancer, diabetes
IL-6, IL-11, leptin, CT-1	JAK2, JAK1, TYK2	STAT1, STAT3, STAT5	Inflammation, platelet production, obesity, cardiovascular disease

Table 1.  
Differential activation of JAK/STAT pathway upon ligand binding.

phosphatases (PTPs), which negatively regulate the signaling of the pathway by dephosphorylating the JAK-associated receptor and/or JAK itself. Furthermore, the protein inhibitors of activated STATs (PIAS) constitute another classical group of JAK/STAT negative regulators. This family of proteins can inhibit STAT signaling

and function by directly preventing STAT from binding DNA or indirectly inhibiting STAT dimerization [23, 24]. But doubtlessly, the most broadly studied group of negative modulators of JAK/STAT signaling is the family of Suppressor of Cytokine Signaling (SOCS) proteins [25]. The family comprises eight members (SOCS1–7 and CIS) of 20–30 kDa, which show different structural domains including a N-terminal domain of variable length, little conserved; a central Src homology region that contains an extended SH2 sequence that leads to SOCS binding to tyrosine-phosphorylated residues either on the associated receptor or at JAK protein; and a highly conserved C-terminal domain, called SOCS box [25, 26]. Furthermore, SOCS1 and SOCS3 share a small kinase inhibitory region (KIR) located at their N-terminal region, which is implicated in the inhibition of JAK-catalytic activity. SOCS proteins exert a negative feedback loop mechanism, so that activated STATs induce the expression of SOCS, which then control STAT transduction signaling (**Figure 2**). The mechanisms by which SOCS proteins suppress JAK/STAT signaling include (1) binding to JAK catalytic site and subsequent inhibition of its kinase activity; (2) competition with STAT for the binding sites on the associated receptor; and (3) proteasomal degradation [23].

### 3. JAK in hematopoiesis

Hematopoiesis is a multistep process by which blood cells, which have a limited life span, are continuously renewed. It is initiated in the bone marrow with the proliferation and differentiation of pluripotent hematopoietic stem cells, which undergo asymmetric divisions and differentiate into lineage-committed progenitors that eventually give rise to specialized blood cells [9]. Deregulation in hematopoiesis leads to the accumulation of intermediate progenitors or mature cells in the bone marrow, blood, or lymphoid tissues driving hematological malignancies [9]. Hematopoietic cytokines including erythropoietin (EPO), thrombopoietin (TPO), granulocyte colony-stimulating factor (GM-CSF), among others, tightly regulate hematopoiesis. They maintain regular levels of blood cells or induce their production according to physiological needs. These cytokines bind to their cognate receptors at the cell membrane, which generally (except some tyrosine kinases such as c-KIT, FLT-3, or GM-CSF receptor) lack intrinsic enzymatic activity at their intracellular part. Nevertheless, these receptor chains are constitutively associated with a JAK kinase, which mediates cytokine-induced signaling [9]. During myelopoiesis, JAK2 has been found to respond upon EPO, TPO, G-CSF, GM-CSF, IL-3, and IL-5 binding, mediating myeloid cell proliferation and differentiation [9], whereas in lymphopoiesis are mainly JAK1 and JAK3, which cooperate by binding to specific cytokine receptors (IL-2R, IL-4R, IL-7R and IL-15R). It has been suggested that JAK1 functions as the primary signaling effector since JAK3 is a JAK1 scaffold [9]. Gene disruption studies have confirmed the essential role of JAK proteins in hematopoiesis. JAK1-deficient mice showed perinatal lethality and defective lymphoid development [27]. Lack of JAK2 expression resulted in an embryonic lethality due to a block in erythropoiesis but with intact lymphoid development [27]. JAK3 deficiency revealed severe combined immunodeficiency with low functional T and B cell numbers and aberrant myelopoiesis [27].

### 4. Aberrant JAK signaling and hematological cancer development

The multifactorial process of tumorigenesis is characterized by cellular fail in sensing and repairing DNA damage, loss of regulation of cell cycle progression and

apoptosis, and expression of aberrant patterns of growth signaling and angiogenesis [28, 29]. Numerous studies have provided strong evidence for the key role that JAK kinases play in hematologic cancer genesis and progression. This is not surprising considering the close relation between JAKs and cytokine and growth factor signaling, hematopoiesis, proliferation, apoptosis, and immune response, processes that, when deregulated, contribute to tumor development [29, 30]. Either gain-of-function mutations in JAKs, cognate JAK tyrosine kinases, or JAK associate receptors, the generation of fusion proteins, or the loss of negative feedback regulation of JAK signaling can contribute to constitutive and aberrant STAT signaling and therefore to oncogenesis [18]. The first evidence of the strong implication of JAK kinases in HCs was the identification of oncogenic fusion proteins involving JAK kinase domain (e.g., TEL/ETV6-JAK2) [31]. Subsequently, other JAK2 fusion proteins and JAK2 gene amplifications have been identified. However, although they were more recently discovered, JAK point somatic mutations are the most common JAK deregulations found in hematological tumors, being the mutation JAK2 V617F found in more than half of all classical myeloproliferative disorders (MPDs) [32]. Besides, other JAK mutations are associated to hematological malignancies, such as JAK1 mutations, found in 10–20% of T-ALL, and other JAK2 mutations associated to ~20% of Down syndrome (DS)-associated B-ALL [32] (**Table 2**). Interestingly, the discovery of all these mutations has highlighted JAK proteins as potent drug targets and biomarkers for HCs.

4.1 JAK2 mutations

4.1.1 JAK2V617F mutation in myeloproliferative disorders

Myeloproliferative disorders (MPDs) are a group of chronic clonal malignancies arising from the expansion of mature hematopoietic progenitor cells [33]. The World Health Organization (WHO) distinguishes two MPDs subtypes: (a) chronic myelogenous leukemia (CML) involving the Philadelphia (Ph) chromosome, frequently associated to BCR-ABL fusion oncoprotein and (b) a set of Ph-negative MPDs syndromes mainly referred to polycythemia vera (PV), essential thrombocythemia (ET), and idiopathic myelofibrosis (IMF) [34]. Two key features of this second group are the ability of cytokine-independent blood colony formation [33, 35] and hypersensitivity to numerous cytokines [36, 37]. However, each subtype is characterized by the clonal production of different hematologic lineages. PV and ET present, for example, an increased production of platelets and red cells. Accumulating evidences over the last decade establish that Ph-negative MPDs frequently carry a JAK2 single point somatic mutation at chromosome 9p24, exon 14

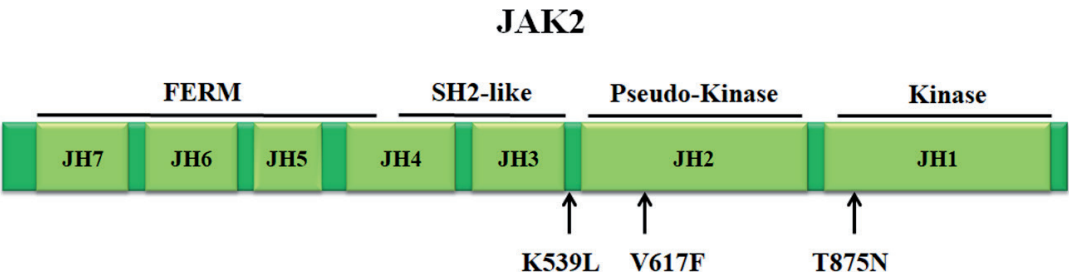
JAK	Mutation	Associated disease
JAK2	V617F	MPDs
	K539L	PV
	T875N	Acute megakaryoblastic myeloid leukemia
	Deletion of IREED	ALL
JAK1	A634D	T-ALL
	T478S, V623A	AML
JAK3	A572V, V722I, P132T	Acute megakaryoblastic myeloid leukemia

**Table 2.**  
*Mutation in human JAKs and disease association.*



(JAK2V617F—Val617Phe) [38, 39]. This genetic abnormality has stem cell nature, affecting all cells of the myeloid lineages [36, 40], whereas clonal involvement of the lymphoid lineage is controversial, and its effects are less understood. Larsen and colleagues detected the JAK2V617F mutation in both B lymphocytes and T lymphocytes in a subgroup of patients with Ph-negative MPDs. Their results suggested an early stem cell origin with both lymphoid and myeloid differentiation possibilities [41]. JAK2V617F mutation is present in 50–60% of patients with ET and IMF and in most of cases of PV [35, 36, 40]. Some reports have also related JAK2V617F mutation to other myeloid malignancies like chronic myelomonocytic leukemia (CMML), myelodysplasia (MD), and, in rare cases, acute myelogenous leukemia (AML) [42]. Additionally, in other less frequent leukemias like mediastinal B cell lymphoma and Hodgkin lymphoma, both with amplification of the JAK2V617F mutation, researchers were conscious about an epigenetic role of aberrant JAK2 kinase, leading to histone H3 phosphorylation, thereby promoting gene expression [43]. The origin of JAK2V617F mutation is localized within the pseudokinase domain, JH2 of JAK2 gene [36]. JAK2 activation requires Y1007 phosphorylation [33] and its activation is crucial for cytokine-mediated signaling from the EPO receptor and other type I cytokine receptors [44]. In this sense, JAK2V617F somatic mutation is phosphorylated at Y1007, conferring constitutive activation of JAK2 tyrosine kinase by decreasing the autoinhibitory effect of JH2, thereby recapitulating cytokine receptor downstream signaling pathways, among these STAT5 and ERK (extracellular signal-regulated kinase) [33, 35, 45] (**Figure 3**). The discovery could be performed by tyrosine kinase gene sequencing in MPD patients [35, 36] and by assessing the role of JAK2V617F mutation in different in vitro studies. Cellular transformation of cytokine-dependent cell lines like Ba/F3, Ba/F3-EpoR, and FDCP-EpoR with JAK2 mutant variant led to cytokine-independent signaling triggered by JAK2 constitutive phosphorylation and induced erythrocytosis; whereas concomitant wild-type JAK2 overexpression restored or alternatively decreased the effects of the mutation in vitro [35, 40]. Lower levels of JAK2V617F required coexpression of dimeric type 1 cytokine receptor as a scaffold for the independence of hormone signaling status in Ba/F3 cells [46]. Retroviral transplant mouse models have evidenced that JAK2V617F presence is enough for reproducing PV and IMF diseases in vivo [33, 35, 46]. However, its related effects on ET remained insufficiently understood [45], exposing no sufficient JAK2V617F influence on platelet number [44, 47].

Three hypotheses have been suggested for explaining the causes of phenotype variability exhibited by JAK2V617F: gene dosage background, unidentified mutations, and receptor interaction with JAK2 during myeloid and erythroid differentiation [35, 42]. In the first case, mice genotyping of the JAK2V617F gene showed increased expression of this protein in homozygote samples, leading to PV or IMF like diseases. Homozygous form of this single-point mutation is found in at least 30% of PV patients, probably due to mitotic recombination [36, 40]. On the other



**Figure 3.**  
*JAK2 point mutations.*



hand, heterozygous mice might drive the ET phenotype. In fact, data point out ET as the most heterogeneous MPD. The second hypothesis suggests that the precedence or upcoming sequence of nonidentified mutations following JAK2V617F may drive the acquisition of one or another phenotype [40, 42], thus showing genetic heterogeneity [35]. Finally, Funakoshi and colleagues proposed that cellular context-specific receptor's interaction with JAK2V617F expression levels would determine the activated phenotype [48]. From another perspective, JAK2V617F mutation in Ph-negative MPDs leads to constitutive phosphorylation of JAK2 in the absence of EPO [36]. This event is closely linked to downstream STAT3/5 proteins phosphorylation. PV patients exhibit high STAT5 and STAT3 phosphorylation; ET patients exhibit high STAT3 but low STAT5 phosphorylation; and myelofibrosis patients exhibit both low STAT5 and STAT3 phosphorylation. Different STAT3/5 phosphorylation patterns allow the discrimination among Ph-negative MPDs [49]. As we can see, constitutive activation of JAK2–STAT5 or JAK2–STAT3 signaling is a major driver of PV, ET, and IMF [36, 49]. In short, JAK/STAT signaling pathway is demonstrated to be essential for hematologic stem cells differentiation. Focusing on JAK2 as a therapeutically valid target remains an attractive option for MPDs treatment.

#### 4.1.2 JAK2K539L mutation (exon 12 mutations) in polycythemia vera

The JAK2V617F mutation discovery was followed by other different JAK2 gene gain-of-function mutations identification [33, 38, 50–52]. As we have already described above, most PV patients express JAK2V617F [36, 40, 52]. Nevertheless, less frequently (3–5%) PV cases harbor several exon 12 JAK2 mutations present in the linking region of JH2 and JH3 domains, encompassing a highly conserved amino acid region F537–E543 in the absence of V617F mutation. This leads to a distinct clinical syndrome with isolated erythrocytosis [43, 53]. Three of the cluster of different JAK2 exon 12 mutations [43, 51, 52] included a substitution of leucine for lysine at position 539 (539L) of JAK2 in JAK2V617F-negative PV patients or idiopathic erythrocytosis: F537-K539delinsL, H538QK539L, and K539L. They are reported to be acquired, thus explaining why they appeared in peripheral-blood granulocytes but are absent in T lymphocytes [43, 51]. Functionally, K539L exon 12 mutations modify JH2 domain, resulting in aberrant growth factor responses in Ba/F3 cells *in vitro*. This cell line was able to proliferate without the addition of IL-3 and demonstrated to have an increased phosphorylation of JAK2, ERK1/2, and STAT5, in comparison to murine cells transduced by wild-type JAK2 or V617F JAK2 [39, 51, 54]. Furthermore, these mutations discharged a myeloproliferative phenotype in a murine model, resulting in higher levels of phosphorylated JAK2 compared to those with the V617F mutation. The described consequences as well as kinetics exhibited by K539L mutations were not distinguishable from those observed for cells with the V617F mutation [51]. From a genetic point of view, unlike JAK2V617F-positive PV patients, JAK2 exon 12-mutated PV patients are often heterozygous. However, they share a similar clinical outcome [39, 51].

#### 4.1.3 JAK2T875N mutation in acute megakaryoblastic myeloid leukemia

Acute megakaryoblastic myeloid leukemia (AMKL) is a rare subtype of acute myeloid leukemia (AML) that presents different genetic characteristics and morphological phenotypes. AMKL appears frequently in childhood but is also common in adults in their 50s or 60s [55]. Some cases are developed after chemotherapy or are the result of leukemic transformation of chronic myeloproliferative neoplasms [56]. Diverse cytogenic abnormalities are associated to AMKL that differs between children and adults. The most commonly seen aberrations in adulthood are inv(3)(q21;q26), deletions of chromosomes 5 and 7, and t(9;22)(q34;q11) [55]. Children that develop

this disease are subdivided in two groups. The first one presents constitutional trisomy 21 (Down syndrome) associated to a somatic mutation in *GATA-1* [57]. The second is represented by 1(1:22)(p13;q13) translocation that encodes a fusion protein OTT-MAL (RBM15-MKL1) [58]. Despite all these genetic factors, the fact that DS children spontaneously experiment disease remission, in most cases [57] together with the fact that models of GATA1 mutation fail to reproduce AMKL leukemogenesis [59], suggests that there should be several mechanisms contributing to AMKL promotion.

Merchel et al. were interested in the STAT5 hyperactivation observed in AML, which, in most cases, is the result of activating mutations in tyrosine kinases. In 2006, they identified a novel mutation in JAK2 studying AMKL cell lines such as CHRF-288-11, M07e, or UT7. DNA sequencing of all JAK family members in CHRF-288-11 detected a single homozygous JAK2C2624A allele. This mutation leads to a substitution of a threonine for an asparagine at position 875 of the JAK2 JH1 kinase domain (**Figure 3**). Based on the crystal structure of JAK2, T875 lies within the loop between strands  $\beta 2$  and  $\beta 3$ , which could alter JH1-JH2 interface [56]. However, studying full-length JAK2 crystal structure is necessary to better comprehend the mechanism of constitutive activation of JAK2 mutants [60]. The other cell lines studied, M07e and UT7 (6-month-old and 64-year-old AMKL patients, respectively), did not express hyperactivated STAT5, which is consistent with the heterogeneity of this disorder [56]. Although the frequency of this mutation in patients remains unknown, everything points to an important role of JAK2T875N in AMKL. Indeed, this mutation constitutively activates JAK2 kinase and its downstream effectors in naturally carrying JAK2T875N mutation cells *in vitro* [56] and Ba/F3 cells transduced with EpoR or TpoR. Interestingly, this mutation conferred Ba/F3 cells the capacity of IL-3 independent growth [56, 60]. Moreover, comparative studies of Ba/F3 stably expressing JAK2 wild type or JAK2V617F, JAK2K539L, JAK2T875N mutations showed that the highest kinase activity is associated with JAK2T875N mutation followed by JAK2V617F [60]. Also, JAK2T875N expression was accompanied by significantly increased activation of pathways induced by cytokines and growth factors compared with the other mutations [60]. However, these differences were not detected in HEK293 cells expressing the same JAK2 mutants, which could be result of differences in the transduced cell type [61]. Surprisingly, the higher activation of JAK2-associated JAK2T875N mutant was not linked with the capacity of transforming erythroid progenitors in bone marrow, which showed to be the lowest among the other JAK2 mutations [60]. Moreover, expression of JAK2T875N in a murine bone marrow transplant model was able to reproduce myeloproliferative disease with some AMKL characteristics, except thrombocytosis, insinuating that other genetic events could be involved in the promotion of the disease [56].

#### 4.1.4 JAK2 deletion of IREED (682–686) in acute lymphoblastic leukemia

Children with Down syndrome have an increased risk of developing ALL apart from AMKL, but unlike AMKL favorable outcomes, Down syndrome-ALL undergo higher toxicity of chemotherapy, leading to increased morbidity and mortality compared with non-Down syndrome ALL patients [62]. Activating JAK2 mutations are detected in approximately 20% of Down syndrome-ALL patients [63]. For this reason, Malinge et al. analyzed 90 cases of acute leukemia of myeloid or B-cell origin to screen activating gene mutations based on high level gene expression. This technique allowed them to discover a novel JAK2 mutation in a Down syndrome 4-year-old patient with B-cell precursor acute lymphoblastic leukemia (BCP-ALL). This JAK2 mutation encodes a protein that lacks five amino acids (682–686), JAK2 $\Delta$ IREED. They confirmed constitutive activation of JAK-STAT, ERK, and AKT signaling pathways in Ba/F3 cells artificially harboring JAK2 $\Delta$ IREED and

JAK2V216F mutations. As observed for other JAK2 mutations, EpoR expression was necessary for JAK2 $\Delta$ IREED to transform Ba/F3 cells to growth factor independency. Remarkably, these cells were sensitive to the JAK inhibitor I. In addition, a bone marrow transplant in mice revealed that this mutation promoted MPD in the model, with increased platelet, granulocytic, and red blood cell counts. Intriguingly, EpoR, myeloproliferative leukemia (MPL), and G-CSF receptor are not transcribed in the patient's cells. Hence, which cytokine receptor chain expressed in the leukemic cells is likely to associate with the mutated JAK2 is still unclear [64]. Another important source of information was the study performed by Bercovich et al. that analyzed JAK2 DNA mutations on diagnostic bone marrow samples of 88 Down syndrome-ALL patients and 216 patients with sporadic ALL. They identified acquired somatic mutations of JAK2 in 18% of Down syndrome-ALL patients. Five different alleles were detected, affecting the same evolutionary conserved arginine residue (R683), which is predicted to be located at the pseudokinase to Src homology 2 domain interface. These mutations presented associated genotype-phenotype specificity. Jak2 mutant expression in Ba/F3 EpoR and TpoR cells conferred cytokine independent growth and constitutive activation of JAK2 and STAT5. They also described pro-B cells transduced with the R683S JAK2 as sensitive to pharmacological inhibition of JAK/STAT pathway [63]. Supporting these findings, another group recently performed a genetic study of 83 BCP-ALL cell lines, detecting activating JAK2 mutations in YCUB-5 cell line (JAK2 R683I) and KOPN49 cell line (JAK2 R683G) accompanied by RAS mutations, which point out the involvement of RAS pathway apart from JAK/STAT in the progression of the disease [65]. Furthermore, some reports showed that JAK2 and P2RY8-CRLF2 (cytokine receptor-like factor 2) mutations are rare in Japanese non-Down syndrome ALL and Down syndrome-ALL patients, while in Western countries, CRLF2 is overexpressed in approximately 50–60% of Down syndrome-ALL patients. JAK2 mutations and CRLF2 seem to act in conjunction in leukemogenesis. For this reason, it is being suggested that these genetic aberrations are related to ethnicity [63].

## 4.2 JAK3 mutations

As we mentioned above, JAK3 is involved in lymphocyte development and function, and to carry out its functions, JAK3 interacts with the common gamma chain of some interleukin receptors, including interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15, and IL-21 [5, 66]. Recently, JAK3-activating mutations have been reported in different lymphoproliferative disorders [66–68]. Mutations within the FERM domain, essential for binding of JAK to its receptor, and defects in gamma chain of receptors involved in JAK3 signaling pathway are associated with severe combined immunodeficiency (SCID) [5] and X-linked SCID (XSCID) [69], respectively. There are several activating mutations of JAK3, which have been validated in Ba/F3 cells, including P132T, L156P, R172Q, E183G, Q501H, M511I, A572V, A573V, R657Q, and V722I [67]. Among these transforming mutations, some of them have been more extensively studied because of their frequency and pathological consequences.

### 4.2.1 JAK3A572V, V722I, P132T mutations in acute megakaryoblastic leukemia

In acute megakaryoblastic leukemia (AMKL), AMKL cells present constitutive STAT5 phosphorylation, which indicates an upstream tyrosine kinase activation. The identified candidate responsible of STAT5 activation was JAK3, which carried an A572V mutation in the pseudokinase JH2 [70] that negatively regulates the JH1 kinase activity. Analysis of the entire coding sequence of JAK3 in AMKL patients allowed



for the identification of two additional JAK3 mutations: V722I substitution in the JH2 pseudokinase domain and P132T change in the JH6 domain of the receptor-binding region. These mutations resulted in constitutive activation of JAK3 and phosphorylation of STAT5 and made Ba/F3 hematopoietic cell line cytokine grow independently [66, 70, 71]. However, JAK3 A572V summarized some, but not all, of the phenotypic characteristics of AMKL in a murine bone marrow transplant model, suggesting that other mutations may cooperate in complete AMKL transformation [70].

#### 4.2.2 JAK3A572V and A573V mutations in natural killer/T cell lymphoma

Natural killer/T cell lymphoma (NKTCL) is a localized (areas of Asian and South America) aggressive subtype of non-Hodgkin lymphoma, with molecular characteristics and pathogenesis quite unknown. JAK3A572V and JAK3A573V mutations, located at exon 12 in the JH2 domain, have been described associated to this disease [67, 72]. NK cells need interleukin (IL)-2 to proliferate and be activated and this cytokine mediates JAK1 and JAK3 phosphorylation. In NKTCL, JAK3A572V and JAK3A573V mutations were identified in NK-S1 and MEC04 cell lines [67, 72]. These mutations were shown to trigger constitutive phosphorylation of JAK3, STAT3 [72], and STAT5 [67], respectively, in these cell lines and the ability of IL-2 to independently proliferate in cell culture [67].

#### 4.2.3 JAK3M511I mutation in AML

AML is associated with different karyotype anomalies, and these aberrations are determinant of prognosis. An array-based analysis of human leukemia exemplars could identify the JAK3 M511I mutation [73]. It is located between the SH2 domain and the pseudokinase domain of JAK3. When JAK3M511I is introduced in 32D mouse cell line, which depends on interleukin-3 (IL-3) to grow, cells are able to survive in the absence of the cytokine and they do not differentiate in the presence of G-CSF [73]. Moreover, mice with hematopoietic stem cells infected with retrovirus encoding JAK3M511I showed a marked lymphocytosis in peripheral blood and spleen expansion, developing T-ALL [73, 74].

### 4.3 JAK1 mutations

Considering its important role in lymphopoiesis, JAK1-activating mutations have also been described in several lymphoid neoplasms, with highest frequency (7–27%) in T-ALL, but also in B-ALL and T cell prolymphocytic leukemia, and more rarely in ALL and AML [9]. Most of these mutations occur within the pseudokinase domain of JAK1. Certainly, the oncogenic potential of JAK1 pseudokinase domain disruption had been previously predicted since introduction of a V658F mutation in JAK1 (homologous to the V617F mutation in JAK2) led to its constitutive activation [75]. Recently, the mutation JAK1A634D was identified in adult T-ALL, and it was shown to lead to constitutive JAK1 activation when overexpressed in JAK1-deficient cell lines. Furthermore, A634D was shown to induce the autonomous growth of the cytokine-dependent Ba/F3 cell line, whereas it protected the murine ALL cell line BW5147 from dexamethasone-induced apoptosis. A recent study discovered another JAK1 mutation called JAK1S646P, showing that it is an activating mutation both *in vitro* and *in vivo* in ALL [76]. The first group in reporting somatic JAK1 mutations in AML (JAK1T478S and JAK1V623A) exposed that these mutations may function as disease-modifying mutations in AML, since they do not directly induce cell transformation, but rather modify the activation of downstream signaling pathways in response to external stimuli [77].



Fusion proteins	Disease
TEL-JAK2	T-ALL
BCD-JAK2	Atypical CML
PCM1-JAK2	AML, T-ALL
RPN1-JAK2	PMF
SSBP2-JAK2	B-ALL
PAX5-JAK2	B-ALL

**Table 3.**  
*Most common JAK2 fusion proteins in hematological cancer.*

4.4 JAK fusion proteins

Historically, the identification of oncogenic fusion proteins involving JAK kinase domain entailed the first evidence of the key role of JAK kinases in HCs [31]. After this finding, acquired lesions involving JAK1, JAK2, and JAK3 (but not TYK2) have been reported in both AML and ALL. Interestingly, artificial chimeric TEL-JAK1, TEL-JAK3, and TEL-TYK2 proteins are able to sustain cytokine-independent growth in Ba/F3 cells [78] in which the expression of TEL-JAK2 protects Ba/F3 cells from IL-3 withdrawal-induced apoptotic cell death and leads to IL-3-independent growth. Furthermore, mice transplanted with bone marrow cells containing the ETV6-JAK2 fusion have been shown to develop leukemia [79]. There is no patient-derived chromosomal translocation that fuses the kinase domain of JAK1, JAK3, or TYK2 to a dimerizer described so far. This is probably related to an intrinsic genetic instability of the JAK2 locus, which can otherwise also be subject to amplifications in 30–50% of Hodgkin lymphomas and primary peripheral B-cell lymphomas [80–82]. The chromosomal translocation [t(9;12) (p24;p13)] is associated with T cell childhood ALL and results in the production of the fusion protein TEL-JAK2 (also known as ETV6-JAK2), which contains the JAK2 catalytic domain (JH1) and the oligomerization domain of TEL, one of the Ets transcription factor family members [31, 83]. The TEL subunit facilitates homodimerization of TEL-JAK2 molecules, thus facilitating transphosphorylation and activation of the JAK2 kinase domains. Several analogous JAK2 fusion proteins have since been described in ALLs or AMLs, including PCM1-JAK2 [84], BCR-JAK2 [85], RPN1-JAK2 [86], SSBP2-JAK2 [87], and PAX5-JAK2 [88] (Table 3). In all cases, the mechanism of JAK2 activation is thought to be similar, with the JAK2 fusion partner promoting dimerization and constitutive activation of the JAK2 tyrosine kinase component of the fusion protein, which constitutively triggers several downstream signal transduction pathways, such as STAT3, STAT5 [31, 89, 90], MAP kinase [91], PI3-kinase/Akt [92, 93], and NF-κB [94] independent of the presence of anchoring receptors.

5. JAK inhibitors and hematological cancer treatment

The starting point for the development of JAK inhibitors is located in 2005 when the JAK2V617F mutation was identified as the main cause of the majority of BCR-ABL1-negative myeloproliferative neoplasms (MPNs). Subsequently, the search for JAK inhibitors, and its development, continued with the discovery of other driver mutations (calreticulin (CALR) and myeloproliferative leukemia (MPL) virus onco-gene) that also produce a constitutive JAK2 activation and, thus, aberrant JAK-STAT

signaling [35, 36, 40, 95–97]. JAK inhibitors could be classified into different groups depending on their mechanism of action/region targeted in JAK: type I (they target the ATP-binding site of JAKs in the active conformation of the kinase domain), type II (they target the ATP-binding pocket of kinase domain in inactive conformation), and allosteric inhibitors (they bind to a different site from the ATP-binding site) [98]. All JAK inhibitors that have been clinically tested are type I, so this section of the present book chapter will focus on them.

## 5.1 Type I inhibitors

These compounds may be differentiated according to their specificity for each JAK. Most often they target JAK2, JAK1, and other kinases, such as TYK2 (e.g., ruxolitinib and momelotinib (CYT-387) or JAK3 and JAK1 (tofacitinib)). Some of them can inhibit all JAKs (e.g., gandotinib and peficitinib) and less frequently they specifically target JAK2 (e.g., pacritinib, NS-018 and CEP-33779), JAK1 (e.g., filgotinib and itacitinib) or JAK3 (e.g., decernotinib and JANEX 1) [98–100]. Type I JAK2 inhibitors are commonly used in MPNs, such as myelofibrosis (MF), polycythemia vera (PV), and essential thrombocythemia (ET) [101–104]. However, type I JAK inhibitors that target JAK1 and/or JAK3 are utilized to treat inflammation and autoimmune diseases [105]. Toxicity of type I inhibitors is also related to their specificity for the different JAKs: hematologic dyscrasia/immune suppression for JAK2 inhibitors [106] and immune suppression for JAK1 and JAK3 inhibitors [107]. At this point, it should be kept in mind that JAK2 cannot be completely long term inhibited because this will produce a severe cytopenia and even lead to aplastic anemia, since wild-type JAK2 (WT-JAK2) is indispensable for normal hematopoiesis. Thus, these inhibitors may be therapeutically used because they only partially inhibit JAK2 *in vivo*.

### 5.1.1 Ruxolitinib

Nowadays, ruxolitinib is the only type I JAK2 inhibitor that has been approved by the US Food and Drug Administration (FDA) to be used in the treatment of MF and hydroxyurea (HU)-resistant or HU-intolerant PVs [101, 108, 109]. Approval for MF was due to the two key phase 3 studies: Controlled Myelofibrosis study with Oral JAK inhibitor Treatment I and II (COMFORT-I and II) [108, 109]. In both studies, ruxolitinib was very effective in reducing spleen size and improving MF-general symptoms with dose-dependent anemia and thrombocytopenia, due to JAK2 inhibition, as the most frequent hematological side effect. However, anemia was well managed with dose adjustments and/or red blood cell transfusions [108]. Moreover, in both trials, ruxolitinib significantly reduced the risk of death [110]. In HU-refractory PVs, ruxolitinib effectively controls hematocrit, reduces spleen volume, and decreases JAK2V617F allele burden [101, 111]. Combined therapy with ruxolitinib and other JAK2 inhibitors may provide novel therapeutic strategies for the treatment of MPNs. Notably, it has been recently reported that combinations of ruxolitinib and vorinostat, a histone deacetylase (HDAC) inhibitor that down-regulates JAK2 expression, acted synergistically to reduce tumor growth in several hematological cancer cell lines (B cell lymphoma, multiple myeloma, anaplastic cell lymphoma, chronic B cell leukemia, and Hodgkin lymphoma) [112]. Moreover, this synergic effect on tumor cell growth was related to reduced glucose metabolism and induced ROS production and apoptosis [112]. These findings provide the rationale to support future clinical trials evaluating ruxolitinib-vorinostat combinations in patients. This combinatorial strategy has proved effective even in CML (BCR-ABL<sup>+</sup> myeloproliferative neoplasm). Thus, it has been shown that synergic combinations of ruxolitinib and nilotinib (a direct BCR-ABL inhibitor) profoundly inhibit JAK2

and STAT5 phosphorylation and induce apoptosis in primary CML CD34<sup>+</sup> cells. These effects contribute to an effective elimination of these cells *in vitro* and *in vivo* and support the current utilization of ruxolitinib/nilotinib combinations in clinical trials to eradicate persistent disease in CML patients [113]. In fact, a phase I and a phase I/II clinical studies are already underway to evaluate the potential synergic effects of ruxolitinib-tyrosine kinase inhibitors combinations, such as nilotinib/imatinib, on eradicating CML stem/progenitor cells (ClinicalTrials.gov identifiers: NCT01702064 and NCT01751425).

#### 5.1.2 Momelotinib

Given its potential clinical relevance, there are other type I JAK inhibitors that should be highlighted: momelotinib (CYT38) is a dual JAK1/2 inhibitor that, similar to ruxolitinib, reduces spleen size and MPN general related symptoms in intermediate or high-risk MF patients [114, 115]. Relevant, momelotinib has been shown to reduce anemia, which is a major issue in MF, so this drug might be an alternative to ruxolitinib for MPN patients with anemia. However, two phase-3 studies, SIMPLIFY-1 and SIMPLIFY-2, have reported that momelotinib does not seem to have major advantages over ruxolitinib, although it was related to less transfusion requirement [116, 117]. These findings have caused that momelotinib development has been stopped.

#### 5.1.3 Pacritinib

Pacritinib (SB1518) is a JAK2-selective inhibitor (it does not inhibit JAK1) that also inhibits FLT3 (FMS-like tyrosine kinase 3, a key target in the therapeutics of acute myeloid leukemia), colony-stimulating factor 1 receptor (CSF1R) and interleukin-1 receptor-associated kinase 1 (IRAK1) [118]. In phase I/II studies, pacritinib, at a recommended dose of 400 mg/day, showed a good activity in MF patients with gastrointestinal alterations being the most frequent side effect [119, 120]. After these promising results, two phase-3 clinical trials (PERSIST 1 and 2) were initiated testing different pacritinib concentrations [121]. However, in 2016, FDA carried out a full clinical hold on these trials due to a suspected excess of mortality in treated patients caused by intracranial hemorrhage and cardiac events. This clinical hold was lifted by the FDA on January 2017 [121] and subsequently CTI Biopharma announced PAC203, a new trial in which different doses of pacritinib are being evaluated in MF patients with thrombocytopenia.

#### 5.1.4 NS-108

NS-108 is a potent JAK2-selective inhibitor that also inhibits Src kinases [122]. This compound showed selectivity and high potency for JAK2V617F mutant in mouse models without producing anemia or thrombocytopenia [122]. NS-108 has been tested in a phase II trial at a recommended dose of 300 mg/day in MF patients. As previously described for other JAK2 inhibitors, NS-108 significantly reduced spleen size and improved general MF-related symptoms. However, this product was not able to significantly reduce the amount of JAK2V617F mutant cells [123].

#### 5.1.5 Gandotinib

Gandotinib (LY2784544) is a selective and potent inhibitor of JAK2V617F [124]. This drug has been evaluated in a phase I trial for safety, tolerability, and pharmacokinetic parameters in patients with MF, PV, and ET. Treatment with this compound

at 120 mg/day (oral recommended phase II dose) was associated with an acceptable safety/tolerability and with clinical improvements in MPN JAK2V617F patients [103]. These findings provide rationale for further gandotinib clinical testing.

## 5.2 Type II inhibitors

Two type II JAK2 inhibitors (NVP-BBT594 and NVP-CHZ868) have been developed. NVP-BBT594 was effective in MPN cellular models [125] and NVP-CHZ868 in preclinical mouse MPN models. However, JAK2 inhibition caused by type II inhibitors is more effective and powerful than that produced by type I inhibitors, which in turn may induce profound cytopenia, limiting its future development and clinical use.

## 5.3 Allosteric inhibitors

In this group are the so-called type III (they bind to a site close to the ATP-binding site, e.g., LS104 [126]) and type IV inhibitors (they bind to an allosteric site distant from the ATP-binding site, e.g., ON044580 [127]). Since these inhibitors do not target the ATP pocket, hypothetically, they are more specific than type I/II JAK inhibitors due to the high homology shown by the ATP-binding sites. Taking this into account, JAK allosteric inhibitors would be particularly indicated to treat MPNs related to JAK mutations (especially JAK2V617F) as an efficient inhibition of WT-JAK2 will always produce a profound cytopenia. Nowadays, there is no a JAK allosteric inhibitor in clinical development.

## 6. Conclusions

In summary, JAK kinases are key proteins in the development of hematological malignancies, since different genetic alterations including fusion protein formation, gene amplification, and point mutations have been discovered in a wide array of hematological malignancies. Particularly, JAK somatic point mutations have been detected in a high proportion of HC patients. Furthermore, detection of JAK mutations is beginning to provide prognostic information. For all these reasons, manipulating JAK activity currently constitutes an interesting therapeutic strategy and an interesting biomarker in hematological cancer. A great effort has been made by researchers in the last decade to find and characterize novel JAK inhibitors that might be clinically used, and, in fact, some of them have already reached clinical evaluation. However, more efforts are needed in order to identify more JAK mutations that lead to develop more accurate therapies against specific malignancies.

## Acknowledgements

We thank all the authors who contributed to the understanding of the cross-talk between the JAK kinase and hematological cancer. We apologize to those whose work deserves to be cited but unfortunately are not quoted because of space restriction. The research program in the author's lab was supported by grants-in-aid from the Spanish Ministry of Economy and Competitiveness (MINECO) with the funding of European Regional Development Fund-European Social Fund (SAF2015-65113-C2-2-R), and Alfredo Martin-Reyes Foundation (Arehucas)-FICIC. CR and MMG were supported by postdoctoral grants-in-aid from the MINECO-Juan de la Cierva Program and ULPGC, respectively.



## **Conflict of interest**

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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