Mutations and chromosomal rearrangements of JAK2: not only a myeloid issue


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Until today, JAK2 alterations have been mainly associated with myeloid malignancies among which they play a key pathogenic role in chronic myeloproliferative neoplasms. More recently, aberrations involving the JAK2 gene have also been reported in lymphoid diseases, including acute leukemia and lymphomas. In addition, the constitutively activating JAK2V617F mutation has been identified in some patients affected by B-chronic lymphocytic leukemia with a concomitant myeloproliferative neoplasm. Interestingly, these cases could help us to better understand the pathogenesis of these myeloid and lymphoid diseases and reveal if they share a common ancestral progenitor or just coincide. The involvement of JAK2 in lymphoid neoplasms may suggest the possibility of new therapeutic approaches broadening the use of JAK1-2 inhibitors also to these malignancies.

KEYWORDS: acute leukemia ● B-chronic lymphocytic leukemia ● JAK2 ● JAK-STAT pathway ● JAK2 inhibitors ● lymphoma ● myeloproliferative neoplasms

Janus kinases (JAKs) are a family of intracellular tyrosine kinases (TKs) that are associated with cytokine receptors and are involved in signal mediation by the JAK-STAT pathway. Four JAK family members have been identified (JAK1, JAK2, JAK3 and Tyk2), which share a similar structure of the protein domains: a kinase domain, a pseudo-kinase domain (which lacks TK enzymatic function and assumes a regulatory role) [1], a SH2 domain and a FERM domain [2]. The JAK-STAT signaling pathway is crucial in normal hematopoiesis [3] and may be altered by several genetic alterations and various studies reported that JAK genes are mutated in human cancers and, in particular, in different hematologic malignancies. In 2005, the JAK2V617F mutation was recognized as a key molecular event in the pathogenesis of chronic myeloproliferative neoplasms (MPNs) including polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis (MF) by five different groups independently [4–8]. The JAK2V617F mutation confers to the hematopoietic progenitor cells growth-factor independent proliferation due to the constitutive activation of the enzyme which initiates the downstream cascade of the JAK-STAT pathway. There is also strong evidence that the hematopoietic precursor bearing this mutation acquires a proliferative and survival advantage [9,10]. In 2007, Scott et al. described different mutations (small insertions and deletions located in the JAK2 exon 12) conferring different types of gain of function in some patients affected by a JAK2V617F-negative PV [11]. Further evaluations confirmed the presence of an alteration of JAK2 within the exon 12 in about 4% of the patients affected by a PV which were characterized by peculiar clinical features such as higher hemoglobin levels and lower levels of platelets and leukocytes compared with patients with JAK2V617F mutation [12]. More recently, the deletion of the entire exon 14 of JAK2 was described in a small portion (0.5%) of PV patients. This aberration is probably due to an alteration of the splicing processing of mRNA that results in the synthesis of a truncated JAK2 protein (Δexon 14) [13]. However, it is still not clear the functional role of exon 14 deletion which most likely has no pathogenic relevance. 3All in all, the alterations of the JAK2 gene result in a constitutive activation of JAK-STAT signaling which is likely to be a key event for the development of a neoplastic proliferation of hematopoietic cells [14]. Additional studies have described the presence of JAK2 mutations in...
other myeloid neoplasms and myelodysplastic syndromes (MDS) suggesting that JAK2 might be implicated in the pathogenesis of these diseases. Finally, several reports now indicate the involvement of JAK2 in several lymphoid malignancies including acute lymphoblastic leukemia (ALL), Hodgkin’s lymphoma (HL) and non-Hodgkin’s lymphoma (NHL) and B-cell chronic lymphocytic leukemia (B-CLL). In this article, we will review the most frequent genetic lesions or functional abnormalities involving the JAK2 gene in these latter malignancies.

**Table 1. Chromosomal rearrangements involving JAK2 in acute leukemia and lymphoma.**

<table>
<thead>
<tr>
<th>Chromosomal rearrangement</th>
<th>Type of ALL or lymphoma</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAX5-JAK2</td>
<td>B ALL</td>
<td>[23,24]</td>
</tr>
<tr>
<td>PCM1-JAK2</td>
<td>Secondary AML and early pre-B ALL T-cell lymphoma</td>
<td>[20,22]</td>
</tr>
<tr>
<td>SEC31A-JAK2</td>
<td>CHL</td>
<td>[45]</td>
</tr>
<tr>
<td>SSBP2-JAK2</td>
<td>Pre-B ALL</td>
<td>[16]</td>
</tr>
<tr>
<td>ETV6-JAK2</td>
<td>Early pre-B ALL</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>Pediatric T-ALL</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>Pediatric T-ALL</td>
<td>[15]</td>
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</table>


**Translocations involving JAK2 in acute leukemias**

Acute leukemias have been the first type of hematologic malignancies in which a JAK2 genetic lesion was identified [15]. The JAK2 gene has been found involved in several chromosomal translocations with different partner genes in patients affected by hematological malignancies, although they are a rare phenomenon, with only a few cases described in literature (Table 1). The breakpoints in JAK2 and each of its partner genes are variable, with no apparent disease specificity. Despite this variability, all chimeric proteins contain a common attribute: a domain capable of dimerization which leads to the autoactivation of JAK2 even in the absence of the growth factor stimulation [16]. A detailed biochemical characterization of most of these fusion products has not been reported yet, mainly because of the rarity of these events [17]. In 1997, two different groups characterized a chromosomal translocation that resulted in the fusion of JAK2 and ETV6 genes in a pediatric T-cell ALL [15,18]. The investigators proved that this translocation generated a fusion between the ETV6-specific oligomerization domain and the JAK2 catalytic domain resulting in a constitutive TK activity [15]. In the same year, Peeters et al. described the presence of the ETV6-JAK2 chromosomal translocation also in a patient affected by an atypical chronic myeloid leukemia (aCML) and in another patient affected by early pre-B ALL [19]. Later on, in 2005, PCM1, which had already been described in other tumor fusion genes, was identified as another partner for JAK2 in patients with aCML/chronic eosinophilic leukemia, secondary acute myeloid leukemia (AML) and pre-B ALL carrying the chromosomal translocation t(8;9) (p22;p24). The PCM1-JAK2 fusion protein preserves the PCM1 coiled motifs that seem to act as dimerization domain to induce constitutive activation of the JAK2 kinase [20–22]. In 2009, Poitras et al. identified SSBP2 as another fusion partner of JAK2 derived from a t(5;9)(q14.1;p24.1) in a 39-year-old female affected by a pre-B ALL. Even if the normal function of SSBP2 is poorly understood, the presence in its amino-terminal part of the LisH motif, which has been found to play an important role in dimerization of the platelet-activating factor acetylhydrolase IB subunit alpha, suggested that the molecular mechanism for JAK2 constitutive activation is similar to the others previously described [16]. Moreover, a study conducted by the Groupe Francophone de Cytogénéétique Hématologue on a cohort of 153 adult and pediatric B ALL patients carrying karyotypic abnormalities at chromosome 9p identified a PAX5-JAK2 fusion transcript in a del(9)(p13;p24) case. This aberration resulted in an in-frame chimeric transcript, which fused exon 5 of PAX5 to exon 19 of JAK2. The PAX5 gene encodes a transcription factor belonging to the family of paired-box domain transcription factors and was described to be involved in several leukemia-associated rearrangements [23]. The authors supposed that the deletion occurred before/ or concomitant with JAK2 inversion since the two genes are in opposite orientation on the 9p arm [24]. In conclusion, JAK2 rearrangements are rare events in acute leukemias and their biochemical consequences are poorly understood.

**Point mutations & deletions of JAK2 in acute leukemias**

The original identification of the JAK2V617F mutation in MPNs prompted several investigators to look for this mutation in other hematological malignancies. In 2005, Levine et al. sequenced exon 14 of the JAK2 gene and reported that the JAK2V617F point mutation occurs in other myeloid malignancies including chronic myelomonocytic leukemia (CML) and BCR-ABL-negative aCML. Moreover, these investigators identified the molecular alteration in two of 48 patients affected by a MDS (~4%) and in four of 219 patients affected by AML (~2%) [25]. Three of these AML patients were characterized by a previous history of MPN while in one case no evidence of a pre-existing MPN could be documented. The presence of the JAK2V617F mutation in BCR-ABL-negative aCML (three/16 patients, 19%), megakaryocytic AML (two/11 patients, 18%), CML (seven/52 patients, 13%) and MDS (one/68 patients, 1%) was confirmed in the same year with a more sensitive technique [26]. Confirming these results, JAK2V617F was considered frequent in AML secondary to a preceding MPN (~6%) but a rare finding in de novo AML (only three patients among 162 AML cases) [27]. Furthermore, Lee et al. studied the JAK2 gene in 113 AML patients discovering that a single patient presented a 1821G-C transversion in exon 14 giving a lysine to asparagine substitution in a pseudokinase domain conserved residue [28]. Until now, there is no evidence of
JAK2V617F mutation in ALL [25,26,29]. In 2006, Kratz et al. confirmed this rare incidence also in an analyzed cohort of 286 children affected by ALL. Nevertheless, in this group of patients an isolated case carrying a different point mutation that causes a leucine to serine substitution at the 611 amino acid of the JAK2 gene was described. This mutation was identified in the DNA extracted from bone marrow at diagnosis but it was absent in remission samples and it is thus likely to represent a pathologic mutation [30]. Other JAK2 somatic mutations were reported in pediatric ALL patients affected by Down syndrome (DS-ALL). In 2007, a small deletion within the JAK2 gene in a child affected by DS-ALL has been described by Malinge et al. This alteration causes the synthesis of a mutated JAK2 protein lacking five amino acids (from 682 to 686) called JAK2ΔIREED. In vitro experiments conducted by the same group introducing the JAK2ΔIREED construct in a murine hematopoietic IL-3-dependent Ba/F3 cells demonstrated a constitutive activation of the JAK-STAT pathway as a consequence of this deletion [31], comparable with the results obtained in the control Ba/F3-JAK2V617F cells. Moreover, Bercovich et al. sequenced from exon 10 to 25 of the JAK2 gene in 88 DS-ALL patients and pointed out that 16 patients (18%) presented an acquired missense mutation on the highly conserved amino acid 683 that caused constitutive JAK-STAT pathway activation and cytokine-independent growth [32]. Considering these robust data, we can assume that JAK2V617F mutation is a pro-tumorigenic of myeloid diseases but other alterations of the JAK2 gene (translocations, deletions and single nucleotide mutations) might be involved in the pathogenesis of ALL.

The role of JAK2 in lymphomas

The presence of the JAK2V617F mutation has been investigated in HL and primary mediastinal B-cell lymphoma (PMBL) but all cases proved negative [33]. By single strand conformation polymorphism analysis, the absence of the JAK2V617F mutation was also reported in 117 cases of NHL disease [34]. Although other JAK2 point mutations have been described in lymphomas, some chromosomal translocations as well as copy number alterations inducing a pathological activation of the JAK2 signaling and variations in JAK2 expression profile have been reported [35]. In HL, about 95% of patients are affected by the classical form of the disease (cHL), while 5% of patients are affected by nodular lymphocyte-predominant HL (lpHL) [36]. Molecular studies on cell lines derived from HL proved that the JAK-STAT is one of the many deregulated signaling pathways in this disease. The altered JAK-STAT signaling pathways may provide the Hodgkin and Reed-Sternberg lymphoma cells (HRS) with additional proliferation signals and protection against apoptosis. Since HRS cells usually account for only 1% of the total tumor mass, their rarity make it difficult to understand the genetic and molecular biology of HL [37]. To overcome this limit, Joos et al. collected pools of 30 CD30+ Hodgkin cells from 11 cHLs and 1 lpHL and tested the obtained DNA by PCR amplification and a subsequently comparative genomic hybridization [38]. The results reported that 53 imbalances were detected in the Hodgkin cells pools in 11/12 of the analyzed tumors and gains were much more frequent than losses. These gains involved mainly chromosomal arms 2p, 12q and 9p. Interestingly, amplified DNA sequences were identified on 9p23-24 and the candidate gene for this site is JAK2 [39]. The chromosomal region 9p23-24 is recurrently gained in about 33% of HL as well as in the 50% of PMBL [39,40]. The PMBL is a rare disease with peculiar features that may resemble those observed in the more frequent cHL that share many characteristic hallmarks. It is the case of the frequent gain of 9p24 involving JAK2, as just showed, and also the gain of 2p13-p16 involving the REL gene [39]. In a following study, Joos et al. demonstrated that the JAK2 oncogene had an increased copy number in three different HL-derived cell lines (HDLM-2, KM-H2 and L428) and that the JAK2 gene was also targeted by a translocation breakpoint in the HDLM-2 cell line [41]. Additionally, also in gray zone lymphoma, a peculiar disease with overlying morphologic and immunophenotypic characteristics between the nodular sclerosis subtype of cHL and PMBL [42], 9p24 was described to be affected by genetic aberrations. The JAK2/REL locus in 9p24.1 is exposed to gains or amplifications in about 50% of cases. Patients with evident mediastinal involvement have a higher 9p24.1 aberration incidence, but there is no statistical significance compared with the cases without mediastinal involvement [43]. Only recently, a novel JAK2-involving t(4;9) (q21;p24) translocation has been identified by a cytogenetic study in a case of cHL with a complex karyotype. A FISH analysis showed that the breakpoint mapped on 9p24 between exons 9 and 18 of the JAK2 gene, while the partner breakpoint mapped on the 4q21 region harboring SEC31A gene, which encodes for a subunit of the coat protein complex II (COPII)-coated vesicles, essential for protein secretion [44]. This translocation produced an in-frame fusion of exon 22 of SEC31A to exon 17 of JAK2. A further attempt to identify additional cHL cases harboring the SEC31A-JAK2 fusion gene was made preliminarily on 14 selected cases with increased number of HRS cells and subsequently on 131 unselected cHL cases by interphase FISH analysis. A total of four cases with JAK2 rearrangements were recognized, among which two cases presented the SEC31A-JAK2 fusion product and other two cases had novel unknown JAK2 fusion partners, indicating that JAK2 rearrangements are recurrent in cHL and may occur in about 3% of cases (Table 1) [45]. As other rearrangements, also SEC31A-JAK2 results in the constitutive activation of JAK2 and its downstream signaling, even if it is not clear in this case which SEC31A domain drives the activation. Of note, the SEC31A-JAK2 was introduced in Ba/F3 cell line to demonstrate its implication in oncogenic transformation. Indeed, this fusion gene is able to transform Ba/F3 cells making their in vitro growth cytokine independent. Interestingly, the SEC31A-JAK2 sensitivity to JAK2-inhibitors tested in SEC31A-JAK2 Ba/F3 transformed cells showed a dose-dependent decrease of proliferation and JAK2 phosphorylation. The effectiveness of the JAK2 inhibitors on SEC31A-JAK2 could be of great clinical
interest for targeted therapy of lymphomas carrying JAK2 rearrangements [45].

The focus on JAK2 in lymphomas is not only related to the presence of rearrangements or mutations, but also to variations in its expression. The suppressor of cytokine signaling gene (SOCS-1) has a critical role in these cases as it mediates a negative feedback loop that downregulates JAK2 activity and its protein expression. Abrupt silence of the SOCS-1 gene has been described in mantle cell and follicular lymphoma [46] and deletions within SOCS-1 have been detected in PMBL [47]. Moreover, point mutations or in-frame insertions/deletions, particularly in the coding region of SOCS-1 gene, were detected also in immunodeficient patients affected by NHL, suggesting that altered SOCS-1 protein activity may play a role in the lymphomagenesis in this subgroup of patients [48]. Since SOCS-1 was found mutated in the PMBL-derived MedB-1 cell line that shows a delayed JAK2 protein degradation and a constitutively activated JAK-STAT pathway, SOCS-1 status was investigated also in cHL that biologically resembles PMBL [49]. The mutation analysis showed that the SOCS-1 gene is frequently mutated both in HL-derived cell lines (three out of five HL cell lines) and in HRS cells from cHL patients (eight out of 19 cases). The genetic alterations detected in SOCS-1 were different in the mutated cell lines and had different zygosity status. The mutations recognized in the patients samples were similar to the ones described in cell lines and in the majority of cases (seven out of eight) out-of-frame deletions were identified affecting the SH2 domain in the SOCS-1 gene and resulting in an accumulation of phospho-STAT5 [33]. By immunohistochemistry analysis, a high JAK2 expression has been observed in the majority (40/47 cases) of lpHL [50]. In a further investigation on the activation status of STAT3, STAT5 and STAT6, no activation of STAT3 and STAT5 was observed, but STAT6 was phosphorylated in 49% of cases (21/43). Given that SOCS-1 performs a negative-feedback loop for JAK2 regulation, the investigators analyzed the SOCS-1 gene and identified different somatic hypermutations among this gene, while no mutations were found within JAK2 [50]. Novel chromosome 9p24 abnormalities have been also reported affecting other lymphoid neoplasms including a t(8;9)(q13;24) involving JAK2 in a patient affected by diffuse large B-cell lymphoma (DLBCL) and add(9)(p24) in patients affected by mantle cell lymphoma (n = 1), plasma cell leukemia (n = 1) and DLBCL (n = 2) [51]. Interestingly, in vitro expression studies on cell lines derived from human DLBCL proved that the disease is characterized by a persistent STAT3 activation [52], but the mechanism of such an activation is presently unknown. Further studies using the DLBCL-derived DHL2 cell line showed that the JAK-STAT pathway is specifically stimulated by IL-10 that may lead to JAK2 hyperphosphorylation. Notably, high serum levels of IL-10 in DLBCL patients correlate with clinical aggressiveness and shorter event-free survival. In these cases, a treatment with JAK2 inhibitors, known to be able to block JAK2 phosphorylation and IL-10 secretion in MPNs [53], could be an innovative therapeutic approach.

**JAK2 mutations in B-cell CLL**

B-CLL is the most common type of leukemia in the western countries with an incidence of 4.2/100,000/year with a median age at diagnosis of 72 even though about 10% of B-CLL patients are younger than 50 [54]. The clinical course of this disease is heterogeneous ranging from very indolent with a life expectancy virtually identical to unaffected individuals with a delayed or no need of treatment to rapidly progressive leading to death despite early and frequent therapies [54, 55]. In the last few years, several studies were assessed to identify biological markers that can be helpful to define the prognosis of the disease and the most appropriate treatment. Since JAK2 is a TK that transduces signals from various cytokines involved in the pathogenesis of B-CLL [56], the presence of the JAK2V617F mutation in this disease has been investigated by different groups. The screening for the detection of JAK2V617F mutation by direct sequencing of the JAK2 exon 14 or by allele-specific PCR (ASO-PCR) with Bsa XI restriction analysis proved negative in two cohorts of 45 and 78 patients, respectively [25, 56]. However, there are few case reports describing the coexistence of B-CLL and PV, ET or MF published from the 70s only with descriptive intention. More recently, a retrospective analysis on a series of 46 patients simultaneously affected by B-CLL and a Ph-positive or -negative myeloproliferative disease suggested the indolent clinical behavior of the lymphoproliferative disease. In addition, prior chemotherapy for B-CLL did not increase the risk to develop the myeloproliferative disease and hydroxyurea used for MPN did not affect the course of B-CLL [57]. Since 2006, some case reports described also the JAK2 mutational status in these particular cases. In 2009, Tabaczewski et al. reported the coexistence of a B-CLL and a MPN illustrating the cases of two old patients affected by B-CLL and ET. Both patients proved positive for the presence of JAK2V617F mutation and were characterized by a high expression of ZAP-70, a biological marker predicting a poor prognosis in B-CLL. In this report, it was not specified in which cellular compartment the JAK2V617F mutation was detected [58]. Up to now, few additional papers reported the simultaneous presence of PV or ET in B-CLL patients in which the JAK2V617F mutation has been investigated in different cellular compartments (Table 2). In three cases, it was clearly shown that the JAK2V617F mutation was detectable in myeloid but not in circulating B [59] and T lymphocytes [60, 61]. Hussein et al. also searched for the JAK2V617F mutation in two different B-lymphocyte fractions, CD5+ and CD5- leukemic B cells, but they were found equally negative. Interestingly, in one additional case report it has been described a patient with a simultaneous ET (with a high JAK2V617F allele burden in myeloid cells only) and B-CLL who had two relatives with a chronic lymphoproliferative disorder both bearing a JAK2V617F mutation with a low JAK2V617F allele burden and no hematologic evidence of a myeloid disease. The authors

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**Table 2**

<table>
<thead>
<tr>
<th>Case Report</th>
<th>JAK2V617F Status</th>
<th>Cellular Compartment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>Positive</td>
<td>Myeloid</td>
</tr>
<tr>
<td>Case 2</td>
<td>Positive</td>
<td>B cells</td>
</tr>
<tr>
<td>Case 3</td>
<td>Positive</td>
<td>Both myeloid and B</td>
</tr>
<tr>
<td>Case 4</td>
<td>Negative</td>
<td>Myeloid</td>
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</table>

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**References**

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2. [46]...
3. [47]...
4. [48]...
5. [49]...
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8. [52]...
9. [53]...
10. [54]...
11. [55]...
12. [56]...
13. [57]...
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20. [60]...
21. [61]...
speculated that the presence of the JAK2V617F mutation may represent an increased risk factor for a future development of this type of disease [62]. Furthermore, Swierczek et al. studied three patients affected by concomitant PV and B-CLL: granulocytes resulted positive for the JAK2V617F mutation, but by contrast B and T lymphocytes were negative [63]. Similarly, Wei et al. performed a JAK2V617F PCR analysis on a coexisting (CD5+) B-CLL and ET patient identifying the mutation in peripheral blood nucleated cells and neutrophils, while they could not detect any mutation signal in B- and T-lymphocyte populations [63]. More recently, Stijns et al. reported the cases of two patients with JAK2V617F-positive Philadelphia-negative PV that developed B-CLL. The group performed an allelespecific competitive blocker-PCR analysis for the JAK2V617F mutation in granulocytes, CD20+/CD5+ B cells, CD20+/CD5− B cells, T cells, NK cells and monocytes. The granulocytes positivity for the mutation confirmed the PV diagnosis. At the same time also CD20+/CD5− B cells, T cells and NK were found JAK2V617F positive while the mutation was not detected in the CD20+/CD5+ B cells. On the base of these results, the author concludes that there is no evidence that the proliferative behavior of B cells is mediated by the JAK2V617F mutation even if the B-CLL and the JAK2V617F-positive MPN do coexist [64]. On the contrary, Kodali et al. reported a case of one patient who had both a JAK2-mutated MPN and B-CLL and studied the status of the mutation in stem cells, myeloid and lymphoid cell lines. The JAK2V617F PCR and enzymatic restriction analysis resulted heterozygous for the mutation in myeloid progenitor cells, megakaryocytes, neutrophils and B lymphocytes but negative in T lymphocytes [65]. All in all, taking also into account that some groups claimed the presence of the JAK2V617F mutation in some healthy individuals [66,67], at this time it is not yet possible to define the precise frequency of the JAK2V617F positivity in B-CLL cases for at least two main reasons including the lack of predefined criteria to select cases and most importantly the different sensitivity of the molecular method used to perform such a screening. Indeed, from the initial identification of the JAK2V617F in 2005, several molecular assays have been developed for the detection of this mutation and their performances compared in a comprehensive work by the European LeukemiaNet and MPN&MPN-Net-EuroNet study groups [68]. Many efforts are still being done to obtain a reproducible and highly sensitive method that allows to increase the JAK2V617F detection rate thus identifying a low-level clone and monitoring the response to treatment for MPNs. A high level of sensitivity (under 1% JAK2V617F) can be reached using primers and probe designed specifically for the mutation detection [69]. In particular, for this kind of assay facing to detect a mutation of a single nucleotide it is very important to verify the specificity using a series of negative controls, usually DNA derived from healthy donors. Recently, we developed a novel method for the detection of JAK2V617F based on an innovative non-PCR technique using a Loop-mediated isothermal AMPlification (LAMP) to specifically detect the JAK2 mutated allele (AS-LAMP). This assay detects within 1 h and under isothermal conditions, the JAK2V617F mutation with a remarkable sensitivity (down to 0.01%) and specificity [70]. This technique has been further optimized by the addition of an intercalating dye (Yo-Pro-1, Invitrogen) for fluorescent real-time monitoring and by the introduction of an internal control (endogenous Ableson gene) [71]. Taking advantage from this approach, we analyzed 158 B-CLL patients leading us to detect the JAK2V617F mutation in four cases (~2.5%). The main clinical findings of these patients are summarized in Table 3. In two of these cases (cases 1 and 3), no evidence of myeloid proliferation could be detected at diagnosis and during the clinical follow-up and the JAK2V617F allele burden was very low (around 0.5%) as established by an accurate comparison with adequate calibrators (Figure 1) [70]. In patient two (bearing a low JAK2V617F allele burden), a thrombocytosis was present at diagnosis and during the follow-up (from 475 × 10^9/l to 510 × 10^9/l) while patient four had a diagnosis of PV which was posed 20 years in advance of the subsequent B-CLL. In this latter case, with an allele burden higher than 50%, an ASO-PCR analysis was performed on the genomic DNA obtained on immuno-magnetic purified myeloid and lymphoid subpopulations. The CD3+ and CD19+ cells, proved negative for the JAK2V617F mutation, while the myeloid cells containing fraction was positive (Figure 2).

A recent study conducted on a total of 250 cases reported that the combination of a MPN with a B-CLL accounts for a 50% of the double-disease patients analyzed [72]. Moreover, in these cases the evaluation of JAK2V617F in different lineages is of increasing interest as it could help to understand if the myeloproliferative and lymphoproliferative disorders are both derived from the same pluripotent stem cell or just coincide. Concerning this issue, several independent investigators

<table>
<thead>
<tr>
<th>Cases (n)</th>
<th>JAK2V617F status in the lymphoid compartment</th>
<th>Concomitant MPN</th>
<th>Ref.</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>Negative PV</td>
<td>ET</td>
<td>[60]</td>
</tr>
<tr>
<td>2</td>
<td>Positive in CD5− B lymphocytes and NK.</td>
<td>PV</td>
<td>[64]</td>
</tr>
<tr>
<td>3</td>
<td>Negative PV</td>
<td>ET</td>
<td>[61]</td>
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Table 2. JAK2 mutational status in the lymphoid lineage in B-CLL patients with a co-existing MPN.

B-CLL: B-cell chronic lymphocytic leukemia; ET: Essential thrombocythemia; MPN: Myeloproliferative neoplasms; NK: Natural killers cell; PV: Polycythemia vera.
identified a JAK2 haplotype that has an increased risk of acquiring mutations in this locus. In particular, comparing the two parental JAK2 alleles more than 80% of JAK2V617F mutations were acquired on GGCC or 46/1 haplotype, showing a non-random distribution of this mutation [73-75]. The same remarkable consideration was subsequently done also for the mutations within JAK2 exon 12 [76]. Thus, this sequence variant of JAK2 confers susceptibility to both JAK2V617F mutated [73] and unmutated MPNs [77]. On the contrary, a study conducted in 2011 demonstrated that the risk of developing lymphoid malignancies is not associated with the GGCC haplotype [78]. In the same year, Rumi et al. studied 22 patients affected by a MPN who presented also a further lymphoid neoplasm and they concluded that MPN patients have a higher risk to develop a lymphoid malignancy than the general population but there was no statistically significant association between the GGCC haplotype and the possibility to have the simultaneous existence of lymphoid and myeloid neoplasms. However, these results were based on a small number of cases since the occurrence of both diseases is a rare phenomenon [79]. Indeed, there are still many questions whether MPNs and chronic lymphoproliferative diseases, such as CLL, are clonally related or have independent pathogenesis. Recently, Swierczek et al. tried to answer to this open issue, studying the case of a

Table 3. Clinical features of B-CLL patients found positive for the JAK2V617F mutation.

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
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<td>55 (M)</td>
<td>65 (M)</td>
<td>54 (M)</td>
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<td>B-CLL</td>
<td>B-CLL</td>
<td>PV</td>
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<td>Lines of previous hematologic treatments</td>
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<td>None</td>
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<td>16</td>
<td>14.7</td>
<td>14.5</td>
</tr>
<tr>
<td>WBC count, ×10⁹/l</td>
<td>37</td>
<td>23</td>
<td>48.8</td>
<td>19.4</td>
</tr>
<tr>
<td>Platelet count, ×10⁹/l</td>
<td>28</td>
<td>510</td>
<td>200</td>
<td>295</td>
</tr>
</tbody>
</table>

The clinical and hematologic data refer to the time when the peripheral blood was collected and analyzed for the JAK2V617F mutation.

B-CLL: B-cell chronic lymphocytic leukemia; M: Male.

Figure 1. Annealing results of the AS-LAMP assay. The AS-LAMP assay specific for the amplification of JAK2V617F was performed on DNA obtained from mononuclear cells of a B-CLL patient (case one). The assay was performed in duplicate and in parallel with a standard curve created using four calibrators (0.5, 1, 10 and 100% JAK2V617F allele). DNA from HL60 cell line was used as a negative control while the Abelson gene was chosen as a quality control of DNA. The reaction products, obtained by LAMP and characterized by distinct melting temperature, were submitted to an annealing analysis. The case#1 presented a very low JAK2V617F allele burden (around 0.5%) as established by an accurate comparison with the peaks obtained by calibrators. A direct relationship between the amount of mutant target and the height of JAK2V617F peaks obtained after annealing analysis has been consistently observed. The height of ABL and JAK2V617F peaks are inversely proportional. B-CLL: B-cell chronic lymphocytic leukemia.
B-CLL patients may suggest the presence of additional genetic lesions that may probably play as contributing events in the molecular pathogenesis of two different diseases.

Five-year view
As a matter of fact, the knowledge of the crucial role of JAK2 in cytokine signaling and the discovery of the JAK2V617F mutation in MPNs contributed to understand the molecular pathogenesis of these diseases. Furthermore, the characterization of TKs alterations is very attractive because these mutated kinases could represent a therapeutic target, as demonstrated by the great efficacy of TK inhibitors in the treatment of CML. These considerations prompted the development of several inhibitors of JAKs, such as ruxolitinib, SAR302503, CYT387, BMS911543 and CEP701 for the treatment of MPNs [80-82].

Recently, the JAK1/JAK2 inhibitor ruxolitinib was approved by the US FDA for intermediate and high-risk MF [83], while SAR302503 (TG101348), a small molecule specific for JAK2 inhibition, is currently under clinical investigation. However, these new drugs may represent a therapeutic option also in other hematologic malignancies in which JAK2 is involved, like those we have described. Up to now, some studies tried to investigate the potential of JAKs inhibitors in these diseases. In the last year, Lierman et al. studied the efficacy of ruxolitinib against Ba/F3 cells transfected by PCM1-JAK2, ETV6-JAK2 and SEC31A-JAK2 fusion genes observing a suppression of growth and confirming the results obtained in SEC31A-JAK2 Ba/F3 transformed cells by Van Roosbroeck et al. [45]. Moreover, they treated a patient affected by a chronic eosinophilic leukemia characterized by the same chromosomal translocation was successfully treated with ruxolitinib that allowed the achievement of a robust clinical and cytogenetic response after 15 months [84]. Interestingly, a second patient with a similar clinical phenotype (eosinophilia), bearing the same chromosomal translocation was successfully treated with ruxolitinib that allowed the achievement of a robust clinical and cytogenetic response [85]. The clinical activity of ruxolitinib in these two patients seems to be significantly more impressive than observed in MF patients who do not bear translocations or rearrangements involving the JAK2 gene.

In another recent work, Chase et al. assessed ruxolitinib as therapy for patients with JAK2-rearrangement associated MPNs. The group used primary patient-derived colonies showing that ruxolitinib had a significant activity against cells harboring JAK2 gene rearrangements and presented evidence for potential activity against cells with JAK2 amplification [86]. These preliminary data are promising and the feasibility of the treatment with ruxolitinib may be extended to lymphoid neoplasms. Indeed, studies on DLBCL-derived DHL2 cell lines treated with TG101348, a JAK2 inhibitor already used in clinical trials for MPNs, showed a block of JAK2 and STAT3 phosphorylation, of IL-10 secretion and an induction of apoptosis in this cell line [53]. Intriguingly, several studies using JAK2 inhibitors have been conducted in lymphoid neoplasms. Refractory HL cell lines treated with lestaurtinib, a multikinase inhibitor that suppresses JAK2 activity in MPNs, display a dose-dependent inhibition of cell-growth, an increment of

79-year-old Caucasian female diagnosed for a PV in 1998 and for a CLL in 2000 with a 50% JAK2V617F allele burden. Genotyping of individual endogenous erythroid colonies showed that ten/13 colonies were heterozygous for JAK2V617F, one/13 was homozygous and two/13 did not bear the mutation, demonstrating that the JAK2 alteration may not have been the initial pathogenic event in this case. Moreover, the analysis of chromosome X inactivation on the two different compartments proved that PV and B-CLL clones used different active X chromosomes, suggesting that the two diseases derived from two different hematopoietic stem cells [63].

Expert commentary
The discovery of JAK2V617F mutation has significantly contributed to understand the molecular pathogenesis of MPNs, even if it raised the question of how a single DNA base mutation can characterize diseases with such a different phenotype. Several studies reported the presence of the JAK2V617F mutation with a low occurrence rate in other myeloid malignancies. More recently, mutations and rearrangements of JAK2 have been identified also in patients with acute and chronic lymphoid malignancies. Although in these latter malignancies these genetic alterations are rare, an altered JAK2-related signal transduction pathway is described less rarely and, at least in part, it may account for the abnormal proliferation and differentiation of these malignancies. Taken together, these findings suggest that JAK2 could have a key role also in the pathogenesis of lymphoid neoplasms through alternative mechanisms compared with those well described in MPNs. Intriguingly, some recent papers describe the coexistence in the same patient of lymphoid and myeloid neoplasms with JAK2 involvement and these cases can represent a model of great interest for this purpose. The coexistence of a chronic myeloproliferative disorder in some B-CLL patients may suggest the presence of additional genetic

Figure 2. Allele-specific PCR analysis of JAK2V617F. Purified lymphoid T and B cells (CD3+ and CD19+) of a B-cell chronic lymphocytic leukemia patient carrying a concomitant myeloproliferative neoplasm (case four) proved negative for the JAK2V617F mutation, while the myeloid enriched fraction (CD3- and CD19-) was positive. CT is the negative control (a pool of mononuclear cells from eight healthy donors); CT+ is the positive control (the SET-2 cell line) and NTC is the no template control.
apoptosis, an inhibited phosphorylation of JAK2, STAT3 and STAT5 and a reduced mRNA expression of the downstream target Bcl-xL. Treatment in lymph nodes from few cHL patients showed a decrease in cell viability at 24 h of treatment [87]. Furthermore, HL-derived cell lines treated with AZD1480, an inhibitor of JAK2 and STAT phosphorylation, demonstrated that this drug regulates proliferation and immunity suggesting that it might be tested alone or in combination with other therapies for HL [88]. In addition, in the last year a Phase I dose-finding and pharmacokinetic/pharmacodynamic study on SB1518, a macrocyclic pyrimidine-based selective JAK2 inhibitor, proved its potential therapeutic contribution in patients affected by a relapsed lymphoma, obtaining a remarkable decrease in tumor size in half evaluable cases and a partial remission in about 10% of the patients [89]. All together, these data provide a molecular rationale for evaluating JAK2 inhibitors in HL or NHL such as PMBL even though other therapeutic strategies recently proved to be extremely active in these diseases [90,91]. Over the next 5 years, additional efforts should be done to evaluate the efficacy of JAK2 inhibitors either alone or in combination with other drugs in the wide variety of patients who show some kind of JAK-STAT pathway alterations. In this context, future studies should consider also the identification of biomarkers able to predict the response to JAK2 inhibitors. The presence of a molecular marker related to a JAK2 mutation or translocation could be also useful to assess minimal residual disease, even though other competitive laboratory strategies, such as the immunoglobulin gene rearrangement analysis and next-generation sequencing, may be equally or even more informative for this purpose. Taken into consideration that MPN patients have a higher risk to develop a lymphoid malignancy than the general population, the research activity should focus on the identification of new susceptibility loci that may confer a higher risk for developing an additional hematologic malignant disease. Finally, the coexistence of two diseases could be an interesting model to study if there is an ancestral clone shared by MPN and lymphoid neoplasm, that precedes the acquisition of further mutations which may lead in the end to the development of each neoplasm. Since it is possible now to have access to a rapid whole genome sequencing, it seems relatively feasible to purify DNA from CD34+ cells as well as from non-pathologic tissue to identify genetic lesions which confer genetic instability.

Key issues

- The JAK-STAT signaling pathway was described to be crucial in normal hematopoiesis and its alteration has been proved in several hematologic neoplasms.
- The JAK2V617F mutation was recognized as a key molecular event in the pathogenesis of chronic myeloproliferative neoplasms (MPNs) and in other myeloid malignancies albeit with a lower incidence.
- The JAK2 gene has been found involved in several chromosomal translocations with different partner genes in acute lymphoblastic leukemia (ALL). Point mutations, different from JAK2V617F, or small deletions are also present in ALL, most of all in children with an associated Down syndrome.
- The JAK-STAT pathway is implicated also in lymphomas, not only due to the presence of rearrangements or mutations involving the JAK2 gene, but also to variations in its expression. The SOCS-1 protein, which mediates a negative feedback loop on JAK2 activity and expression, presents alterations in these diseases.
- On account of the fact that JAK2 transduces signals from various cytokines involved in the pathogenesis of B-cell chronic lymphocytic leukemia (B-CLL), the presence of the JAK2V617F mutation in this disease has been under investigation.
- The lack of predefined criteria for the selection of cases and the range of different sensitivities characterizing the molecular methods used for screening make it difficult to define the actual frequency of the JAK2V617F presence in B-CLL.
- In cases of MPN with a coexistent B-CLL, the JAK2V617F was studied in different cellular compartments with diverging results. These cases are an interesting model to study if there is a common ancestral clone shared by B-CLL and lymphoid neoplasm.
- The presence of a molecular marker of the disease, represented by JAK2 mutations or translocations, could be useful to assess minimal residual disease detection for the evaluation of the response to treatment and the risk of relapse.
- JAK2 inhibitors are being tested as innovative therapeutic approaches in lymphoid diseases with promising preliminary results.

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No writing assistance was utilized in the production of this manuscript.
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- of interest
- of considerable interest


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- Concomitant MPNs and B-cell chronic lymphocytic leukemia (B-CLL) have been demonstrated to arise from distinct hematopoietic stem cells.


- The 46/1 JAK2 haplotype predisposes to the development of JAK2(V617F)-associated MPNs and provides a model of constitutional genetic factor associated with an increased risk of acquiring a specific somatic mutation.

80 The risk of developing lymphoid neoplasm of MPNs patients was found to be higher than observed within the general normal population.

- This paper provided evidence that ruxolitinib is a promising therapy for the treatment of patients with JAK2 fusion genes.