The role of *JAK2* abnormalities in hematologic neoplasms

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Abstract

In 2005, an activating mutation in the Janus kinase 2 (*JAK2*) was identified in a significant proportion of patients with myeloproliferative neoplasms, mainly polycythemia vera, essential thrombocythemia and primary myelofibrosis. Many types of mutations in the JAK-STAT pathway have been identified, the majority are related to *JAK2*. Currently *JAK2* mutations are important in the area of diagnosis of myeloid neoplasms, but its role beyond the confirmation of clonality is growing and widening our knowledge about these disorders. In addition to that, clinical trials to target JAK2-STAT pathway will widen our knowledge and hopefully will offer more therapeutic options. In this review, we will discuss the role of *JAK2* abnormalities in the pathogenesis, diagnosis, classification, severity and management of hematologic neoplasms.

Introduction

For decades the diagnosis, classification and management of hematologic neoplasms was based on the clinico-pathological features of these disorders. Major progress in both the therapeutics and our understanding of these diseases did not occur until 1960 when Philadelphia chromosome was found in bone marrow cells of patients with chronic myelogenous leukemia (CML), followed by the identification of the molecular defect by the fusion of *BCR* and *ABL* genes in 1982. Fifteen years later imatinib, an ABL tyrosine kinase (TYK) inhibitor, was developed in the late 1990s. Nowadays the diagnosis of CML is dependent on the identification of t(9;22) (q34;q11) or *BCR/ABL* fusion gene and imatinib is one of its first line therapeutics. Other hematologic neoplasms are tracking the footprints of CML. *BCR/ABL* negative myeloproliferative neoplasms (MPNs), particularly the classical polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF), are currently drawing the attention of many scientists in the fields of hematology, oncology, pathology, genetics and pharmacology, after the identification of Janus kinase 2 (*JAK2*) mutation in a significant number of patients diagnosed for these disorders in 2005.[1]

In this article, our aim is to review the role of *JAK2* abnormalities in the pathogenesis, diagnosis, classification, severity and management of hematologic neoplasms.

Classification of myeloid neoplasms

As *JAK2* abnormalities are mainly identified in myeloid neoplasms, we will summarize the current classification scheme for these disorders. In general they are divided into 3 major groups: acute leukemia, chronic leukemia and myelodysplastic syndrome. Chronic leukemia can be sub-divided into *BCR/ABL* positive or negative, those *BCR/ABL* positive will be labeled as CML regardless of their clinico-pathological features unless it is presenting as acute leukemia, while those *BCR/ABL* negative will be divided further into classical MPNs, non-classical MPNs with or without dysplasia, and myelodysplastic syndrome (MDS) (Figure 1). The diagnosis and classification of these disorders are based on peripheral blood counts, blast percentage, type of myelosis, presence of significant dysplasia, extent of fibrosis, clinical features, biochemistry and most importantly genetics.[2-4]

JAK2 abnormalities are not only associated with most of the classical myeloid neoplasms but they are also seen in association with other myeloid neoplasms except *BCR/ABL* positive CML and acute lymphoid leukemia (ALL) where it is only rarely reported as we will see later on.

One of the interesting things regarding the clinical features of myeloid neoplasms is their tendency to transform to acute leukemia; the classical MPNs, beside their pre-leukemic behavior, progress and regress to each other (Figure 2).[3-5]

So, why does an abnormal gene give rise to different disorders? Why do classical MPNs progress and regress? What are the roles of *JAK2* abnormalities in the pathogenesis of hematologic neoplasms?

JAK family

Janus kinase is a family of intracellular non-receptor tyrosine kinases that transduce cytokine-mediated signals. At present, it consists of 4 members: JAK1, JAK2, JAK3 and TYK2.[6]

Janus kinases was named after Janus or *Ianus* who in Roman mythology was believed to be the God of gates, beginnings and endings. He was imagined as having two faces or heads facing in opposite directions.[7]

Indeed, Janus kinases are located just beneath the cellular receptors to control the signal transmission downstream and have seven domains, two of which are structurally similar. One of these (JH1) is an activating domain while the other (JH2) seems to exert an inhibitory effect (Figure 3).

Upon ligand binding to its specific receptor, JAK protein will be activated and it will then phosphorylate the downstream signaling molecules like STATs, which will be actively transported to the nucleus where it will activate transcription factors (Figure 4).

Abnormalities in *JAK1* have been reported in ALL of mainly T cell type where it is found in nearly 20% of the cases,[8] *JAK2* is found in myeloid neoplasms and rarely rearranged in ALL,[6] *JAK3* have been reported in more than 50% of transient abnormal myelopoiesis in Down syndrome patients, acute myeloid leukemia (AML) megakaryoblastic type (M7) and in some cases of severe combined immune deficiency (SCID),[9-11] and TYK2 might have a role in lymphoid neoplasms and natural killer cell functional defects.[12]

JAK2

JAK2 was mapped on the short arm of chromosome 9p24 in 1992 by Pritchard and his colleagues,[13] It has 140 kb spanning 25 exons to form 1132 aminoacid JAK2 protein.[14] It works as a signaling molecule for many cytokines including: INF-γ,[15] erythropoietin (EPO),[16] prolactin,[17] thrombopoietin (TMP), G-CSF, GM-CSF[18] and IL-3[19] via activating many signaling pathways like: MAPK, PI3,[16] ERK[20] and STATs.[14] *PRV1* (CD177) and *NF-E2*

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also appear to be activated and over-expressed by JAK2.[6] One of the most important pathways that are activated by JAK2 is STAT5 followed by activation of *BCL-XL* and finally upregulation of *BCL2* where the cell will gain a survival advantage.[6]

Types of *JAK2* abnormalities and pathogenesis

In general we can divide these into 4 categories:[4,21-25]

A. Rearrangements

JAK2 can be rearranged to:

- *TEL/ETV6*: t(9;12) (p24;p13) reported in some CML like MPNs and T-cell ALL;
- *BCR*: t(9;22) (p24;q11.2) reported in some CML like MPNs;
- *PCM1:* t(8;9) (p22;p24) reported in some MPNs, AML and ALL;
- *NF-E2*: der(9) t(9;12) (p24;q13) reported in some cases of MDS.

B. Point mutations

- V617F G >T at nucleotide 1849 on exon14, reported in mainly the classical MPNs;
- T875N reported in AML (M7);
- R683G and less frequently other R683 point mutations reported in 18-28% of ALL in Down syndrome patients and 10% of a high-risk cohort of childhood ALL in patients without Down syndrome.

C. Deletions/Insertions

- Exon12: there are more than eight reported mutations including deletions and insertions in 538 to 543 codons report ed in 4% of PV cases;
- IREED del which is a five amino acids deletion in JH2 pseudokinase domain reported in B-cell ALL in Down syndrome patients.

D. Numerical

- It can present as trisomy (+9) or be over-expressed due to amplification.

The majority of these abnormalities are affecting the JH2 domain leading to loss of inhibitory effects on JH1 domain, hence the later will be auto-activated. Suppressors of cytokine signaling (SOCS) 1 and 3 are negative regulators for JAK2 kinase, these suppressors will also be phosphorylated and stabilized by the hyperactive tyrosine kinase.[26] SOCS3 promoter methylation is another mechanism that is found in a group of patients.[27]

It is reported that 5-10% of MPN patients have at least one relative who is affected by this disorder, and in familial MPNs the risk of developing these disorders increases 6-fold.[6,28] Recent studies suggest that particular single nucleotide polymorphisms (SNP) are associated with a higher risk of developing *JAK2* V617F mutation. Out of about 659 SNPs, rs10974944 and rs12343867 are reported in 77% and 85% in association with *JAK2* V617F mutation

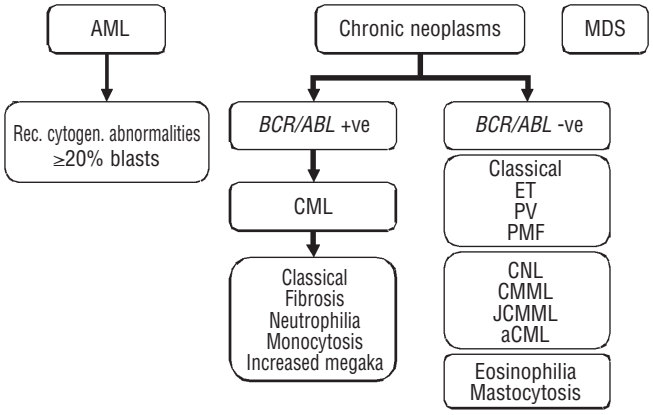

Figure 1. The current classification of myeloid neoplasm. CNL: chronic neutrophilic leukemia, CMML: chronic myelomonocytic leukemia, JCMML: juvenile myelomonocytic leukemia, aCML: atypical chronic myelogenous leukemia.

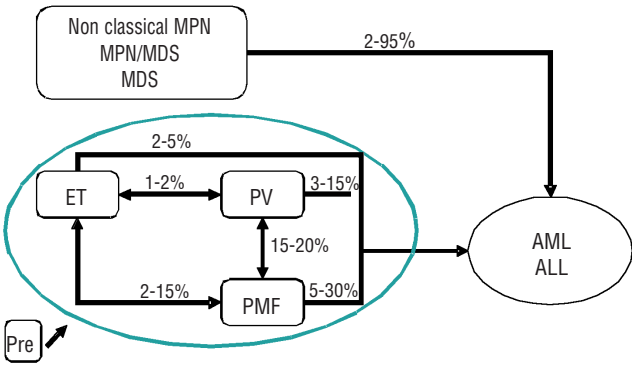

Figure 2. The rate of progression of chronic myeloid neoplasms to leukemic phase. Pre: pre-fibrotic stage of PMF and unexplained thrombotic events prior to the development of MPN.

Figure 3. The structure of Janus kinases

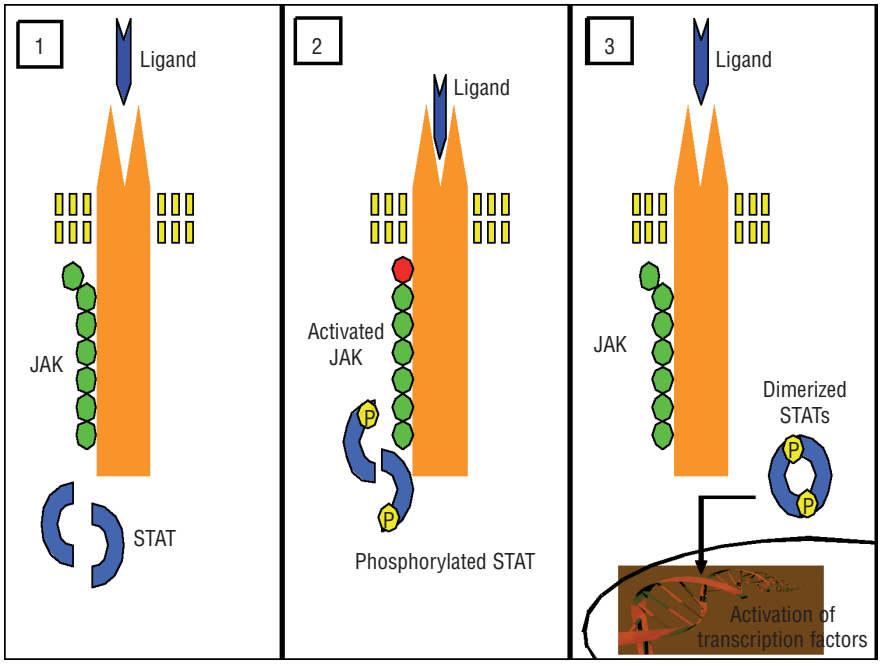

Figure 4. Steps of JAK-STAT pathway.

respectively, with a significant difference if compared to wild-type *JAK2*.[29]

Prevalence of *JAK2* abnormalities in hematologic neoplasms

JAK2 V617F mutation is reported only in myeloid neoplasms with a high frequency in PV, ET, PMF and refractory anemia with ring sideroblast and thrombocytosis (RARS-t). It is rarely reported in CML,[30] but not in ALL or molecularly characterized eosinophilic neoplasms and mastocytosis, i.e. those with abnormalities in *PDGFRA, PDGFRB, FGFR1* or *KIT*. *JAK2* rearrangements are seen in some cases of AML, atypical CML and ALL, while exon 12 mutations are only reported in PV (Table 1).[3,4,31,32]

Screening and quantification tests

Detecting *JAK2* mutations, determination of hetero-or homozygosity, and the allele burden, i.e. the ratio of the mutant allele to the wild-type, are the aims of carrying out molecular studies. Variable screening techniques are available and these are basically utilizing direct sequencing, allele specific polymerase chain reaction (PCR) analysis or ultra sensitive PCR techniques with variable sensitivities that can detect mutant gene at the levels of 20%, 3% and 0.01%, respectively. However, quantification techniques are preferred in order to estimate the mutant allele burden, to monitor the disease course and effect of therapeutics. Peripheral blood and bone marrow samples, frozen plasma and paraffin-embedded trephine bone marrow biopsies can all be used.[2,33] Eric Lippert and his colleagues studied the concordance of assays designed for the quantification of *JAK2* V617F and reported the highest sensitivity by using Taqman allele specific PCR with reverse and forward primers with detection sensitivity up to 0.2% and 0.15%, respectively. But they found these techniques laborious, having false positive results and they were, as expected, capable of detecting only the mutation of interest. On the other hand, they found pyrosequencing and direct DNA sequencing to be the least sensitive, limited to the level of 2% and 5%, respectively, but still having the advantage of detecting new mutations.[34] It has recently been suggested that immunoprecipitations and Western blotting to test for SOCS3 tyrosine phosphorylation may be a novel bio-marker of MPNs resulting from a *JAK2* mutation and a potential reporter of effective JAK2 inhibitor therapy currently in clinical development.[26]

The roles of *JAK2* abnormalities in hematologic neoplasms

Confirmation of clonality

The long list of congenital, secondary or reactive causes for cytosis, cytopenia and dysplasia is creating difficulties in labeling cases for neoplastic conditions without ruling them out, a process that requires extensive investigation steps not to mention the time needed to confirm persistence. Confirming clonality can bypass all these issues and can solve the problematic cases in which a neoplastic condition is co-existing with a secondary or congenital cause. Many methods are used to detect clonality but the most dependable is by performing cytogenetic, fluorescence *in situ* hybridization (FISH) and/or molecular tests. Abnormal immunophenotype and loss of X-linked polymorphism have many limitations in MPNs. It is currently widely accepted that detecting JAK2 mutation, particularly V617F mutation, is a major criterion in diagnosing myeloid neoplasms, especially MPNs. It is now incorporated in the WHO 2008, Nordic 2007, and BCSH 2007 classifications.[3,35,36]

Phenotype and lineage determination

JAK2 abnormalities are seen in many types of hematologic neoplasms as mentioned earlier, and also are reported in 70-80% of cases with unexplained hepatic venous thrombosis without overt neoplasms.[37] Not only, V617F mutation alone is seen in a wide range of myeloid neoplasms. This fact has created much hypothesis regarding the pathophysiology of these neoplastic conditions to the extent that some authors suggest that some disorders are in fact phases of a single disease. Many factors possibly play a role (Figure 5).

The type of abnormality

Some abnormalities are only reported in myeloid neoplasms. These are *JAK2* rearrangements to *BCR*: t(9;22) and *NF-E2*: der(9)t(9;12), V617F and T875N point mutations and exon 12 mutations. On the other hand, R683G and IREED del are reported in lymphoid disorders. While rearrangements to *TEL/ETV6*: t(9;12) and *PCM1*: t(8;9) are reported in both lineages. Some of these abnormalities are only reported in a single entity (Table 1).

The targeted cell or receptor

It is suggested that the phenotype is determined by the ability of the affected stem cells to differentiate into different lineages, and on the receptor affected by the mutation. For example, EPO, TMP, G-CSF or GM-CSF receptors are targeted to give PV, ET, PMF or chronic myelomonocytic leukemia, respectively.[38] Another example is the identification of homozygous *JAK2* V617F mutation in the endothelial cells in the vessels of PV patients with Budd-Chiari syndrome.[39]

Table 1. Prevalence of JAK2 abnormalities in hematologic neoplasms.

Neoplasms	JAK2 V617F mutation	Other JAK2 abnormalities
Polycythemia vera	>95%	4% *JAK2* exon12 mutation
Essential thrombocythemia	50-60%	
Idiopathic myelofibrosis	40-50%	
Chronic neutrophilic leukemia	20%	
Chronic myelomonocytic leukemia	3-13%	
Juvenile chronic myelomonocytic leukemia	20%	
Atypical chronic myeloid leukemia	20%	*JAK2* rearrangement, t(8;9), t(9;12), t(9;22)
MPN/MDS-U	12-20%	
MPN/MDS (RARSt)	50-70%	
Myelodysplastic syndrome (RAEB,-5q)	1-7%	*JAK2* rearrangement, t(9;12)
Secondary AML	4%	*JAK2* rearrangement, t(8;9)
AML (M6)	Few reported cases	
AML (M7)	15%	*JAK2* T875N
Mastocytosis	0-25%	*JAK2* rearrangement, t(8;9), t(9;12)
Eosinophilic neoplasms	0-2%	
Chronic myelogenous leukemia	Rare reported cases	
ALL	Non	*JAK2* rearrangement, t(8;9), t(9;12), *JAK2* R683G and less frequently other R683 point mutations
ALL in Down syndrome patients	Non	*JAK2* R683G, IREED del, other point mutations, insertions and deletions

Genetic background of the patient

Gender is one of the possible determinants, as ET tend to occur in females more than males, while the opposite is true of PV. Iron metabolism is another factor, as iron depletion will lead to ET phenotype rather than PV. The opposite picture will be seen in relation to EPO bioavailability. SNPs are another possible participant as rs10974944 is significantly seen in patients with PV if compared to ET.[4,29,38]

Dosage effect

This appears to be the most important factor, and it can be explained by different thresholds of JAK2 kinase activity at which variable receptors, cytokines and proteasomes need to react. This is supported by the reported results of homozygous *JAK2* V617F mutation occurring in 25-30% PV compared to 2-4% in ET. Few cases are reported that demonstrate dosage dependent phenotype, like those transforming from ET to PV with increasing *JAK2* V617F mutation burden, or from PMF to PV with burden reduction after treatment with hydroxyurea (HU).[40] We have also encountered a case of PMF that transformed to ET after treatment with HU (K Alkhairi, KM Alayed, MK Alabdulaali, unpublished data, 2007) By monitoring allele burden it is found that *JAK2* V617F mutation burden is increasing significantly from ET, PV to PMF.[6,38,40-44] The levels at which the burden is found seem to correspond to a specific phenotype of 25%, 55%, 50% and 60% for ET, PV, PMF and post ET or PV myelofibrosis, respectively.[43] Hypermethylation of the *SOCS3* promoter was identified in nearly 1/3 of patients with myelofibrosis and in both JAK2 mutation positive and negative cases,[27] but not in other types of MPNs; this again will strengthen the role of dosage effect.

Pre-*JAK2* mutation event

Several findings raise the possibility of a pre-*JAK2* mutation event leading or participating in the development of neoplasia: the presence of *JAK2* mutation negative MPNs with 25% abnormal cytogenetics, the finding that 50% of *JAK2* mutation positive cases are seen with recurrent cytogenetic abnormalities, and more interestingly, that the majority of JAK2 mutation positive cases develop *JAK2* mutation negative leukemic blasts when they progress to AML.[4,6,38,45] Some of the cytogenetic abnormalities are suspected to present more in association with *JAK2* mutation to develop a specific phenotype like del 20q in cases with PV.[4]

Clinical severity and progression

Thrombosis, bleeding, splenomegaly, bone marrow failure and evolution to acute leukemia are the main complications of MPNs. In ET, many studies suggested that *JAK2* mutation positive cases have a higher risk of developing venous thrombosis compared to patients with wild-type *JAK2*. In a systematic literature review, Panayiotis D. Ziakas analyzed 17 studies with 2,905 ET patients, of whom 1,646 (65.7%) patients were *JAK2* V617F mutation positive. Thrombotic events occurred in 523 (31.8%) of mutation positive cases and in 255 (20.3%) of patients with wild-type JAK2. Finally, he concluded that JAK2 V617F patients have a 2-fold risk of developing thrombosis (OR 1.84, 95%CI 1.40-2.43).[46] Presence of inherited thrombophilia can increase the realtive risk of the development of thrombosis in patients <60 years of age with ET and JAK2 V617F mutation from 2.23 (95%CI 1.57-3.18) to 7.66 (95%CI 2.66-22.03).[47] High mutation dosage are significantly associated with larger spleen size, higher WBC count, higher lactate dehydogenase (LDH) level and higher risk of developing venous thrombosis or cardiovascular events in patients with ET or PV. However, these findings are not sufficient for chemical cytoreduction intended risk stratification which is still dependent on age, prior thrombotic event and cardiovascular event-related risk factors.[2,4,41,48]

It is still debatable whether an increased *JAK2* mutation burden is associated with increased risk of evolution to acute leukemia. However, there is good evidence of its association with progression from ET to PV to PMF. If evolution to leukemia then occurs, in the majority of cases the leukemic cells will be *JAK2* mutation negative as mentioned earlier. The overwhelming blasts will, therefore, reduce the allele mutation burden which might be considered a sign of evolution if it is not related to chemical cytoreductive therapy (Figure 6).[41,44]

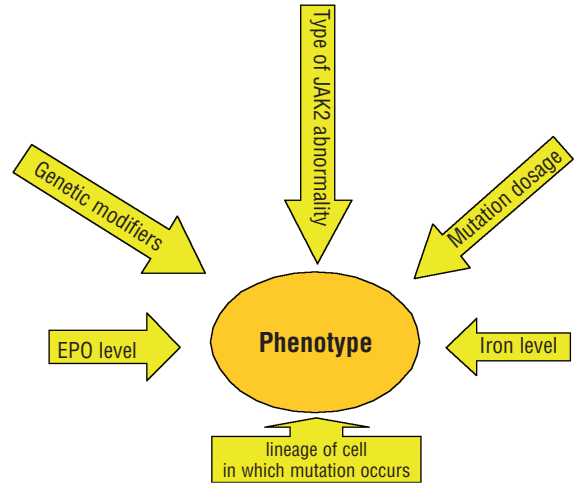

Figure 5. Factors that might determine the phenotype of JAK2 mutation positive disorders.

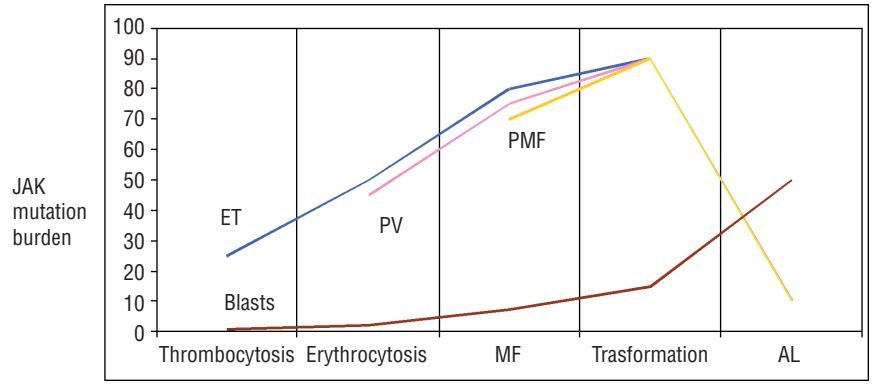

Figure 6. *JAK2* mutation burden during progression of MPNs. AL: acute leukemia, ET: essential thrombocythemia, MF: myelofibrosis, PMF: primary myelofibrosis, PV: polycythemia vera.

JAK2 as a therapeutic target

For decades, the treatment options for patients with MPNs was limited to palliation and preventive measures against the development of thrombosis using aspirin, phlebotomy, splenectomy, splenic radiation or the use of steroids, androgens or EPO in patients with PMF.[1] Since the middle of the 20th century, aims are widening and touching the areas of prevention of leukemic transformation and cure with the use of cytoreductive medications like busulfan, HU and interferon-alpha (INF-α),

together with the trials of stem cell transplantation. But the use of these options always carries the risk of leukemogenesis. So, given the fact that MPNs are very slow progressing disorders and most of the patients are kept under strict control with non-chemical approaches, the use of these options is limited to high-risk patients.

In the last decade, the use of tyrosine kinase inhibitors is expanding in the management of hematologic neoplasms, especially after the successful results of imatinib in CML patients. However, the use of imatinib in MPNs is only achieving limited benefits. It is reported that the use of oral imatinib in PV can reduce the need for phlebotomy and spleen size, while parenteral administration can achieve remission in 22% of cases.[1,4,49] Currently, there are many trials of new agents on patients with PMF, including farnesyl transferase, the aurora family of serine/threonine kinases, vascular endothelial growth factor (VEGF) tyrosine kinase, proteasome and fibrogenesis inhibitors, VEGF neutralizing antibodies and GX15-070MS, an antagonist of the BH3-binding groove of the Bcl-2 family.[1,4]

Many specific JAK2 inhibitors, like INCB018424, TG101209, TG101348, XL019 and TG10134841, are currently in phase I/II trials on advanced stages of MPNs l such as PMF, post-ET or PV myelofibrosis. Recent reports from the trials with INCB018424 are showing significant improvement in splenomegaly, constitutional symptoms, control of myeloproliferation and reduction in allele burden. Non-specific JAK2 inhibitors are also in clinical trials. These include: CEP-701 (an FLT3 inhibitor), tipifarnib (a farnesyltransferase inhibitor), ITF2357 (an HDAC inhibitor) and hypomethylating agents. The main drawbacks of JAK2 inhibitors are hematologic toxicity like neutropenia and thrombocytopenia, non-hematologic side effects which are mainly immunological and endocrinological. Beside that, currently used therapeutic options are well tolerated, and pegylated INF-α and parenteral imatinib are reported to achieve 18-22% remission rates, respectively. Another unpromising issue is the limitation in curing the disease or preventing evolution in the presence of a pre-*JAK2* mutation event and *JAK2* mutation negative leukemic transformation. The development of resistance *in vitro* is another worrying issue.[1,4,6,48-50] For the anticipated toxic effects of JAK2 inhibitors, few agents targeting component downstream of JAK2 are currently investigated like those inhibiting BCL-XL or SOCS1 mimetics.[1]

Conclusions

JAK2 abnormalities are important contributors but not the sole events in the development of hematologic neoplasms particularly MPNs. Its mutations, rearrangements or del are important to confirm clonality, and their type and dosage can help in classifying hematologic neoplasms. Currently, *JAK2* abnormalities are mainly utilized in confirming clonality in diagnosing classical *BCR/ABL* negative myeloproliferative neoplasms; however, we believe that their role will expand to be added in more hematologic neoplasms, to include the type of *JAK2* abnormality in the sub-classifications criteria and, possibly, to consider *JAK2* mutation burden in the process of classification and in the assessment of transformation.

The JAK2 V617F positive MPNs are more closely related and appear with variable clinico-pathological phenotypes in response to different modifiers. They seem to have a more severe clinical course but there is still not sufficient available data to include this mutation in the risk stratification.

JAK2/STAT pathway is an important target for new therapeutics, but more studies are needed to minimize their toxicity.

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