

Molecular markers guide diagnosis and treatment in Philadelphia chromosome-negative myeloproliferative disorders (Review)

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Abstract. The Philadelphia negative chronic myeloproliferative neoplasms are hematological disorders with several diagnostic challenges. Due to recent molecular findings, the WHO classification of Tumors of Hematopoietic and Lymphoid Tissue 2008 reorganized the field of chronic myeloproliferative diseases. Thus, specific molecular markers provide important information for current diagnostic strategies. This review highlights the important diagnostic tools in classical and atypical myeloproliferative neoplasms mainly the JAK2V617F mutation, the Mpl receptor, Polycythemia rubra vera 1 (PRV1), platelet-derived growth-factor receptor α (PDGFRA), platelet-derived growth-factor receptor β (PDGFRB), fibroblast growth-factor receptor 1 (FGFR1) and c-kit tyrosine kinase. A description of the origin, clinical correlations and role in diagnosis and therapy is provided for each of these molecular markers.

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1. Chronic myeloproliferative disorders

Chronic myeloproliferative disorders (CMPD) are a group of heterogeneous diseases with distinct prognostic implications. Until recently, diagnosis and classification were mostly based on clinical presentation, blood cell counts and bone marrow cytology. Diagnosis was difficult and it was often based on exclusion of a multitude of secondary alterations. In many cases, the distinction proved impossible, e.g., between

reactive platelet elevation and essential thrombocytosis. Recently, important discoveries were made with regard to specific mutations in CMPD. These basic research findings were rapidly translated into clinical practice. The most recent World Health Organization (WHO) classification of hematopoietic tumors, published in 2008, brings changes by replacing the term CMPD to myeloproliferative neoplasms (MPN) and by organizing the classical and the atypical myeloproliferative syndromes in new subcategories based on their molecular pathogenesis (1).

This diagnosis-oriented molecular approach was first proposed as semi-molecular classification (see Table I) and implemented by the WHO in 2008 (Table II).

Despite their clinical and phenotypic heterogeneity, CMPD/MPN share similar pathogenic mechanisms that involve the disturbance of tyrosine-kinase receptors or cytokine receptors (2) (Table III).

Therefore, protein molecular markers are associated with different subtypes of myeloproliferative syndromes, with implications in the pathogenesis (Table IV).

Chronic myeloid leukemia (CML) today serves as a paradigm in terms of molecular diagnosis, molecular monitoring and targeted therapy. Elucidation of the pathogenetic mechanisms of CML started in 1973 by the identification of the typical t(9;22) mutation. Much later, the translocated genes were identified and the molecular mechanisms of CML were studied in detail (3-5). The t(9,22) (q34;q11) translocation, (Philadelphia chromosome-Ph1) generates the BCR-ABL chimeric fusion protein. BCR-ABL dyslocalizes the abl tyrosine kinase activity into the cytoplasm. Subsequently activation of multiple cytoplasmic signaling pathways occurs. Main downstream signaling pathways are the Ras/Mitogen-activated protein kinase (Ras/MAPK), phosphatidylinositol-3 kinase/AKT (PI3K/AKT), and signal transducer and activator of transcription (STAT) pathways. These pathways are implicated in mitogenic signaling and enhancement of cellular survival. The development and the world wide use of abl tyrosine kinase inhibitors have dramatically improved CML patients' prognosis.

The WHO reformation of the pH-negative MPN classification emphasizes the importance of the recently discovered genetic mutations (Table I) in the diagnosis of these diseases. The following molecular anomalies imposed themselves as hallmarks in the pathogenesis of these myeloproliferative syndromes: i) acquired point mutations of the gene encoding

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Table I. Semi-molecular classification of chronic myeloproliferative diseases.^a

Main class	Clinical pathogenic entities	Molecular traits
Classic MPS	BCR-ABL positive BCR-ABL negative	Chronic myeloid leukemia (CML) Polycythemia vera (PV) (~100% <i>JAK2V617F</i> ⁺) Essential thrombocythemia (ET) (~50% <i>JAK2V617F</i> ⁺) Myeloid metaplasia with myelofibrosis (MMM) (~50% <i>JAK2V617F</i> ⁺)
Atypical/ borderline MPS	Chronic myelomonocytic leukemia (CMML) Juvenile myelomonocytic leukemia (JMMC): <i>frequent mutations PTP11, NF1 and RAS</i> Chronic neutrophilic leukemia (CNL) (~20% <i>JAK2V617F</i> ⁺) Chronic eosinophilic leukemia (CEL)/myelo- proliferative diseases associated with eosinophilia Hypereosinophilic syndrome Chronic basophilic leukemia Systemic mastocytosis (SM) Unclassified MPS (~20% <i>JAK2V617F</i> ⁺)	PDGFRA gene rearrangements (ex: FIP1L1-PDGFRA) PDGFRB gene rearrangements (ex: TEL/ETV6-PDGFRA) FGFR1 gene rearrangements (ex: ZNF198/FIM/RAMP-FGFR1; 8p11 myeloproliferative syndrome) CEL uncharacterized molecular PDGFRA gene rearrangements (ex: FIP1L1-PDGFRA) KIT mutations (ex: KITD816V) SM uncharacterized molecular mixed/overlapped MDS/MPS (<i>including RARS-T</i>) CML-like syndromes, BCR-ABL negative

^aSee ref. 67. MPS, chronic myeloproliferative syndromes; MDS, myelodysplastic syndromes; RARS-T, refractory anemia with ringed sideroblasts associated with thrombocytosis and megakaryocytic anomalies similar those found in MPS.

Table II. Chronic myeloproliferative neoplasms classification according to the novel WHO criteria.^a

Main category	Subtypes
Myeloproliferative neoplasms	Chronic myelogenous leukaemia BCR-ABL1 positive Chronic neutrophilic leukaemia Polycythaemia vera Primary myelofibrosis Essential thrombocythaemia Chronic eosinophilic leukaemia, NOS Mastocytosis Cutaneous mastocytosis Systemic mastocytosis Mast cell leukaemia Mast cell sarcoma Extracutaneous mastocytoma Myeloproliferative neoplasm, unclassifiable
Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of PDGFRA, PDGFRB and FDGFR-1	Myeloid and lymphoid neoplasms with PDGFRA rearrangements Myeloid and lymphoid neoplasms with PDGFRB rearrangements Myeloid and lymphoid neoplasms with FGFR-1 rearrangements
Myelodysplastic/myeloproliferative neoplasms	Chronic myelomonocytic leukaemia Atypical chronic myeloid leukaemia BCR-ABL1 negative Juvenile myelomonocytic leukaemia Myelodysplastic/myeloproliferative neoplasms, unclassifiable Refractory anemia with ring sideroblasts associated with marked thrombocytosis ^b

NOS, not otherwise specified. ^aSee ref. 1. ^bProvisional entity, supplementary data required.

Table III. Genetic lesions associated with myeloproliferative syndromes.

Genetic defect	Associated disease	Dissemination in population (%)
<i>BCR-ABL</i>	Chronic myeloid leukemia	100
<i>JAK2V617F</i>	Polycythemia vera	95
	Essential thrombocythemia	50-70
	Myeloid metaplasia with myelofibrosis	50-60
	Other myeloid lineage affections	1-5
<i>MPL</i> W515K/L	Essential thrombocythemia	5
	Myeloid metaplasia with myelofibrosis	1
<i>KIT</i> gene mutations	Systemic mastocytosis	
<i>FIP1L1-PDGFR</i>	Eosinophilic leukemia - chronic form	
<i>PDGFRB</i> fusion gene	Chronic myeloid leukemia	Rare
<i>FGFR</i> fusion gene	Chronic myelomonocytic leukemia	Rare
Trisomy 9	JAK gene amplification	Associated to JAK2V617F mutation
Trisomy 8	Chronic myeloproliferative diseases, myelodysplastic diseases, acute myeloid leukemia	Target gene not identified
Trisomy 1q	Determined by duplications, trisomias or unbalanced translocations	
20q deletion	Present in chronic myeloproliferative diseases, myelodysplastic diseases; associated to JAK2V617F mutation, which sometimes precedes	Target gene not identified
5q and 7q deletions	Probable secondary to cytotoxic therapy	Target gene not identified
13q deletion	Associated to myeloid metaplasia with myelofibrosis, the missing piece being also implicated in chronic lymphocytic leukemia	

^aSee ref. 68.Table IV. Protein molecular markers associated with myeloproliferative syndromes.^a

Protein	End effect
<i>BCL-XL</i>	Overexpressed in Polycythemia vera as a result of anti-apoptotic effect of JAK-STAT on the erythroid cells
<i>NFE2</i>	Increased expression in JAK2 positive myeloproliferative diseases, possible implicated in erythroid differentiation
<i>PRV1</i>	Increased ARN levels in Polycythemia vera, without an increase in protein levels
<i>MPL</i>	Decreased protein expression on the cell surface and aberrant glycosylation in the chronic myeloproliferative disease - unclear role in pathogenesis
<i>FIP1L1-PDGFR</i>	Hypereosinophilic syndromes

^aSee ref. 68. FIP1L1, FH interacting protein 1-like 1; PDGFRA, platelet-derived growth-factor receptor α polypeptide; PDGFRB, platelet-derived growth-factor receptor β polypeptide; FGFR, fibroblast growth-factor receptor; BCL-XL, B-cell leukemia/lymphoma 2-like protein X long-transcript variant; NFE2, nuclear factor erythroid-derived 2; PRV1, Polycythemia rubra vera 1; and MPL, thrombopoietin receptor.

Janus Kinase 2 (JAK2) (6-10); ii) mutations of the gene encoding the thrombopoietin receptor (MPL) (11,12); iii) mutations of the genes for the platelet derived growing factor (α -PDGFRA and less frequent β -PDGFRB), represented by deletions in chromosome 4, leading to fusion

gene FIP1L1-PDGFR in hypereosinophilic syndrome (HES) (13); iv) mutations for receptor 1 of the fibroblastic growing factor (FGFR1) (14) or KIT gene mutations (15) encoding stem-cell factor receptor, which occur in systemic mastocytosis.

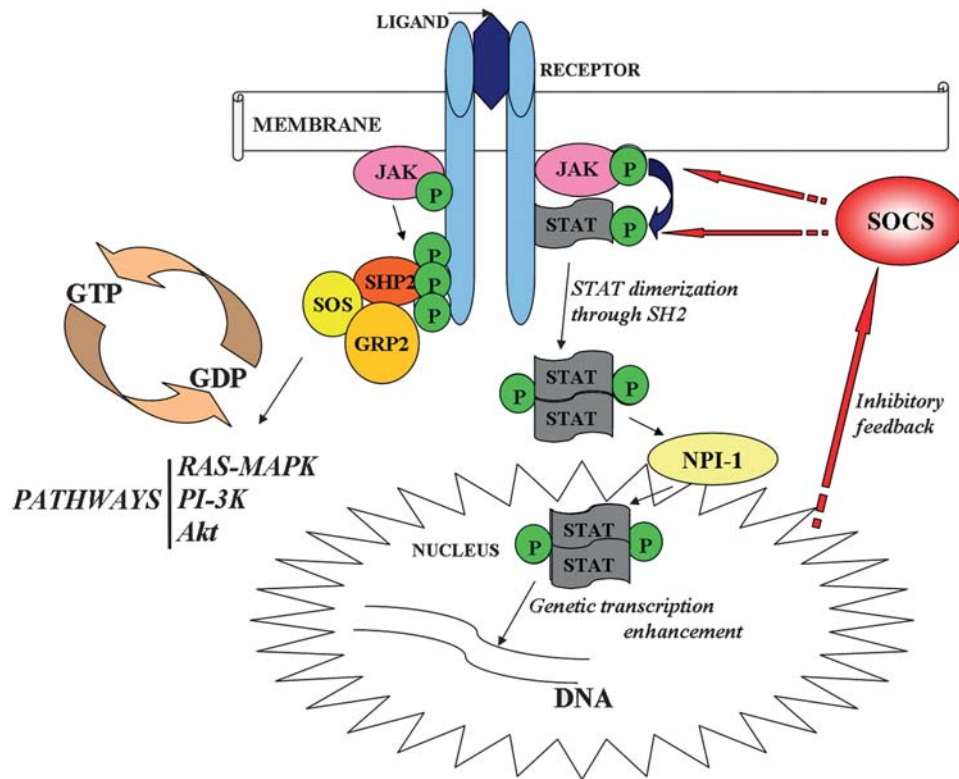


Figure 1. JAK-STAT signalling pathways and the JAK2V617F mutation. The figure depicts main signaling pathways including JAK-STAT signaling (modified after 16).

2. The role of molecular markers in myeloproliferative diseases

JAK2V617F allele. JAK 2 represents one of the 4 janus-kinases that play a role in intracellular signaling. The association of JAK2V617F mutation with myeloproliferative disorders: Polycythemia vera (PV), essential thrombocythemia (ET) and myelofibrosis with myeloid metaplasia (MMM) was first reported in 2005 (8,9) and is considered by far the most common mutation in BCR-ABL negative CMPD. The etiology of the mutation is not known, but it was recently discovered that patients with a specific single nucleotide polymorphism (SNP) pattern at the JAK2 gene harbor a significantly increased risk to develop a JAK2 mutation and concomitant CMPD (Fig. 1).

JAK2V617F is generated by a somatic mutation of guanosine into usually thymidine in the JAK2 gene which results in the substitution of valine with phenylalanine at codon 617. The mutation JAK2V617F activates ligand independent activation of downstream targets. As a consequence, transition from G1 phase to S phase of the cell cycle is accelerated. JAK2V617F induces BCL-XL and cyclin D2 protein expression. The JAK2 mutation also inhibits receptor-mediated apoptosis and leads to a decrease in MPL receptor expression (2). In cell lines, JAK2V617F stimulates cell growth independent of cytokines such as IL3 and erythropoietin, being associated with constitutive activation of JAK2, STAT5 and PI3K pathways.

Two molecules of each protein attach through the FERM domain to the intracellular part of a type 1 heterodimeric

cytokine receptor, like the erythropoietin receptor, G-CSF receptor and MPL or of a type 1 or 2 heterodimeric cytokine receptor such as the receptor family for IL3 or INF- γ , starting a transphosphorylation process between the two components of the receptor. Then JAK2 phosphorylates the receptor's tyrosine residues, and initiates the process of recruiting the next molecules into the signaling chain: PI3K, RAS and STAT5. Each of these molecules is the starting point of some signaling cascades implicated into the regulation of proliferation, differentiation and apoptosis of the cells.

The JAK2V617F substitution is found in the homology domain 2 (JH2) that plays a role in the physiological inhibition of the tyrosine-kinase activity that is maintained in homology domain 1 (JH1). The mutant protein JAK2V617F is constitutively phosphorylated and leads to activation of the signaling pathways STAT5, MAPK/ERK and PI3K/AKT. Full activation is still partially dependent of a ligand interaction with the type 1 homodimeric cytokine receptor. This might explain why the effect of this mutation is seen mainly in erythroid, myeloid and megakaryocytic cell lines that predominantly express these types of receptors.

Several findings clearly indicate JAK2 in PV pathogenesis (2): i) 95% of PV patients show a V617 mutation, and the others might harbor other JAK2 mutations; ii) mice transplanted with mutant JAK2 hematopoietic cells develop a clinical picture and natural history similar to PV: low serum erythropoietin level, splenomegaly with extramedullar hematopoiesis, megakaryocytic hyperplasia with myelofibrosis and late onset of anemia (17). Thrombocytosis is constantly

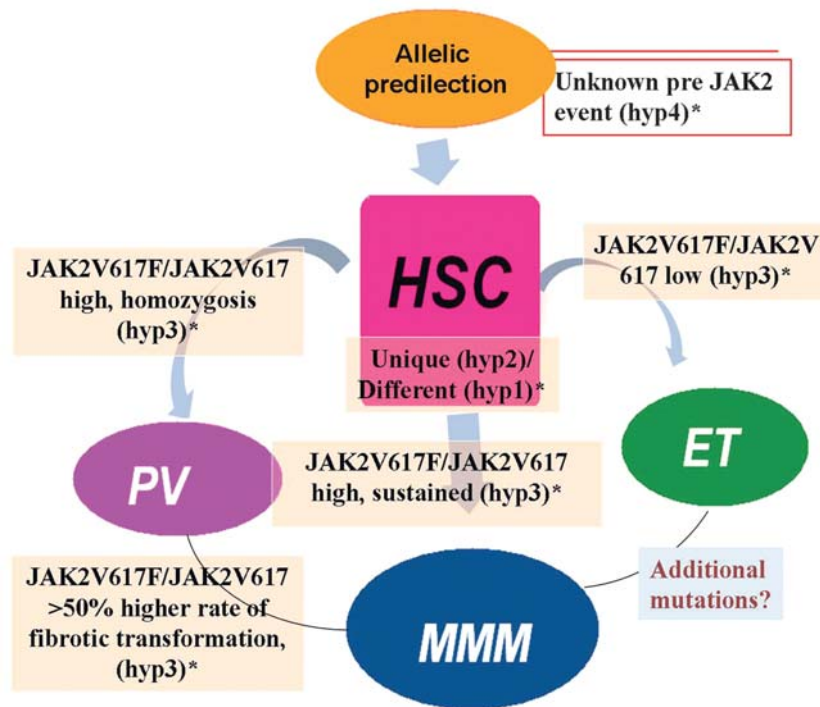


Figure 2. Oncopathogenic role of JAK2V617F mutation in MPN. Several hypotheses exist with regard to the pathogenetic mechanisms of JAK2 mutations. Four main hypotheses are graphically represented. The asterisks and numbers mention the different hypothesis are explained in the text (modified from 31).

absent in PV mice, but it is the main feature in MMM mice associated with MPLW515L mutation. The presence of this mutation explains two features of PV patients - erythropoietin hypersensitivity and terminal amplification of the erythroid lineage.

The JAK2V617F mutation is also detected in other myeloproliferative disorders such as ET in 40-60% of cases and MMM in 50% of cases. There is a particular common phenotype of JAK2 positive mutant MPNs. As an example, JAK2V617F positive ET shares some common features with PV: i) higher level of hemoglobin and neutrophils; ii) erythropoiesis and granulopoiesis are more active in the bone marrow; iii) higher incidence of venous thrombosis and polycythemic transformation; iv) erythropoietin and serum ferritin levels lower than in JAK2V617F negative ET patients.

The JAK2 mutation was also identified in 20-25% of atypical MPN, in a few rare MPN such as HES, chronic neutrophilic leukemia, chronic myelomonocytic leukaemia (CMML); and in myelodysplastic disorders (MDS) or acute leukemia cellular lineages [for example, cellular lineage of humane erythroleukemia (HEL)], and also in few cases of acute myeloid leukemia (AML) (18). In patients with AML that harbor a JAK2 mutation it is possible that the mutation indicates detection of a transformed disease that initially presented as MPN (but was not diagnosed). Alternatively, there might also be cases where JAK2 mutations contribute to an initial disease manifestation. Interestingly, some MPN patients that progress to AML do not show JAK2 mutations in the leukemic blasts. In these cases secondary AML might have developed due to increased susceptibility of the bone marrow or as a consequence of prolonged cytoreductive

therapy (e.g., by hydroxyurea). Kremer *et al* (19) described the JAK2V617F mutation in borderline CMPD/MDS, but could not find it in MDS with fibrosis.

Remacha *et al* (20) identified RARS-T entity (refractory anemia with ringed sideroblasts with thrombocytosis) to have common features with CMPD/MDS: hyper cellular bone marrow with important megakaryocytic hyperplasia and giant forms, with a tendency to evolve into fibrosis. This entity associates JAK2 mutation and it is thought to correlate MDS-type erythropoiesis from RARS subtype, with ET-type myeloproliferative elements.

At present, there are four main hypotheses regarding the involvement of JAK2V617F mutation in the pathogenesis of chronic myeloid neoplasms (21) (Fig. 2): one explanation for the occurrence of different phenotypes as a result of the same oncogenic event is the occurrence of the initial oncogenic event in different hematopoietic progenitor cells. The specific phenotype is therefore a consequence of the properties of the mutated self renewing cell to differentiate into platelets for ET or into erythrocytes for PV. The relevance of this hypothesis is uncertain due to recent discoveries which report other differences in the HSC compartment of PV and MMM patients: JAK2 wild-type for PV and JAK2V617F MMM. These results are obtained in a NOD/SCID mouse model (22).

Another possible theory suggests that the mutation occurs in the same HSC compartment of each patient. The phenotype then differs due to genetic specificity and heterogeneity of each case. This conclusion was first based on experiments in mice in which the same JAK2V617F transduced cells were transplanted in different species of mutation-

specific research mice generating different phenotypes (17,23,24). Recent genotyping of single nucleotide polymorphism (SNP) in the genes for JAK2, G-CSF and receptors for MPL and Epo of patients with MPN showed that certain SNPs of JAK2 and EpoR were particularly associated with PV or ET suggesting that phenotypic variation is the result of the genetic diversity (25).

The 'dosage model' proposes the mechanism of the phenotype diversity as a consequence of the variability in the number of the JAK2 allele copies from one patient to another. It regards all the JAK2V617F MPN as a single disease with different evolution stages due to the JAK2 kinase activity level. A low level of enzyme will generate ET, a high level will cause PV and the long persistence of an increase JAK2 kinase activity might finally induce myelofibrosis (26).

This hypothesis was first generated by Moliterno *et al* (27) in 1998. They reported a 67% level of JAK2V617F in neutrophils of PV patients (range 35-100%), compared to 47% level in neutrophils of ET patients (range 10-63%, $p \leq 0.001$). Vainchenker and his team (2,26) raised the hypothesis that the level of JAK2V617F-kinase activity is crucial in differential diagnosis of different forms of MPN. This hypothesis is also supported by the diagnostic finding that JAK2V617F in PV is often homozygous whereas ET rather presents with heterozygous mutations. In 2008, Tiedt and collaborators showed a strong link between the JAK2V617F/JAK2 wild-type ratio and the MPN phenotype (28).

'The pre JAK2 mutation-event hypothesis' suggests that the different phenotypes would be the result of different genetic events that occur in HSC before the burst of JAK2 mutation. The most striking evidence is the presence of different JAK2V617F MPN in the same families and also the observation that the JAK2 mutation is absent in these patients' B and T lymphocytes, advocating that this mutation is an acquired event and that an unknown germ cell factor predisposes to multiple MPN (29,30).

From a clinical point of view, a general concern regarding the MPN is the risk of developing arterial and venous thrombosis. A study on 806 patients diagnosed with ET reported an association between the occurrence of JAK2V617F mutation and the venous thrombosis (32). Also, an increase in the prevalence of thrombotic events was observed in JAK2V617F MMM patients (33). Multiple studies, identified older age (32-34) higher haematocrit level (32,34,35) and higher white blood cells number (32,34) in relation with JAK2V617F mutation but not obvious risk factors for arterial and venous thrombosis collectively (34). Nevertheless homozygous mutations correlated with a higher risk of fibrosis and a higher level of hemoglobin (36).

Studying a group of 116 patients diagnosed with PV, Vannucchi *et al* (37) reported correlations between clinical parameters and homo/heterozygous status. A significant direct association was found between intracellular JAK2V617F and haematocrit, WBC, LDH, alkaline phosphatase levels, and an indirect relation with medium erythrocyte volume and platelet number.

Patients with a high fraction of mutant JAK2 allele/wild-type JAK2 allele (homozygous) at diagnosis are associated in a higher percentage with splenomegaly and pruritus and they need chemotherapy more often than other patients. A

multivariate analysis including age, leucocytosis, haematocrit, platelet number and therapy options established the above mentioned fraction as an independent risk factor for major vascular events ($p=0.027$) (37).

Another study confirmed these findings (38) in a group of 1306 patients with MPN and established an incidence of thrombosis at diagnosis of 12, 21 and 15%, in wild-type PV, heterozygous and homozygous for JAK2V617F mutation. For ET patients, the percentage of patients with thrombosis at diagnosis were 11, 22 and 50%, and for MMM patients 1, 7 and 3%. The relative risk for thrombosis during disease evolution (adjusted to age, sex and previous thrombosis) was significantly higher in ET patients and in MMM patients.

A recent analysis observed a statistically pertinent correlation between the allele burden (>25%) and the presence of only arterial thrombosis in ET patients (39). The JAK2V617F mutation was also identified as an independent risk factor for pregnancy complications in ET women (40).

Baxter *et al* identified an association of JAK2 in 59% of patients with Budd-Chiari syndrome (BCS) (7). Also, Smalberg *et al* (41) found a 41% prevalence of this mutation in BCS patients, on a group of 40 patients with primary non-malignant BCS. As a consequence, all patients with Budd-Chiari syndrome that lack a clear reason for thrombosis should be analyzed for the presence of an occult myeloproliferative syndrome (42).

Regarding the impact on survival, the results are controversial; there are studies from the Mayo Clinic which did not detect any role of the mutant JAK2V617F status as an independent prognostic factor for the survival in ET (34) or MMM (33). A multicentre European study (43) reported inferior survival in mutation positive MMM and ET patients, possibly related to the higher risk of leukemic transformation. Also, the JAK2V617F mutation in ET patients was associated with the evolution towards PV (34).

The JAK2 mutation also affects the response to treatment. Among ET patients, those with V617F mutation are more sensitive to hydroxycarbamide, but not to anagrelide, compared to patients without mutation. Importantly, treatment response could be evaluated directly by quantitation of JAK2-positive peripheral blood cells. This is especially important for patients undergoing allogeneic hematopoietic stem cell transplantation. The development of specific JAK2 inhibitors might allow disease monitoring similar to BCR-ABL positive CML (44).

In 2008 the 4th edition of the WHO classification of Haematopoietic Tumors established the presence of the JAK2V617F mutation as a clonality marker and a major criteria for diagnosis in PV, ET and MMM.

Mpl - thrombopoietin receptor. Thrombopoietin represents an important regulator of megakaryopoiesis and thrombopoiesis through the thrombopoietin receptor c-Mpl, encoded by the proto-oncogene c-Mpl. This receptor is expressed on CD34⁺ hematopoietic progenitors, megakaryocytes and platelets. Mutations in Mpl and in the erythropoietin receptor were initially identified in familial erythrocytosis and thrombocytosis.

Pikman and his group discovered that Mpl mutations have an activating effect on intracellular JAK2/STAT pathway,

similar to JAK2V617F mutation. Thus, *MPL*^{W515L} mutation, represented by the substitution of tryptophan with leucine at the junction of transmembrane domain with cytoplasmic domain of *MPL* leads to a cytokine-independent cell proliferation and constitutive phosphorylation of JAK2, STAT, AKT and ERK (11).

Transplanting *Mpl* mutant cells in mice induces a lethal MPN characterized by leucocytosis, severe myelofibrosis with megakaryocytic hyperplasia, and severe thrombocytosis, without erythrocytosis. In a group of 1182 patients with different hematological diseases, of which 579 patients were diagnosed with ET and MMM, *MPL* mutation was present in 5.5% of MMM patients and 1.2% of ET patients, but not in PV patients, MDS, AML or CMML patients. The association of mutant *Mpl* with mutant JAK2 was very rare and occurred only in 2 ET patients and 4 MMM patients (11).

On the cell surface, JAK 2 mutation leads to down-regulation of c-*Mpl* expression (45,46) suggesting that the decrease of c-*MPL* expression in PV and MMM is a characteristic of these diseases, being also an element of differential diagnosis between PV and other types of erythrocytosis.

From the clinical and diagnostic point of view the *MPL*W515-positive ET patients versus the *JAK2*V617F-positive ET patients have a higher number of platelets, lower measurement of hemoglobin (47) with higher erythropoietin level and reduced bone marrow general and erythroid cellularity and not erythroid colonies but megakaryocytic colonies growth (48). In addition the *MPL*W515-mutated MMM patients have also a more severe anemia with an increased transfusion support (49).

The thrombotic role of the *MPL* mutation is not well established yet. It was noted as a significant risk factor for microvessel disturbances, probably due to constitutively active *MPL* platelets. This augmented the occurrence of arterial thromboses only in comparison to *MPL* wild-type/*JAK2* wild-type patients (47). In consequence, the *MPL* W515K/L mutation represents a clonality marker considered as major diagnosis criterion for the diagnosis of MMM and ET (WHO classification 2008).

PRV1. PRV1 is a surface protein from the uPAR/Ly6/CD59 receptor family (50). PRV1 mRNA is overexpressed in granulocytes of PV and ET patients (51-54). Depending on the detection technique, independent laboratories have reported PRV1 expression in up to 90% of PV patients (55).

In 2005, a prospective study on 88 patients with MPN from Mayo Clinic (56) has quantified PRV1 expression in neutrophils by RT-PCR. Their results indicated overexpression in 83% of PV patients, 21% of ET patients, 42% of MMM patients and 18% of pseudo-polycythemia patients, compared to 0% in healthy controls. It is important to mention that all the MMM patients with overexpression of PRV1 developed MMM secondary to PV. All of these points suggest that overexpression of PRV1 represents a characteristic of PV which is not affected by the fibrotic transformation of the bone marrow. The diagnostic relevance of PRV1 in the era of JAK2 mutation detection technology is currently still uncertain. It might help in patients with characteristic disease features that lack the defining JAK2V617F mutation.

The level of expression of PRV1 also correlates with the risk of thrombosis. Griesshammer *et al* (57) identified the group of PRV1 positive ET as a separate entity, characterized by common features with PV regarding the thrombotic events and the evolution to PV. The PRV1 positive ET types represent 40% of ET.

Goerttler *et al* (58) identified overexpression of PRV1 as an independent risk factor for thromboembolic complications, while the down-regulation of the c*MPL* expression was not associated with the same risk. In conclusion the WHO classification 2008 did not include the PRV1 as a diagnosis marker for PV.

PDGFRA, PDGFRB and FGFR1. Platelet derived growth factor receptors (PDGFRs) are transmembrane tyrosine kinase receptors that consist of an extracellular ligand-binding domain, a transmembrane domain and a cytoplasmic tyrosine kinase domain (59). They can bind at least five types of active PDGF heterodimers (PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC and PDGF-DD). These factors are released mainly from platelet α -granules and act as potent mitogenic and chemotactic signals for a variety of mesenchymal cells. Affected cells include fibroblasts, vascular smooth muscle cells, glomerular mesangial cells and brain glial cells.

There are two receptor isoforms [PDGF receptor- α (PDGFRA) and - β (PDGFRB) (60)] which also dimerize upon binding of the PDGF heterodimer ligand. Three possible receptor combinations occur ($\alpha\alpha$, $\beta\beta$ and $\alpha\beta$) that activate the tyrosine kinase. The intracellular signaling cascade initiated by these receptors include Ras/Mitogen-activated protein kinase (MAPK) and PI-3 kinase as well as phospholipase- γ (PLC γ) pathways.

The 2008 WHO classification of the Hematopoietic Tumors contains a new category of MPNs associated with rearrangements of PDGFRA, PDGFRB and FGFR1 genes that generate an abnormal tyrosine kinase (1).

PDGFRA related disorders include chronic eosinophilic leukemia (CEL) with an involvement of the mast cell lineage and often the neutrophil lineage. PDGFRA rearrangements were also detected in some rare cases of acute myeloid leukemia and precursor T lymphoblastic lymphoma (T-LBL). The most frequent fusion gene implicated in PDGFRA-associated MPN is FIP1L1-PDGFRB. This mutation is characterized by the loss of around 800 kb DNA sequence at chromosome 4q12 and cannot be detected by conventional cytogenetics. The intra-chromosomal deletion leads to the aberrant chimeric fusion protein FIP1L1-PDGFRB.

This relatively rare (14-16%) (61) form of a hypereosinophilic syndrome was also called the 'myeloproliferative variant of the hypereosinophilic syndrome'. In the absence of genetic evidence it can be suspected in a BCR-ABL negative MPN with splenomegaly and hematological features of CEL, high number of mast cells in the bone marrow, serum tryptase level (>12 ng/ml) and markedly raised B₁₂ vitamin level. From the clinical point of view, CEL presents with a more severe phenotype, with extended organ involvement (1). In these diseases, the heart, lung, gastrointestinal tract, skin and the nervous system (central and peripheral) are commonly affected, as a consequence of the cytokines release. Imatinib inhibits PDGFRA activity and frequently induces

remissions in patients with eosinophilic leukemias. The FIP1L1-PDGFR α gene product is 100 times more sensitive to imatinib than BCR-ABL. Similar to CML management; imatinib resistance may develop as a result of the T674I mutation. This mutation is the equivalent of the T315I mutation in BCR-ABL positive disorders. Other very rarely observed PDGFR α rearrangements (KIF5B-PDGFR α , CDK5RAP2-PDGFR α , STRN-PDGFR α , ETV6-PDGFR α , BCR-PDGFR α) have also responded to imatinib therapy.

In the case of PDGFR β rearrangements, the most frequent clinical finding is chronic myelomonocytic leukaemia with eosinophilia, although aberrant proliferation of mast cells may be a consequence. Very rare cases of transformation in acute myeloid leukemia have been described.

The genetic location of PDGFR β rearrangements is at 5q31-311 chromosome. This anomaly often generates the ETV6-PDGFR β fusion gene [t(5;12)] with clinical features of CMML accompanied by eosinophilia. There are also many exceptions which might mimic a clinical picture of CMML (RABEP1-PDGFR β , NDE1-PDGFR β), of CMML with eosinophilia (KIAA1509-PDGFR β , TP53PB1-PDGFR β , HIP1-PDGFR β , NIN-PDGFR β), of CEL (TPM3-PDGFR β , GIAP1-PDGFR β , WDR48-PDGFR β , GIT2-PDGFR β), chronic basophilic leukemia, atypical CML with eosinophilia, juvenile myelomonocytic leukemia, MPD or MPD/MDS with eosinophilia. Data on a rather small number of cases show that the poor survival of these patients (<2 years) can also be improved by imatinib (62).

FGFR-1 positive diseases often present as T-LBL with eosinophilia. There are also reported cases with this genetic rearrangements and CEL, precursor B lymphoblastic lymphoma/leukemia or AML. The genetic aberrations affect the pluripotent lymphoid-myeloid stem cell and are composed by a variety of translocations at 8p11 breakpoint. In all cases abnormal tyrosine kinase activity is observed for whom no TK inhibitor treatment has been firmly established (1).

c-KIT. This type III receptor tyrosine kinase is involved in a variety of developmental processes. It is the receptor of the stem cell factor (SCF). The gene encodes a five loop transmembrane immunoglobulin-like structure receptor for the stem cell growth factor (SCF), a member of the PDGF and CSF-1 receptor family (63). An intracellular kinase insert splits the catalytic domain into the ATP binding site and the phosphotransferase active site.

The c-kit receptor (CD117) is expressed on mast cells, hematopoietic stem cells, melanocytes and germ cell lineages where it is involved in mediating critical signals for the growth and maturation of these cells (64) especially via the PI3K-Akt pathway (65). Normal kit signaling contributes to hematopoiesis, mast cell development and function, gametogenesis and melanogenesis. Mutations associated with loss of function generate an autosomal dominant condition characterized by depigmented patches of skin and hair named piebaldism. c-kit is altered in several malignancies. Overexpression and mutations of c-kit are found in myeloproliferative disorders, AML, especially core binding factor acute myeloid leukemia and systemic mastocytosis (SM). In addition, c-kit alterations occur in germ cell tumors, gastrointestinal stromal tumors, sino-nasal T cell lymphomas and

myelodysplastic syndromes (66). In patients with mastocytosis, the tyrosine kinase domain of the SCF receptor encoded by the KIT proto-oncogene frequently acquires a somatic point mutation.

The most common site involved is the codon 816 with a change of the amino acid valine to aspartic acid. This induces a self-activated tyrosine kinase which is resistant to classical tyrosine kinase inhibitors such as imatinib (D816V mutation). Sensitive PCR-based laboratory methods identified D816V in ~95% adults with systemic mastocytosis and one third of the children with cutaneous mastocytosis. Several clinical trials are underway that specifically target this mutation. The presence of an activating point mutation at codon 816 of Kit in bone marrow, peripheral blood cells or extracutaneous organs is considered a minor criterion in the newly revised WHO classification of hematopoietic neoplasms.

3. Conclusions

The dramatic therapeutic progress achieved due to inhibition of BCR-ABL in CML has initiated a novel era of genetic and molecular discoveries in hematological disorders. Currently, specific mutations are found in many patients with suspected myeloproliferative disorders. This led to new diagnostic classification. The rapid invention of specific inhibitors for the mutated kinases provides hope that specific therapies can be developed for BCR-ABL negative myeloproliferative syndromes. It can be anticipated that further identification of novel mutations will define novel and so far unrecognized disease entities.

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